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20 YEARS

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Ebola

November 2015

EMERGING INFECTIOUS DISEASES

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Parque dos Continuadores, Maputo, Mozambique.

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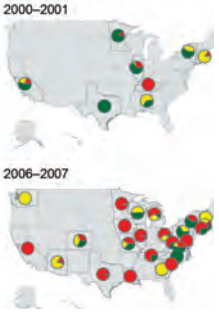

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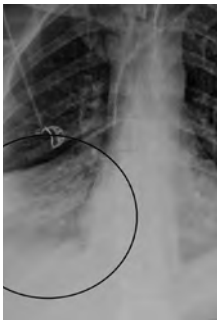

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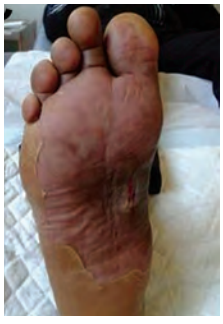

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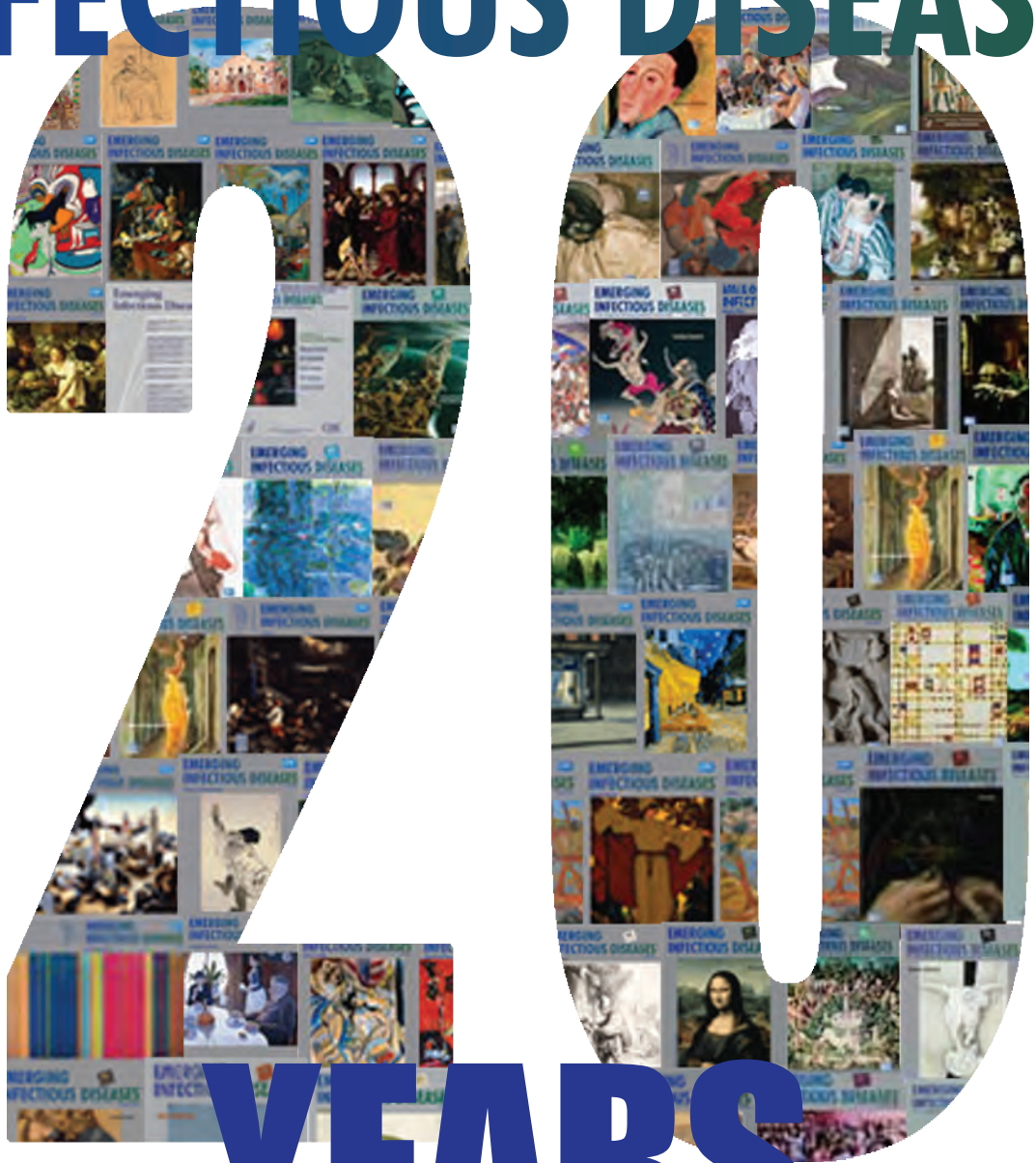

20 YEARS

Presenting the ongoing challenges that emerging microbial threats pose to global health

Ebola in West Africa—CDC's Role in Epidemic Detection, Control, and Prevention

Thomas R. Frieden, Inger K. Damon

Since Ebola virus disease was identified in West Africa on March 23, 2014, the Centers for Disease Control and Prevention (CDC) has undertaken the most intensive response in the agency's history; >3,000 staff have been involved, including >1,200 deployed to West Africa for >50,000 person workdays. Efforts have included supporting incident management systems in affected countries; mobilizing partners; and strengthening laboratory, epidemiology, contact investigation, health care infection control, communication, and border screening in West Africa, Nigeria, Mali, Senegal, and the United States. All efforts were undertaken as part of national and global response activities with many partner organizations. CDC was able to support community, national, and international health and public health staff to prevent an even worse event. The Ebola virus disease epidemic highlights the need to strengthen national and international systems to detect, respond to, and prevent the spread of future health threats.

The unprecedented epidemic of Ebola virus disease (Ebola) in West Africa highlights the need for stronger systems for disease surveillance, response, and prevention worldwide. After a preventable and costly local and global delay, heroic efforts by clinicians and public health personnel and organizations from West Africa and throughout the world broke the cycle of exponential growth of the epidemic and prevented many deaths. As of late 2015, this response, conducted at great expense and personal risk, continues. Here we summarize the experience of the Centers for Disease Control and Prevention (CDC), which complements efforts by the affected countries, the international community, and many partner organizations.

Since Ebola was first reported in West Africa on March 23, 2014, CDC has undertaken the most intensive outbreak response in the agency's history. As of July 2015, >1,200 CDC employees had deployed to the affected countries for >50,000 person workdays; >3,000 CDC staff, including all 158 Epidemic Intelligence Service Officers, have participated in international or domestic response efforts. For context, over the course of more than a decade, ≈300 CDC staff participated in the smallpox eradication program, one of CDC's most notable international responses and most intensive technical collaborations with the World Health Organization (WHO) before the current Ebola response (*1*).

CDC had a team of experts on the ground in Guinea within 1 week after the initial case report. When Ebola resurged and spread, CDC activated its Emergency Operations Center (EOC) (*2*) on July 9, 2014. Since then, CDC has coordinated >1,400 deployments to Guinea, Liberia, and Sierra Leone and sent staff to help Nigeria (*3*), Senegal (*4*), and Mali (*5*) prevent the spread of Ebola. CDC staff also have undertaken development of new diagnostic tests (*6*) and research to evaluate therapeutic drugs (*7*) and vaccine efficacy (*8*,*9*). As of mid-2015, >500 CDC staff continued working throughout the 3 most heavily affected nations (Guinea, Sierra Leone, and Liberia), the West Africa region, and the United States.

At the peak of the epidemic in fall 2014, widespread transmission of Ebola virus was occurring in the capitals of Liberia and Sierra Leone; health care systems had become largely nonfunctional; Ebola cases or clusters occurred in other countries of Africa; and there was a real possibility that Ebola could spread widely and become endemic in some of the poorest and sickest countries of the world. As of late 2015, although the region is not Ebola-free, enormous progress has been made. There is a risk for resurgence and cross-border spread, and because the status of Ebola virus reservoirs is not confirmed and the possibility of sexual transmission from survivors persists, the potential exists for periodic outbreaks.

CDC Response in Heavily Affected Countries

Incident Management

One challenge in responding to complex outbreaks is coordination among partners. CDC's priority in West Africa during summer 2014 was to augment the efficiency of response activities through incident management systems run by national leaders and supported by an EOC reporting to the president of each affected country. These systems were developed in collaboration with WHO and served as the focal point for international assistance. CDC also helped countries establish subnational EOCs in areas with Ebola

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virus transmission in Liberia and Guinea; the United Kingdom similarly played a key role in Sierra Leone. When resources had to be mobilized rapidly, the CDC Foundation, a not-for-profit philanthropic entity authorized by the US Congress in 1992 to help CDC improve its response capacity (*10*,*11*), supported staffing, logistics, data management, informatics, and operations of EOCs.

Epidemiology and Surveillance

Working with governments, nongovernmental organizations, and WHO, CDC epidemiologists assisted national- and district-level staff in each country in identifying cases and contacts and trained in-country staff to perform these essential public health activities. Because clinical, public health, laboratory, and data systems were overwhelmed (*12*), CDC staff assisted with data entry and management, including geographic information systems to track and evaluate disease trends.

Contact Tracing

After the cycle of exponential epidemic growth was broken and personnel could refocus on contact identification, CDC strengthened work with national counterparts and WHO to help improve the quality of contact identification and follow-up, including isolation of symptomatic contacts for clinical assessment and laboratory testing. These activities were vital to reduce Ebola transmission. WHO has played a critical role in improving contact tracing and contact management, particularly in Guinea (*13*).

Laboratory Testing

Global collaboration with laboratories from a European Union consortium made real-time quantitative reverse transcription PCR available in the heavily affected West Africa countries for patients and decedents suspected of having Ebola. CDC experts helped coordinate the laboratory section of the incident management system, supported laboratories in Liberia with the US Department of Defense (DoD) and National Institutes of Health, and operated a field laboratory in Bo, Sierra Leone, that processed >2,000 samples during a 3-week period at the height of the epidemic (*14*); by mid-2015, that laboratory had processed >20,000 samples.

Rapid Isolation and Treatment of Ebola Patients

Rapid isolation and treatment of Ebola patients is a key strategy to stop Ebola outbreaks. Each country had limited capacity to isolate and treat patients, and strategies to do so effectively and safely evolved over time. In collaboration with the US Agency for International Development's Office of Foreign Disaster Assistance (USAID/OFDA), WHO, DoD, and multiple other partners, CDC provided technical support and training to establish Ebola treatment units (ETUs) and community care centers. Beginning in early October 2014, CDC designed and helped implement a strategy of rapid isolation and treatment of Ebola (RITE) in Liberia. This strategy controlled outbreaks faster and supported the care of patients in remote areas, cutting the time to control outbreaks in half (Figure 1) and doubling survival rates (*15*).

Infection Control

In the 3 heavily affected countries, CDC and its partners trained >25,000 health care workers in infection control, including use of personal protective equipment (PPE) (*16*). A 3-day hands-on training course designed by CDC with Médecins Sans Frontières trained >600 US health care providers on Ebola clinical care and infection control before their deployment to West Africa (*17*).

Health Promotion and Communications

In addition to the efforts of partner organizations, CDC field teams included emergency risk communication specialists to generate and disseminate accurate information, address rumors, decrease stigma, reduce unsafe burial practices, and respond to community needs. CDC staff in Liberia and Sierra Leone identified and promulgated burial practices that met community needs for culturally acceptable mourning, thus reducing resistance to safe burials (*18*,*19*). In all countries, community engagement and effective communication were key strategies for successful outbreak control.

Technical Guidance

CDC has issued >200 scientific documents, including >100 technical guidance documents, covering many aspects of the response. CDC staff also worked closely with UNICEF and other partners to develop guidance in related areas, such as safe reopening of schools (*20*).

Mobilization of Partners

During summer 2014, CDC recognized that despite Médecins Sans Frontières' massive response; CDC's own response; and responses of affected countries, WHO, and international partners, the epidemic was spiraling out of control. CDC then advocated to increase involvement by the US government and the global community.

DoD, along with USAID/OFDA's Disaster Assistance Response Team (DART), has been a key partner in this scale-up. Initially focused on researching treatments and vaccines and providing laboratory diagnostics, in September 2014, DoD took the lead on constructing, supplying, and maintaining a field hospital to treat health care workers with Ebola in Liberia. DoD also deployed 3,000 military personnel for logistics and coordination, provision of medical personnel to train health care workers, establishment of additional treatment centers in Liberia, and operation of 3 mobile medical

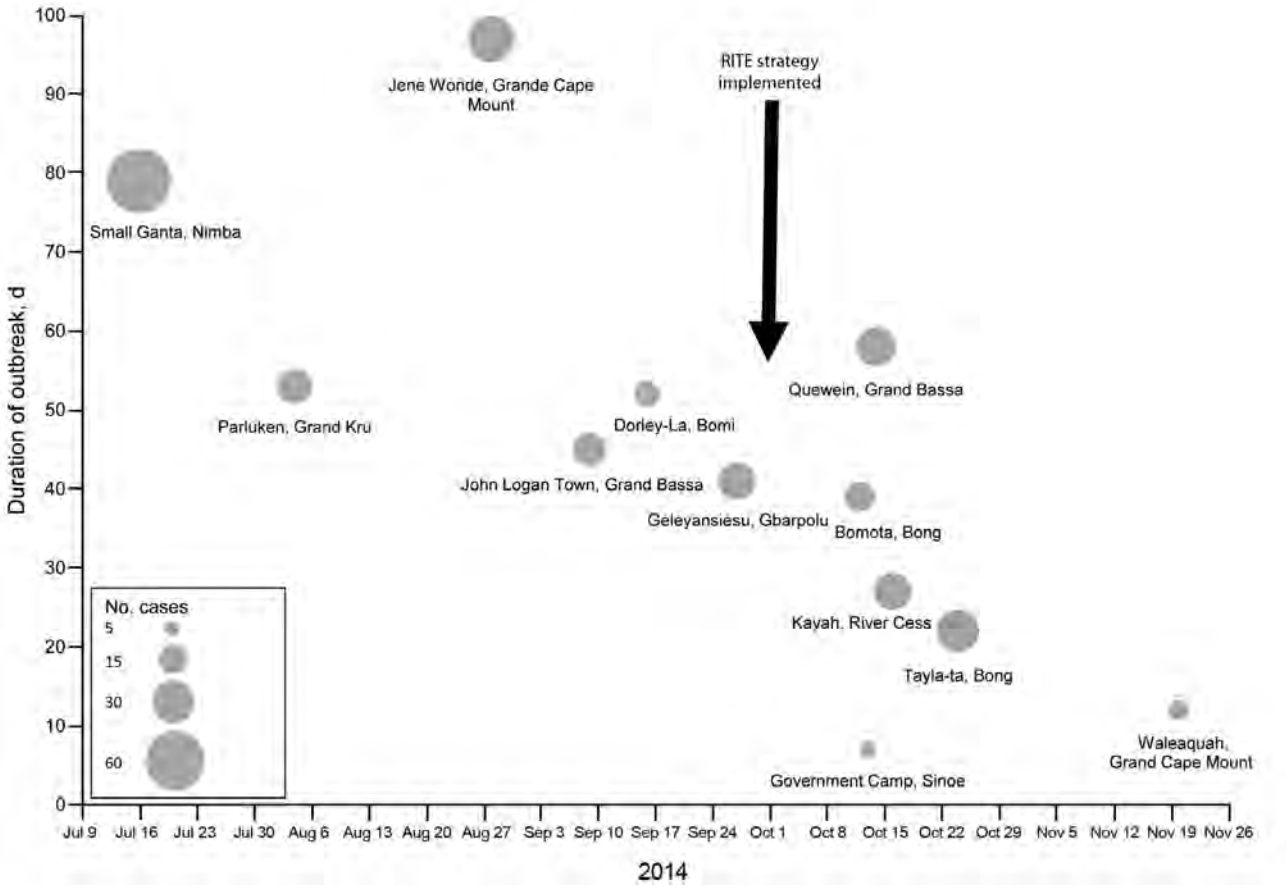

Figure 1. Decreased size and duration of outbreaks in remote areas before and after implementation of the Rapid Isolation and Treatment of Ebola (RITE) strategy, Liberia, 2014. Size of circle is proportional to number of cases in cluster.

laboratories (*21*). The DART provided coordination to rapidly engage partners providing services and supporting response efforts; CDC staff served as the technical lead for health, public health, and medical issues within the DART.

Epidemic Modeling

A CDC model that projected the possible trajectory of the epidemic if the trend of rapid transmission through August 2014 continued unabated was key to increasing the speed and scale of the US and global response (*22*). The worst-case scenarios of the model made clear the need for urgent action and helped stimulate a massive global response.

Analysis from the model provided 4 key findings. First, cases were increasing exponentially, and the response needed was massive and urgent. CDC helped facilitate assistance, including from the African Union, which mobilized nearly 1,000 staff, including doctors, nurses, epidemiologists, and health educators (*23*).

Second, the model predicted a severe penalty for delay; case numbers at the peak roughly tripled for every month of delayed scale-up (Figure 2). Thus, interventions (isolation, treatment, and safe burials) had to be rapid, with action and progress measured in hours and days rather than in weeks and months. In each country, CDC encouraged national leaders, incident managers, health workers, the media, and communities to take action immediately, because even a rapid international response would not be fast enough.

Third, the model identified a tipping point at which the epidemic would plateau and decline if enough (i.e., ≥70%) Ebola patients were isolated effectively and decedents buried safely. This finding led to establishment of community isolation facilities (*24*) and to contracting by USAID/OFDA for burial teams that worked to technical specifications established by CDC, first in Liberia and later in Sierra Leone (*25*). In Liberia, experienced CDC public health specialists conducted detailed planning exercises with community, political, medical, and public health leaders in each county to identify where sick persons could be isolated until ETUs were constructed and how contacts could be monitored and cared for if they became ill.

Fourth, the model predicted that when the tipping point was reached, transmission would decline rapidly.

This prediction was shown to be accurate in the following months in Liberia and Sierra Leone (Figure 3). For Liberia, the model's prediction that if urgent action were taken, there would be 10,000–27,000 cumulative cases by January 21, 2015, closely matched the 8,500–24,000 cases that occurred (Figure 4). The predictions also closely matched the actual case trajectory after effective intervention.

Border Health Security

CDC worked with ministries of health and airport authorities in all 3 heavily affected countries, as well as in other affected countries, to establish screening of travelers leaving the country by air to prevent sick or exposed persons from boarding planes. By mid-2015, >200,000 travelers leaving Guinea, Liberia, and Sierra Leone had been screened. In addition to reducing the likelihood of additional spread of Ebola to other countries, this screening, along with CDC's work with airlines to address air transport industry and flight crew concerns, helped enable humanitarian and public health organizations to sustain travel to affected areas by regular commercial airline flights. CDC staff also provided technical assistance on measures to reduce risk for spread through maritime ports and across land borders.

Innovation

CDC laboratory scientists implemented high-throughput laboratory capacity by using robotics and collaborated with private industry to promote development of lateral-flow assays to detect Ebola in point-of-care settings within 30 minutes after a finger stick or oral swab (*6*). In addition to supporting the National Institutes of Health randomized controlled trials of Ebola treatment (*27*) and vaccines (*28*), CDC staff worked with Sierra Leone authorities to implement a parallel Sierra Leone Trial to Introduce a Vaccine against Ebola (STRIVE), an adaptive, phased-introduction trial of a vaccine candidate among health workers in that country (*8*,*9*).

Support to Other At-Risk Countries

In Nigeria, a cluster of Ebola cases in July 2014 resulted from a traveler from Liberia. CDC deployed disease control experts to Lagos, the country's most populous city, within 72 hours and, in the first week after disease confirmation, supplemented response efforts with 13 Field Epidemiology Training Program (FETP) trainees, graduates, and trainers who had experience in epidemiology and infection control. In the 2 weeks that followed, CDC sent additional agency staff and helped mobilize 40 CDC-trained physicians from Nigeria's FETP. With the Nigerian government and partners, CDC facilitated creation of an effective incident management system, using leadership and staff from the Nigerian Polio Eradication Program and support from the Bill and Melinda Gates Foundation. This incident management system oversaw training of 2,300 health care staff, creation of an ETU in 14 days, and identification of >800 contacts; conducted 19,000 home visits of these contacts to monitor symptoms and temperatures; and screened >150,000 persons at airports. Although 19 secondary cases of Ebola occurred in 3 generations of spread in 2 cities, this rapid action controlled transmission, and Nigeria has been Ebola-free since this incident (*3*).

CDC staff provided similar assistance in Mali after a child arriving from Guinea died of Ebola and again after a cluster of cases occurred from a person from the Mali–Guinea border who had previously undiagnosed Ebola (*29*), and in Senegal after an incident of disease importation (*4*). CDC also collaborated with WHO to increase preparedness in at-risk countries by helping establish EOCs, surveillance for hemorrhagic fever and clusters of deaths, training in contact tracing, laboratory specimen transport and testing, isolation capacity for patients suspected of having Ebola, health communication messages, and border health security.

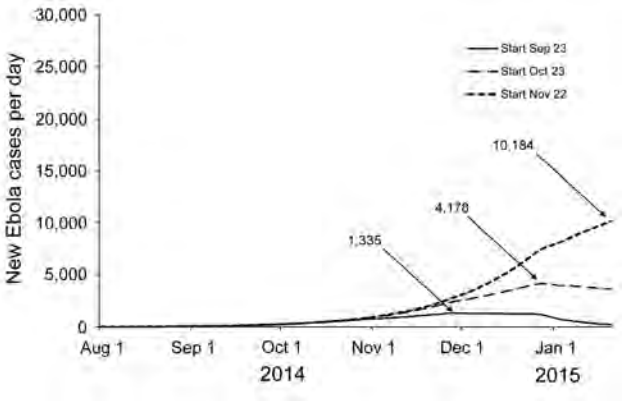

Figure 2. Estimated impact of delaying intervention on daily number of Ebola virus disease cases, Liberia, 2014–2015. The intervention modeled is as follows: starting on September 23, 2014 (day 181 in model), and for the next 30 days, the percentage of all patients in Ebola treatment units increased from 10% to 13%. This percentage was again increased on October 23, 2014 (day 211 in model) to 25%, on November 22, 2014 (day 241 in model) to 40%, and finally on December 22, 2014 (day 271 in model) to 70%. Day 1 in model is March 3, 2014. The impact of a delay of starting the increase in interventions was then estimated by twice repeating the above scenario but setting the start day on either October 23, 2014, or November 22, 2014. When the intervention is started on November 22, 2014, the peak is not reached by January 20, 2015, which is the last date included in the model. Graph based on Figure 10 in Meltzer et al. (*22*).

Ebola in the United States

Before diagnosis of the first case of Ebola imported to the United States, CDC alerted US health care providers to consider Ebola if compatible signs and symptoms manifested within 21 days after a traveler arrived from an affected

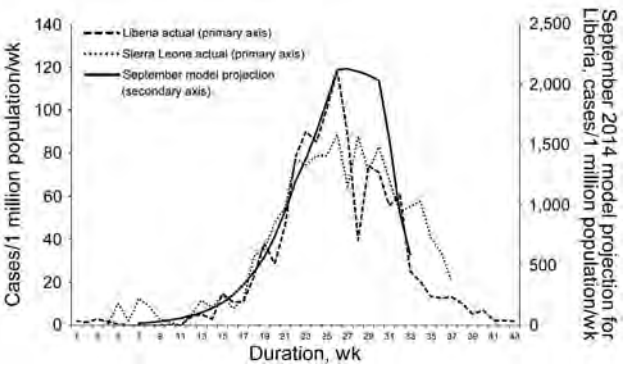

Figure 3. Comparison of estimated weekly Ebola virus disease case rate for Liberia with intervention with actual weekly case rates for Liberia and Sierra Leone. The September 2014 modeled projection curve was based on Figures 9 and 10 in Meltzer et al. (*22*), by using model predictions calculated assuming that interventions started on September 24, 2014. Liberia, week 1 begins May 4, 2014; Sierra Leone, week 1 begins May 25, 2014. The model projected the incidence that would occur if the proportion of Ebola patients who were hospitalized was 25% at week 22, increased to 40% at week 26, and increased again to 70% at week 30, while the proportion in effective home isolation remained constant at 10%. The similarity in the increase and decrease in the actual epidemic curves in both Sierra Leone and Liberia closely match the model after taking into account differences in start dates and population sizes between the 2 countries, implying that the proportion of cases effectively isolated in both countries followed a similar time course as the model.

country (*30*). CDC also issued infection control guidance for hospitals (*31*); strengthened laboratory networks and existing surveillance systems; and disseminated recommendations for travelers on the CDC website, through social media channels, and at US international airports.

The first case of Ebola diagnosed in the United States, imported by a traveler from Liberia, revealed gaps in hospital preparedness and response capabilities (*32*). Ebola was not considered in the patient's initial presentation, despite fever and travel history to Liberia. CDC provided assistance to the state and local health departments and to nearby hospitals. Two nurses caring for the patient were infected, most likely as the result of underprepared processes, lack of training, and suboptimal use of PPE during the first few days of the patient's second hospitalization, before his Ebola diagnosis. CDC subsequently strengthened recommendations for infection control, particularly training, supervision, and specifications of PPE. The second nurse who became ill was allowed to travel by air despite exposure that CDC should have categorized as high-risk to prevent the nurse from flying (*33*). In turn, this measure would have reduced the number of travelers whose health was monitored and the work of public health personnel monitoring contacts.

Recognizing a need for enhanced preparedness and training, CDC staff then visited 81 facilities in 21 states and Washington, DC, helping 55 of these facilities qualify as Ebola Treatment Centers for patients with suspected or confirmed Ebola. CDC also has qualified 56 state, county, and local public health laboratories to perform real-time quantitative reverse transcription PCR for Ebola with a Food and Drug Administration–approved DoD assay developed by the US Army Medical Research Institute of Infectious Diseases (*34*).

CDC established Ebola Response Teams composed of CDC experts in infection control, clinical care, contact tracing, communications, environmental waste management, and other areas to support state and local health departments and to deploy to any hospital in the United States that has a patient under investigation for Ebola (*35*). CDC staff arrived at New York City's Bellevue Hospital before Ebola was confirmed in the patient treated there.

To strengthen protection throughout the United States and to preclude restrictions on travel that could have undermined the response in West Africa and led to surreptitious travel from the region, CDC, together with the US Customs and Border Protection and state and local public health departments, developed a postarrival monitoring program to educate and follow >20,000 travelers arriving in the United States from Guinea, Liberia, and Sierra Leone since October 2014 (*36*). Travelers are met at the airport and provided with Check and Report Ebola (CARE) kits that include health education materials, a thermometer, and ways to connect with their state or local health department, including a prepaid cell phone. Through mid-May 2015, >1,200 travelers were referred to CDC for additional screening because of illness or, more commonly, to assess possible exposures; 28 persons were referred for medical evaluation. Ebola was not diagnosed in any of these persons (*37*).

Nearly 500 persons considered to be at "some or high risk" received direct active monitoring that included daily

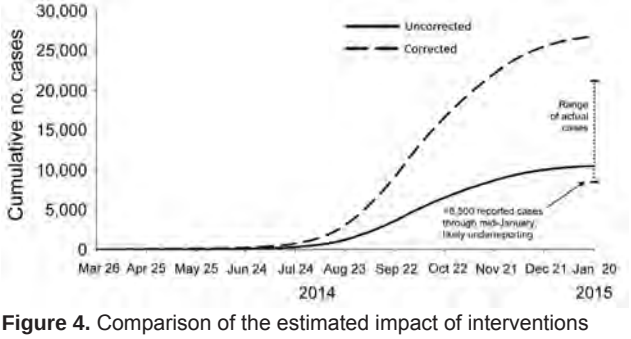

Figure 4. Comparison of the estimated impact of interventions on number of Ebola cases with actual cases reported, Liberia, 2014–2015. The September 2014 modeled projection curve was based on Figure 3 in Meltzer et al. (*22*) by using model predictions calculated assuming that interventions started on September 24, 2014. The corrected curve of projected cases is adjusted for potential underreporting by multiplying reported cases by a factor of 2.5. Actual reported cases are from World Health Organization situation report for January 21, 2015 (*26*).

direct observation of symptoms and temperature monitoring by health workers. More than 20,000 travelers classified as "low but not zero risk" received active monitoring, in which they monitored their own temperature and any symptoms and reported daily to the state or local health department until 21 days after their departure from an Ebola-affected country (an effort that has involved >400,000 cumulative contacts with arriving travelers). Health departments facilitated safe transport to a hospital ready to assess travelers for Ebola if the person developed fever or other symptoms of concern.

Before initiation of the active monitoring program, 1 case of Ebola was detected by self-monitoring; rapid detection and isolation prevented further disease transmission. Every jurisdiction now monitors travelers arriving from the highly affected countries and reports to CDC.

Lessons

The Ebola epidemic in West Africa is unprecedented in size and geographic distribution; it spread in many areas unfamiliar with the disease, including the first large urban outbreaks of Ebola. If the response in West Africa and global assistance had been implemented earlier, faster, and more effectively, far fewer cases and deaths and much less social and economic disruption would have occurred. The epidemic has shown that critical improvements are needed in 2 main areas. First, the ability of every country to quickly identify and respond to a health threat needs to be enhanced. Second, the ability of the global community to rapidly respond to needs in a country overwhelmed by an epidemic must be improved.

For months, the Ebola epidemic spread faster than the international community, including CDC, responded. Critical barriers in the affected countries include limited electronic connectivity (*38*); insufficient numbers of trained staff; inability to surge rapidly enough to provide needed case detection, education, contact tracing, and isolation services; and poorly functioning national health and public health systems with staff who often were unpaid, untrained, and poorly supervised. Surveillance and data management systems were overwhelmed; solutions are needed to manage, track, and support large outbreaks and public health interventions.

Stronger national and international systems for disease detection and control are needed. Paradoxically, the world is better prepared to find and stop emerging health threats than at any time in history, yet also is at greater risk for rapid spread of infectious diseases, which occur more frequently because of encroachment into forest areas, spread of antimicrobial-resistant organisms, and increasing ease of creation of dangerous pathogens, in the context of an increasingly mobile, interconnected, and urban world. The global community must use these lessons to improve response systems for large-scale emergencies, following the principles of the International Health Regulations, while using core staff and facilities on a daily basis to respond to ongoing health problems.

If the 3 highly affected countries had had effective surveillance and containment systems in place before 2014, the outbreaks might have been detected and stopped promptly (*39*). There was an unrecognized need for more effective control in urban areas with mobile populations. The use of the incident command system in this complex scenario was critical for organizing focused efforts to stop chains of transmission at the community level and within the health care system. Trust and coordination had to be established with more diverse communities, many of which were in postconflict environments, than in past outbreaks. In all 3 countries, emergency risk communication was a dynamic process, changing as the outbreak evolved, to promote understanding of nuanced messages of risk. Community engagement and understanding of each local community's beliefs and traditional practices was critical to success of the overall response and particularly important to ensure rapid isolation of infected patients, complete elicitation and monitoring of contacts, and safe burials.

In Uganda, where CDC and others have invested in public health for years, cases of Ebola and Marburg virus disease are now diagnosed promptly, infection control and contact tracing quickly implemented, and outbreaks either stopped rapidly or prevented altogether (*40*). Similarly, leveraging infrastructure and assets developed through the polio eradication efforts in Nigeria enabled an effective rapid response and demonstrated the value of investing in core public health capacities and training epidemiologists through the country's FETP program, which is needed in countries around the world. In contrast, before the outbreak, CDC had limited activities and no offices in any of the 3 heavily affected countries. The Global Health Security Agenda, supported by the United States in partnership with other nations and international organizations, seeks to rapidly improve the capacity of countries throughout the world to find, stop, and, wherever possible, prevent the spread of health threats (*41*,*42*).

Sustainable response capacity of international entities also needs to be improved. There were initial delays in effective response by WHO country offices and initial resistance of these offices and the African Region of WHO to involve CDC and other organizations (*43*). WHO has since mounted an effective response supporting the core public health interventions to stop spread of Ebola and is working to become more effective. The Global Outbreak Alert and Response Network is designed to provide a global response (*44*) but needed more staff with a wider range of skills to be deployed rapidly and for longer periods of time. Organizations that participated in the response needed a broad

range of skills, including expertise not only in laboratory and epidemiologic functions but also clinical care, logistics, health communications, information technology, data management, and anthropology, as well as fluency in English, French, and local languages and substantial knowledge of the cultural, social, and religious sensitivities that need to be addressed to engage communities and stop the spread of disease. CDC, along with communities, health care workers, and leaders in the affected nations and the international community, will continue to respond until the Ebola epidemic ends and is committed to strengthening national capacities in West Africa and elsewhere to prevent similar epidemics in the future.

Acknowledgments

This article provides an overview of the outcomes of a tremendous effort involving thousands of CDC employees, staff, and assignees working in West Africa and in the United States in 2014 and 2015. We specifically recognize technical, logistical, and operational leadership within the response. As Chiefs of Staff, Liz Bell, Sherrie Bruce, and Toby Crafton coordinated general staff activities (logistics, operations, planning, staffing, and situational awareness) to facilitate domestic and international work. Beginning in late March 2015, Dan Jernigan served as Incident Manager. As Deputy Incident Managers, Mark Anderson, Michael Beach, Christopher Braden, Kashef Ijaz, Eric Mast, Toby Merlin, Jordan Tappero, Tracee Treadwell, Chris Van Beneden, and Ian Williams coordinated various aspects of the domestic or international efforts. Clinical and domestic coordination benefited from the leadership of Steven Boedigheimer, Peter Briss, John T. Brooks, Mark Davis, Denise Jamieson, Eric Kasowski, Lisa Koonin, Chris Kosmos, Fleetwood Loustalot, Dana Meaney-Delman, Harald Pietz, Tim Uyeki, and Cynthia Whitney. The Epidemiology and Laboratory Task Force leads, Stephanie R. Bialek, Brian Bird, Maleeka J. Glover, Barbara Knust, Barbara Mahon, Paul Mead, Mark Rayfield, Ute Ströher, Jonathan Towner, and Melinda Wharton, provided metrics and assessments of the response efforts. Manoj Gambhir, Martin Meltzer, and Michael L. Washington coordinated innovative ways to use modeling to help our response efforts. Francisco Alvarado-Ramy, Clive Brown, Marty Cetron, Tai-Ho Chen, Nicole J. Cohen, Andrew Demma, Katrin Kohl, Nicki Pesik, Lisa Rotz, and Steve Waterman provided leadership in the Emergency Operations Center for border and migration issues. Tom Clark, Tamara Pilishvili, Robert Pinner, Stephanie Schrag, Jane Seward, and Marc-Alain Widdowson provided leadership for the development and implementation of vaccine trials. Ray Arthur and Barbara Marston provided stalwart leadership of the international task force. Benjamin Dahl, Kevin De Cock, Rana Hajjeh, Peter Kilmarx, Michael Kinzer, Tom Ksiazek, Frank Mahoney, Joel Montgomery, Oliver Morgan, Pratima Raghunathan, Pierre Rollin, and Brian Wheeler served tirelessly, detailed as in-country leadership for multiple months of the response. Sarah D. Bennett, David Blaney, Joseph Bresee, Rebecca Bunnell, Athalia Christie, Gary Cobb, Brendan Flannery, Chris Gregory, Kathy Hageman, James Ham, Jeffrey Hanson, Sara Hersey, Thomas G. Ksiazek, Scott Laney, Kim Lindblade, Lise Martel, James McAuley, Jonathan Mermin, John Neatherlin, Joshua A. Mott, Sonja J. Olsen, John Painter, John Redd, Laurence Slutsker, John Vertefeuille, Henry Walke, and Desmond E. Williams provided key leadership in the international response efforts. Sara Bedrosian, Joanne Cox, Wendy Holmes, Craig Manning, John O'Connor, and Barbara Reynolds coordinated various health and risk communications efforts. Laura Landers Eastham, Kathy Gallagher, Lauren Hoffmann, Kim Hummel, Susan McClure, Julie Racine-Parshall, Karina Rapposelli, Kerry Stephens, Serena Vinter, and Sara Zeigler coordinated all communication flows with CDC partners and US government interface efforts. Jeffrey Nemhauser coordinated worker health, safety, and well-being, as well as medical evacuations. Stuart Nichol provided coordination as Chief Scientific Officer (and was the first Incident Manager), and David Bell, Dianna Blau, Carolyn Bridges, Thomas Sinks, Jerome Tokars, and Martin Vincent served as Associate Directors for Science. Finally, we thank leaders across the agency for committing personnel to support this effort, specifically Carmen Villar and the Office of the CDC Chief of Staff, Sherri Berger and the Office of the CDC Chief Operating Officer, and Katherine Lyon Daniel and the CDC Office of the Associate Director for Communication, along with CDC Center/Institute/Office directors Beth Bell, Tom Kenyon, Stephen Redd, and Anne Schuchat.

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etymologia

Ebola [eb'o-lə]

Ebola virus, discovered in 1976 during an outbreak in Zaire (now Democratic Republic of the Congo), was first isolated from Myriam Louise Ecran, a 42-year-old Belgian nursing sister working at the Yambuku Mission Hospital who died caring for people with this unknown disease. When the international commission considered the name "Yambuku virus," Karl Johnson and Joel Breman noted that naming the Lassa virus after the Nigerian village where it was discovered brought stigma to the community. Johnson suggested naming the virus after a nearby river, and the rest of the commission agreed. The Belgian name for the river, *l'Ebola*, is actually a corruption of the indigenous Ngbandi name *Legbala*, meaning "white water" or "pure water" (J.G. Breman, L.E. Chapman, F.A. Murphy, P.E. Rollin, pers. comm.).

The Ebola virus, originally described as "Marburg like," was determined to be a related filovirus (from the Latin *filum*, "thread"), named for the elongated, flexible shape. The virus was first described in 3 back-to-back articles in The Lancet in 1977.

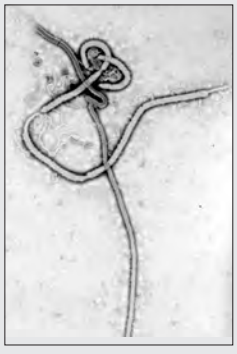

Figure 1. Taken by Frederick Murphy at CDC, this iconic transmission electron micrograph shows the filamentous shape of the Ebola virus. On October 13, 1976, Murphy captured this image and, along with Karl Johnson and Patricia Webb, carried the printed negative, dripping wet, directly to CDC Director David Sencer. At the time, they were among the only persons in the world to have seen this "dark beauty" (F.A. Murphy, pers. comm.).

Figure 2. Ebola River, ca. 1932. Photo courtesy Pierre Rollin.

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SYNOPSIS

Use of Internet Search Queries to Enhance Surveillance of Foodborne Illness

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As a supplement to or extension of methods used to determine trends in foodborne illness over time, we propose the use of Internet search metrics. We compared Internet query data for foodborne illness syndrome–related search terms from the most popular 5 Korean search engines using Health Insurance Review and Assessment Service inpatient stay data for 26 International Classification of Diseases, Tenth Revision, codes for foodborne illness in South Korea during 2010–2012. We used time-series analysis with Seasonal Autoregressive Integrated Moving Average (SARIMA) models. Internet search queries for "food poisoning" correlated most strongly with foodborne illness data (r = 0.70, p<0.001); furthermore, "food poisoning" queries correlated most strongly with the total number of inpatient stays related to foodborne illness during the next month (β = 0.069, SE 0.017, p<0.001). This approach, using the SARIMA model, could be used to effectively measure trends over time to enhance surveillance of foodborne illness in South Korea.

Foodborne illness is a growing public health problem in developing and industrialized nations and a common cause of illness, and sometimes death, worldwide (*1*). However, exact morbidity associated with foodborne illnesses is difficult to determine because many cases of foodborne illness are underdiagnosed or underreported and thus not identified by public health surveillance systems (*2*).

The objective of public health surveillance systems for foodborne illnesses is to identify the causes of foodborne disease so that prevention and control programs can be introduced and, if necessary, strengthened (*3*). The overall quality or validity of a public health surveillance system depends on the quality of the data in terms of the following 3 factors: completeness, timeliness, and consistency (*4*,*5*). To estimate the effect of an illness on a person's overall health, specific information about incidence is required, along with the development of a method for estimating the completeness of reporting. Furthermore, detection of outbreaks necessitates comparison of current reporting with the expected baseline, and timeliness is highly relevant. Finally, to measure trends over time, reporting must be kept consistent, so that the techniques used to detect underlying factors do not change (*4*,*6*,*7*).

M'ikanatha et al. (*8*) and Vogt et al. (*9*) suggested that the benefits of electronic and Web-based reporting systems for infectious disease surveillance data include improved timeliness and completeness. Internet-based public health surveillance is a new approach that can be performed by using syndrome- and disease-specific terms (*10*,*11*). The relative frequency of certain Internet queries is highly correlated with the occurrence of some infectious disease symptoms (*10*–*12*). Internet-based surveillance systems offer a new and developing means of measuring trends over time (consistency) and monitoring the effectiveness of various public health concern interventions, including those for emerging infectious diseases (*13*). To enhance the consistency in public health surveillance systems for foodborne illness, we propose the use of Internet search query data.

Internet availability and use has increased greatly during the past 10 years (*14*). The availability of health-related information on the Internet has changed how persons seek information about health (*15*). Although Internet-based surveillance systems do not have the capacity to completely replace traditional surveillance systems (*16*), they do provide a new means by which to detect and monitor infectious diseases. In a study reviewing Internet-based surveillance systems, Milinovich et al. (*16*) suggested that future research in this area should focus on using data generated through Internet-based surveillance and response systems to bolster the capacity of traditional surveillance systems for emerging infectious diseases.

Pelat et al. (*17*) emphasized the need for query surveillance studies on diseases other than influenza or in languages other than English. Recently, Desai et al. (*10*) compared norovirus outbreak surveillance data with Google Internet query data. Wilson and Brownstein (*18*) performed search-term surveillance of a listeriosis outbreak in Canada. The results of these studies suggest that Internet surveillance tools can assist in the early identification of foodborne disease outbreaks. However, these 2 studies are among relatively few that have addressed possible relationships between Web search queries and foodborne illness, especially bacterial foodborne illness.

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In South Korea, the Health Insurance Review and Assessment Service (HIRA) (*19*) reviews medical fees and evaluates the appropriateness of medical benefits provided to patients. For this purpose, HIRA data have been gathered for all patients in South Korea. These data include foodborne illness and many other infectious diseases and can be used for public health surveillance. Furthermore, by comparing the data generated by the HIRA surveillance system with that collected from Internet search queries, a more comprehensive surveillance system can be created. This combined surveillance system could contribute to the strength of traditional surveillance systems for foodborne illness in South Korea.

To assess whether Internet search query trends can be used to effectively measure trends over time in the spread of foodborne illnesses, particularly those caused by bacteria, we compared Internet query data for 5 foodborne illness syndrome–related search terms from the most popular 5 search engines in South Korea with HIRA data in South Korea. We used time-series analysis, taking into account lagged effects, autocorrelation, and the seasonal fluctuation in incidences of foodborne illness.

Methods

Data on Bacterial Foodborne Illness

We included data about bacterial foodborne illness and infectious enteritis (i.e., acute gastroenteritis) because some bacterial infectious enteritis symptoms are similar to those of bacterial foodborne illness. Foodborne illnesses caused by viruses, protozoa, natural toxins, and chemical agents were excluded because these fell outside the scope of the current study. We collected data on bacterial foodborne illness from HIRA (*19*) for 2010–2012 using a method of Park et al. (*20*). From the total set of patient data, we extracted cases in which bacterial foodborne diseases and intestinal infections had been diagnosed by using the Korean Standard Classification of Diseases (*21*). This classification is based on, and highly similar to, the International Classification of Diseases, Tenth Revision (ICD-10), issued by the World Health Organization but is adapted for use in South Korea. ICD-10 assigns numeric codes to specific illnesses to standardize diagnosis for epidemiology, health management, and clinical purposes (*22*). The 26 ICD-10 codes defining bacterial foodborne illness and infectious enteritis comprise diagnosis codes in the following range: A02.0, A02.8–9, A03.0–3, A03.8–9, A04.0–6, A04.8–9, A05.0–4, A04.8–9, and A32 (Table 1). We included cases that corresponded to these codes and classified them accordingly. Then we grouped cases according to whether they resulted in inpatient stays or outpatient visits and the month and year in which they occurred. Because preanalyses showed a stronger correlation between Internet search queries and HIRA inpatient stays than between Internet search queries and outpatient visits or officially reported data, we used only HIRA inpatient data for the analysis.

Internet Query Data

We analyzed Internet queries submitted to the 5 most popular Internet search websites in South Korea: Naver (http://www.naver.com), Daum (http://www.daum.net), Google (http://www.google.co.kr), Nate (http://www.nate.com), and Yahoo! Korea (http://www.yahoo.co.kr). These websites are written in Korean; the submitted queries were also in Korean. The national market share of these websites during the analysis period (January 2010–December 2012) was 68.2%, 21.1%, 5.3%, 2.7%, and 1.5%, respectively, totaling 98.8% of the Internet search market (*23*). However, we did not compare metrics from different search engines because the most popular 2 occupied 89.4% of the market. According to the Korea Internet and Security Agency (*24*), 72.3% of the Korean population uses the Internet daily. The query data were aggregated nationally.

Although more serious complications and other problems can result from foodborne illness, foodborne illness can be defined as any form of infectious gastroenteritis caused by eating food, including food contaminated immediately before ingestion (*25*,*26*). Thus, we chose to focus on 3 common symptoms of bacterial foodborne illness and infectious enteritis: diarrhea, vomiting, and abdominal pain after food consumption that could not be attributed to other factors, such as advanced pregnancy, drug use, and/or alcohol consumption. In addition, we referenced the Standard Korean Dictionary (*27*) to source a representative keyword that Koreans use to define foodborne diseases. Finally, the queries included the following 5 terms related to foodborne illness (Korean translations in parentheses): "food poisoning" (Sik-jung-dog), "diarrhea" (Seol-sa), "vomiting" (Gutto), "abdominal pain" (Bok-tong), and "gastroenteritis" (Jang-yeum). We collected monthly data on these 5 Internet search query terms from January 2010 through December 2012 because the HIRA data on bacterial foodborne illness are also available monthly. The data collection procedure was conducted by WISEnut Korea (Seongnam, South Korea; http://www.wisenut.co.kr), a company specializing in the collection and analysis of large datasets, by using a Korea-dedicated Web crawler to access the 5 most popular Internet search websites in South Korea.

Data Analysis

The collected Internet query data for South Korea were aggregated for each month. To quantify the strength of associations between incidences of foodborne illness and each search term, we calculated the Spearman *r* correlation, taking into account lead or lag effects, with the variables temporally leading and lagged by up to 2 months. For better

Table 1. ICD-10 codes for causes of bacterial foodborne illness and infectious enteritis and number of inpatient hospital stays for each, South Korea, January 2010–December 2012*

ICD-10 code	Diagnosis	No. inpatient stays		
		All 3 y	3-y monthly average ± SD	%
A02	Nontyphoidal *Salmonella* infections			
A02.0	*Salmonella* Enteritis (salmonellosis)	1,623	45.1 ± 21.3	4.8
A02.8	Other specified salmonella infections	100	2.8 ± 3.1	0.3
A02.9	Salmonella infection, unspecified	1,251	34.8 ± 17.5	3.7
A03	Shigellosis			
A03.0	Shigellosis due to *Shigella dysenteriae*	13	1.3 ± 0.7	0.1
A03.1	Shigellosis due to *Shigella flexneri*	54	2.1 ± 1.5	0.2
A03.2	Shigellosis due to *Shigella boydii*	7	1.2 ± 0.4	0.1
A03.3	Shigellosis due to *Shigella sonnei*	50	2.3 ± 1.2	0.2
A03.8	Other shigellosis	20	1.5 ± 1.1	0.2
A03.9	Shigellosis, unspecified	190	5.4 ± 4.4	0.6
A04	Other bacterial intestinal infections			
A04.0	Enteropathogenic *Escherichia coli* infection	131	3.6 ± 2.8	0.4
A04.1	Enterotoxigenic *Escherichia coli* infection	13	1.2 ± 0.6	0.1
A04.2	Enteroinvasive *Escherichia coli* infection	7	1.2 ± 0.4	0.1
A04.3	Enterohemorrhagic *Escherichia coli* infection	72	2.6 ± 1.9	0.3
A04.4	Other intestinal *Escherichia coli* infection	497	13.8 ± 4.6	1.5
A04.5	*Campylobacter* enteritis	39	2.0 ± 1.1	0.2
A04.6	Enteritis due to *Yersinia enterocolitica*	6	1.2 ± 0.4	0.1
A04.8	Other specified bacterial intestinal infections	3.453	95.9 ± 28.0	10.2
A04.9	Bacterial intestinal infection, unspecified	20,897	580.5 ± 114.1	62.0
A05	Other bacterial foodborne intoxications, not elsewhere classified			
A05.0	Foodborne staphylococcal intoxication	42	2.0 ± 1.2	0.2
A05.1	Botulism (classical foodborne intoxication due to *Clostridium botulinum*)	11	1.4 ± 0.7	0.1
A05.2	Foodborne *Clostridium perfringens* (*Clostridium welchii*) intoxication	60	2.2 ± 1.2	0.2
A05.3	Foodborne *Vibrio parahaemolyticus* intoxication	132	5.5 ± 6.5	0.6
A05.4	Foodborne *Bacillus cereus* intoxication	14	2.8 ± 2.7	0.3
A05.8	Other specified bacterial foodborne intoxications	348	9.7 ± 4.8	1.0
A05.9	Bacterial foodborne intoxication, unspecified	4,069	113.0 ± 40.6	12.1
A32	Listerial foodborne infection	30	1.5 ± 0.8	0.2
Total		33,129	936.4 ± 190.2	100.0

*ICD-10, International Classification of Diseases, Tenth Revision.

prediction, the seasonal autoregressive integrated moving average (SARIMA) model was used to estimate the parameters of the regression model through the preprocessing of a stationary time series. The SARIMA model is used for time-series modeling and forecasting and is based on Box and Jenkins' ground-breaking work, which takes into account the impact of seasonality and autocorrelations on the variables (*28*,*29*). A SARIMA model can be described as an ARIMA (p, d, q) multiplied by (P, D, Q), wherein p, d, q represent ordinary components and P, D, Q represent seasonal components and p is the number of autoregressive terms, d is the number of nonseasonal differences needed for stationarity, q is the number of lagged forecast errors in the prediction equation, P is the number of seasonal autoregressive terms, D is the number of seasonal differences, and Q is the number of seasonal moving average terms. These terms or numbers were determined through the autocorrelation function and the partial autocorrelation function. The Akaike Information Criterion was used to assist the model fits, and the residuals were further examined for autocorrelation by plotting scatter diagrams, as well as the autocorrelation function and partial autocorrelation function (*30*). These processes were conducted with SPSS software (SPSS Inc., Chicago, IL, USA). All the analyses were performed using IBM SPSS version 21.0 (Data Solution Inc., Seoul, South Korea) with a significance level of $p = 0.05$.

Results

During 2010–2012, a total of 33,129 inpatient hospital stays involved diagnoses of bacterial foodborne illness and infectious enteritis (corresponding to 1 of the ICD-10 codes; Table 1). This number represents an average of 936 ± 190 hospital stays per month during the 3-year study period, or 22 inpatient stays per 100,000 persons in South Korea annually. Nearly 62.0% of patients in surveyed inpatient stays had diagnoses of unspecified bacterial intestinal infection (A04.9), and 12.1% had diagnoses of unspecified bacterial foodborne intoxication (A05.9). Of specific diagnoses, *Salmonella* (A02.0, A02.8-9) was the most common (8.8% of patients), followed by pathogenic *Escherichia coli* (A04.0-4) (2.4%) (Table 1).

During the 3 years examined, 2,943,776 queries containing at least 1 of the 5 foodborne illness–related search

terms included in this study were submitted to the most popular 5 Internet search engines in South Korea. Of these, diarrhea was the most frequent (found in 1,401,515 [47.6%] searches), followed by gastroenteritis (574,389 [19.5%]), vomiting (403,406 [13.7%]), food poisoning (293,860 [10.0%]), and abdominal pain (270,606 [9.2%]).

Of the 5 search terms, the prevalence of searches for food poisoning correlated most strongly with the number of inpatient stays related to bacterial foodborne illness and infectious enteritis for all surveyed ICD-10 codes (r = 0.68, p<0.001) (Table 2). Although diarrhea was the most frequently searched of all the terms, its correlation with total hospital stays for all surveyed conditions (r = 0.48, p = 0.003) was weaker than for food poisoning or gastroenteritis (r = 0.52, p = 0.001). Abdominal pain (r = 0.38, p<0.022) and vomiting (r = 0.34, p = 0.040) showed the weakest correlations with total hospital stays for all surveyed conditions.

In most cases, the number of Internet search queries for a term was high in 1 month and then the next month the number of related hospital stays was high (Figure 1; Table 2). Searches for food poisoning correlated most strongly with inpatient stays for diagnostic code A04.8-9 (other bacterial intestinal infections) in the next month (r = 0.67, p<0.001). However, these terms correlated even more strongly with the total number of hospital stays for all surveyed conditions in the next month (r = 0.70, p<0.001). For all specified pathogens, hospital stays related to *Salmonella* (A02.0, A02.8-9) correlated most strongly with Internet searches for food poisoning in the previous month (r = 0.63, p<0.001). Except for *Campylobacter* (A04.5), bacterial foodborne pathogens were weakly or not correlated (p>0.05) with most search queries (Table 2). Internet searches for terms included in the study from the 2 months before and 2 months after were more weakly (in some cases negatively) correlated with hospital stays for all conditions. Internet searches for food poisoning occurred 1–2 months before the inpatient hospital stays (Figure 1), which suggests the possibility of a strongly lagged relationship.

The best regression model that had the highest correlation value for Internet searches relating to food poisoning and the total number of hospital stays for all conditions surveyed in the next month was SARIMA (1, 0, 0) (1, 0, 0)$_{12}$ with Akaike Information Criterion 433.6; that is, first-order (seasonal) autoregressive model (p and P = 1, respectively). The parameters estimated by the best SARIMA model are shown in Table 3. The significant parameters in the model include first-order autoregression of the number of inpatient hospital stays and seasonal autoregression, as well as the Internet search query "food poisoning" 1 month earlier (β = 0.045, SE 0.017, p<0.05). With regard to goodness of fit, residuals were randomly distributed with no autocorrelation among them. The incidence of the food poisoning query was positively associated with the number of inpatient hospital stays for total bacterial foodborne illness and infectious enteritis for the next month (Figure 2). This association that Internet search queries can be used to track trends over time in relation to foodborne illness.

Discussion

We assessed relationships between Internet query data for foodborne illness syndrome–related search terms and inpatient hospital stays in which bacterial foodborne illness and infectious enteritis were diagnosed in South Korea. The search query data in the month before hospital stay can be used as early indicators to measure trends over time in foodborne illness in South Korea.

Effective initiation of public health intervention measures depends on early and rapid identification of infectious disease outbreaks (*19*). Early detection of disease activity after a rapid response can reduce the effect of the disease on the general public and is one way to improve early detection monitoring health-seeking behavior in the form of queries entered into Internet search engines (*11*). Ginsberg et al. (*11*) investigated how Google search queries correlated with reports of an influenza epidemic, and Polgreen et al. (*12*) used a Yahoo! query log to investigate the same topic; Hulth et al. (*13*) used the query log of a Switzerland-based Web search engine. This approach obtained more data than did traditional disease surveillance (*19*).

Most existing surveillance systems for foodborne illness are based on disease reporting or on laboratory-based

Table 2. Spearman *r* correlation between number of inpatient hospital stays for types of bacterial foodborne illness and infectious enteritis and number of Internet searches for food poisoning with lead and lag times of up to 2 mo, South Korea, January 2010–December 2012*

Diagnosis (ICD-10 code)	Previous 2 months	Previous 1 month	Same month	Following 1 month	Following 2 months
Salmonellosis (A02.0, A02.8–9)	–0.173	0.218	0.546†	0.629†	0.618†
Campylobacteriosis (A04.5)	–0.200	0.298	0.523†	0.545†	0.366‡
Other bacterial intestinal infections (A04.8–9)	–0.126	0.211	0.587†	0.671†	0.535†
Other bacterial foodborne intoxications (A05.8–9)	–0.080	0.268	0.678†	0.641†	0.395‡
Total bacterial foodborne illness and infectious enteritis (all of the codes in Table 1)	–0.112	0.254	0.679†	0.701†	0.545†

*ICD-10, International Classification of Diseases, Tenth Revision.
†p<0.01.
‡p<0.05.

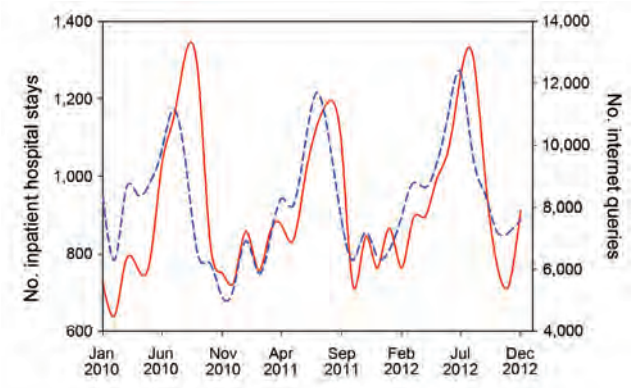

Figure 1. Number of Internet search queries for food poisoning (short dashed blue line) and estimated number of inpatient hospital stays for bacterial foodborne illness and infectious enteritis (solid red line), South Korea, January 2010–December 2012.

surveillance, which also provide crucial information for assessing foodborne disease trends and enable assessment of data trends over time (*31*) but are passive and record only a minor proportion of all cases in the population (*32*). To estimate the true incidence of and monitor trends over time in foodborne illness, population-based prospective studies as active surveillance have been conducted, such as FoodNet in the United States (*33*,*34*) and OzFoodnet in Australia (*35*), but these population studies are expensive and time consuming (*32*). Data generated through Internet-based surveillance can be used to strengthen the capacity of traditional disease surveillance systems (*16*) in foodborne illness.

In determining trends over time in foodborne illness, Internet search queries will greatly aid existing systems. Public health surveillance systems are under development. By monitoring trends in the incidence and proportion of different types of foodborne illness over time, these Internet-based surveillance systems will provide critical information for evaluating the impact of measures to prevent foodborne illness. Consistent Internet-based surveillance systems with an early warning function will thus benefit the development of future foodborne disease prevention measures.

Numerous studies have used online health-seeking behavior to monitor disease incidence by using various methods, for example, correlation analysis or regression modeling. In this study, we used SARIMA models to analyze incidences of inpatient stays in relation to Internet search queries. SARIMA modeling is a statistical approach used to model and forecast nonstationary time series and instances wherein observations are seasonally dependent and autocorrelated (*28*). SARIMA models have previously been used to quantify the relationship between infectious diseases and other variables (*36*,*37*). However, the SARIMA model used in this study can be applied only minimally in relation to the effect of Internet queries on enteric infection. The SARIMA model we developed did not show a perfect goodness of fit because of the unavailability of data; however, results indicate that it could effectively quantify the relationship between data relating to Internet queries and enteric infections, including foodborne illness.

Of the 5 Korean search terms included, Internet searches for food poisoning were the most effective in predicting inpatient stays. However, we used only 5 syndrome-related search terms because our study was designed to assess a general tendency regarding the relationship between Internet searches and the changing rates of foodborne illness. Flint et al. (*38*) suggested that many episodes of foodborne illness are marked by acute gastroenteritis; however, because not all cases of acute gastroenteritis are caused by organisms found in food, gastrointestinal symptoms do not necessarily indicate a foodborne illness. Thus, if more keywords were selected to reflect terms most likely associated with foodborne illness symptoms, and a filtering procedure was conducted, correspondence between Internet searches and hospitalizations for specific conditions could be closer. Moreover, temporal associations between foodborne illnesses caused by specific pathogens and Internet query data related to symptoms should be studied further.

We used HIRA data on the number of inpatient hospital stays in which bacterial foodborne illness and infectious enteritis were diagnosed, rather than officially reported foodborne disease data. Officially reported foodborne disease data probably fail to capture a substantial proportion of foodborne illness because only cases that are identified and reported are included (*39*). However, South Korea has implemented a mandatory health insurance system managed by HIRA. Therefore, HIRA data represent the total patient population in South Korea, including those who have had foodborne illness. Consequently, the number of inpatient hospital stays, as indicated in the HIRA data, more accurately indicates the trend or prevalence of foodborne illness, which was the focus of this study.

Table 3. Parameters estimated by the seasonal autoregressive integrated moving average (1,0,0)(1,0,0) model regarding effects of Internet searches for food poisoning on number of inpatient hospital stays for total bacterial foodborne illness and infectious enteritis in the next month, South Korea, January 2010–December 2012

Variable	β	SE	p value
First-order autoregression	0.4059	0.1546	0.0133
First-order seasonal autoregression	0.5715	0.1447	0.0004
Food poisoning queries 1 mo. earlier	0.0450	0.0176	0.0157
Constant	557.1300	152.6156	0.0010

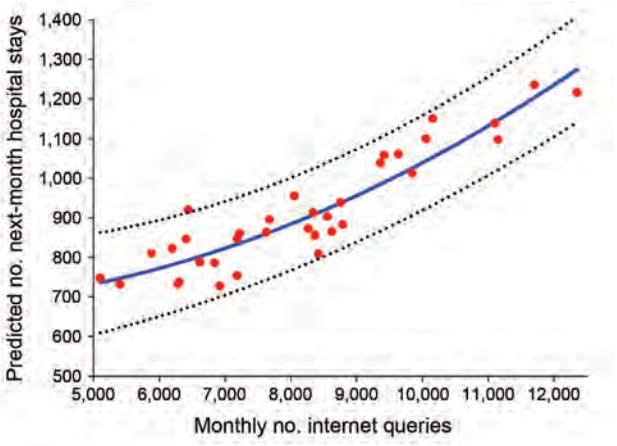

Figure 2. Relationship between the monthly number of Internet search queries for food poisoning and predicted number of inpatient hospital stays for total bacterial foodborne illness and infectious enteritis for next month by seasonal autoregressive integrated moving average model, South Korea, January 2010–December 2012. Red dots and blue line represent actual and predicted numbers of inpatient hospital stays, respectively. Dotted lines indicate 95% CIs ($R^2 = 0.71$).

Some weaknesses are associated with the HIRA data. Because the data source we selected is for inpatient hospital stays in which bacterial foodborne illness and infectious enteritis was diagnosed, we do not know what proportion represents cases in which the patient's illness was actually caused by food. The proportion of pathogen-specific illnesses resulting from eating contaminated food is difficult to accurately estimate (*40*). ICD-10 code A05 specifically refers to foodborne illness, so we can be reasonably confident that most hospital stays in which this diagnosis was made represent actual cases of foodborne illness. However, the proportion of cases in which other diagnoses were made (A02-4) represents illnesses transmitted by food is unclear. Because of these limitations, the actual number of inpatient hospital stays related to foodborne illness for each month might differ from those on which we based our analysis. However, we believe that estimates obtained in this way will be related to Internet query data in a similar fashion to the true values for rates of foodborne illness during the same period. On the basis of this belief, we showed that Internet query data can predict rates of bacterial foodborne illness over time.

Internet-based surveillance systems should not be viewed as an alternative to traditional surveillance systems but rather an extension; therefore, future research needs to focus on how to use Internet-based surveillance systems to complement existing systems (*12*,*13*,*16*). Researchers should preferably validate data from Internet surveillance systems against a body of real events and develop methods that can be used for this purpose—for example, by comparison with national surveillance case data or against data on foodborne outbreaks reported to public health authorities during the same period.

In conclusion, our results showed that search query data can be used to predict changes in the incidence of bacterial foodborne illness over time to a large extent with time-series analysis (SARIMA model). According to the Korea Internet & Security Agency (*24*), the rate of Internet use was 82.1% in South Korea in 2013, compared with 65.5% in 2003. These data suggest that Internet use is increasing substantially and is likely to continue to increase. Therefore, use of Internet search data to predict the incidence of foodborne illness will become a more viable approach and could help to develop a stable and consistent platform to assist foodborne illness surveillance in South Korea.

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Achievements in and Challenges of Tuberculosis Control in South Korea

Ji Han Kim, Jae-Joon Yim

After the Korean War (1950–1953), nearly 6.5% of South Korea's population had active tuberculosis (TB). In response, South Korea implemented the National Tuberculosis Program in 1962. From 1965 to 1995, the prevalence of bacteriologically confirmed pulmonary TB in South Korea decreased from 940 to 219 cases per 100,000 population. Astounding economic growth might have contributed to this result; however, TB incidence in South Korea remains the highest among high-income countries. The rate of decrease in TB incidence seems to have slowed over the past 15 years. A demographic shift toward an older population, many of whom have latent TB and various concurrent conditions, is challenging TB control efforts in South Korea. The increasing number of immigrants also plays a part in the prolonged battle against TB. A historical review of TB in South Korea provides an opportunity to understand national TB control efforts that are applicable to other parts of the world.

Despite the hardships resulting from the Korean War during the early 1950s, South Korea accomplished rapid economic growth and now enjoys one of the world's largest economies and a high standard of living. In parallel with the economic prosperity, South Korea achieved admirable control of tuberculosis (TB) in the past half century. TB in South Korea is a classic example of how a country that was once one of the poorest in the world can drastically reduce its disease burden through national disease control efforts. However, TB incidence in South Korea remains the highest among high-income countries: as of 2013, TB incidence in South Korea was 7 times higher than the average incidence for member countries of the Organisation for Economic Co-operation and Development (*1*). To understand the interaction of disease with major social changes, we discuss how the economic transitions in South Korea during the 20th century shaped the country's TB burden. We also address the major problems in TB control in South Korea today and current endeavors to control the disease.

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Part I. TB in South Korea during the 20th Century

Although historical accounts suggest that TB has existed on the Korean Peninsula for centuries, it is unclear when the causative agent, *Mycobacterium tuberculosis*, was first introduced to the region. TB, as described in modern medicine, did not appear in the official records of Korea until the turn of the 20th century. We therefore begin our discussion of TB at the beginning of the 20th century, during the time of Japanese colonization.

The Colonial Period (1910–1945)

The Japanese Empire of the late 19th century strived for military growth. After victory in the war against Russia of 1905, the Empire of Japan integrated Korea as a protectorate, and in 1910 Korea was officially annexed as a colony. The Korean Peninsula had strategic importance because of its geographic location, and it also provided the empire with raw materials and a labor force. As industrialization in Korea began to expand, the population soared, especially in the cities. Population growth, overcrowding, and malnutrition increased, creating a more favorable environment for the spread of infectious agents.

In 1918, the colonial government implemented several strategies to control TB. To contain the spread of TB, the Japanese Governor-General of Korea installed bowls at various locations throughout cities and villages and ordered residents to spit their sputum into these containers. Patients who were found to have TB were isolated, and their possessions were cleaned. The colonial government also established health clinics equipped with x-ray devices to better diagnose TB (*2*). Western missionaries arrived in Korea, in cooperation with or independent from the colonial government, to help persons with TB. Dr. Sherwood Hall, a Methodist medical missionary from Canada, founded a sanatorium for TB care in 1928 (*3*).

Limited data are available to accurately evaluate the epidemiologic status of TB in Korea during its colonial occupation by Japan. However, evidence suggests that TB death rates gradually increased during the second quarter of the 20th century. From 1926 to 1942, the Japanese imperial government expanded its political influence over Korea, China, and other parts of Southeast Asia. Records on deaths from TB during this period in Korea indicate that the

death rate increased from 18.5 to 71.1 per 100,000 population (Figure 1) (*1*,*4*–*6*).

This increasing trend in TB-associated deaths was in contrast to the trend in most Western Europe countries during the same historical period. Most of these countries were recovering from the repercussions of World War I (1914–1918). For example, in England and Wales, the TB death rate reached its peak (135.8 deaths/100,000 population) when the war ended in 1918, but then the rate steadily decreased from 76.7 to 53.6 per 100,000 population during 1926–1942 (*7*,*8*). North America followed nearly the same trend as that of Western Europe. According to records of the city of New York, New York, USA, the death rate for TB decreased from 126 cases per 100,000 population in 1920 to 49 cases per 100,000 population in 1940 (*9*).

The Korean War (1950–1953)

Korea gained its independence from Japan after World War II in 1945. The country was subsequently divided into North Korea and South Korea, which were protected by 2 competing superpowers, the Soviet Union and the United States, respectively. In 1950, the Korean War broke out and continued for 3 years. By the time an armistice agreement was signed in 1953, most social infrastructure in North and South Korea had been physically damaged or was dysfunctional.

After the Korean War, the number of deaths from TB increased drastically. Data collected in 1954 was used to estimate that 1.3 million of South Korea's 20 million residents had active TB, and death rates ranged from 300 to 400 per 100,000 population (*5*). The last available data on TB before the Korean War were from 1942, when the death rate was 71.7 per 100,000 (*5*). A TB death rate that escalated 5-fold within a decade strongly points to the possibility that war was the strongest causative factor for the TB epidemic in South Korea. It has been well documented that armed conflicts increase deaths from TB for reasons that include malnutrition, overcrowding, and lack of access to health care (*7*,*10*).

By the 1950s, the first effective antimicrobial drugs against TB (i.e., streptomycin, para-aminosalicylic acid, and isoniazid) had been developed. However, it was difficult to deliver the drugs to TB patients in a war-torn country. It was not until 1955 that the Korean National Center for Tuberculosis began the first official distribution of anti-TB medications to 4,000 TB outpatients. The recommended regimen during this period was combined therapy with 2 or 3 of the drugs (*5*).

Economic Growth (1965–1995)

During this period, South Korea experienced phenomenal economic development. This historical achievement

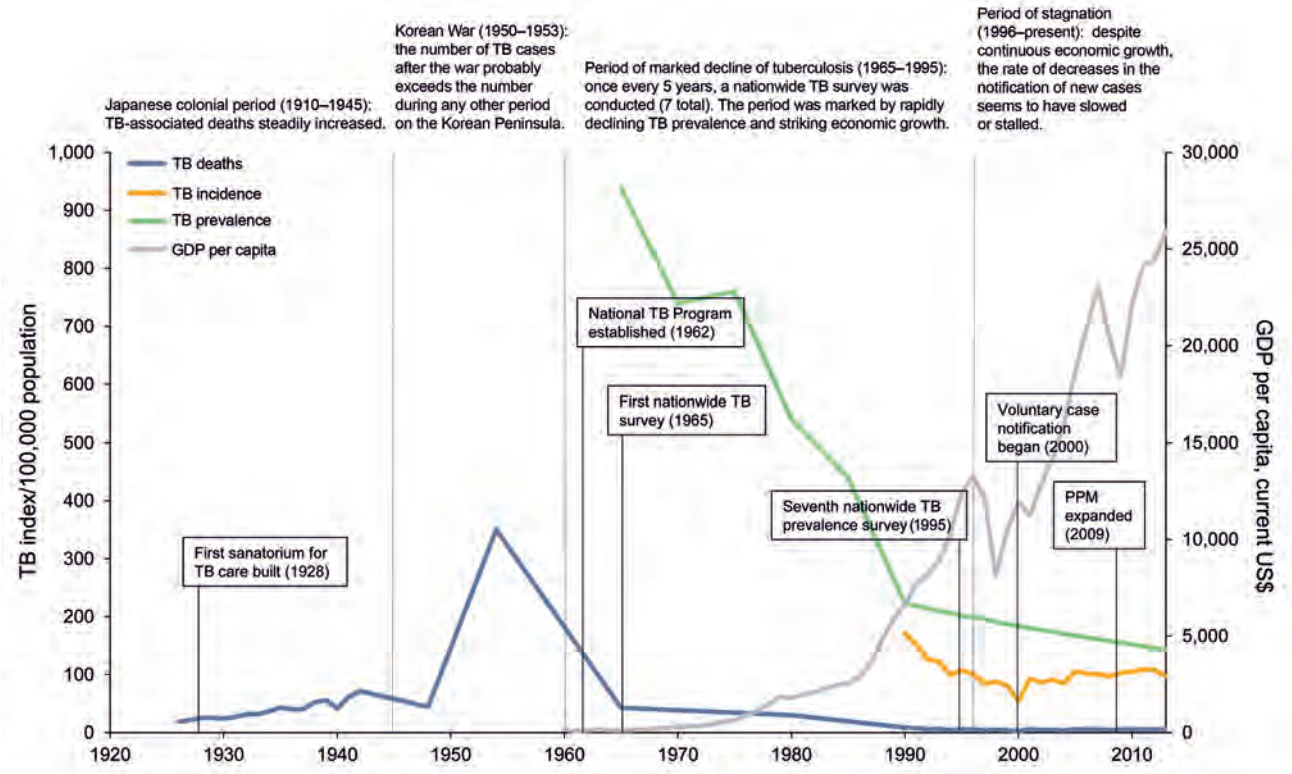

Figure 1. Number of tuberculosis (TB) cases per 100,000 population in South Korea, 1926–2013. Major periods are demarcated by dotted lines. Historical tuberculosis milestones for each period are briefly described. Notable tuberculosis control efforts are summarized in the boxes. GDP, gross domestic product; PPM, public–private mix. Sources: (*1*,*4*–*6*).

is represented by an increase in gross domestic product (GDP) per capita from US $106 in 1965 to US $11,000 by 1995 (*6*). As the economy grew, so did the South Korean national capacity for TB control. The South Korean government established the National Tuberculosis Program 1962. A cornerstone of the program was to provide bacillus Calmette-Guérin (BCG) vaccination to the entire population. Since legislation of this plan, the infant BCG vaccination coverage rate increased dramatically, from 1% in 1965 to 79% by 1990 (*11*). National Health Insurance was enacted in 1963 and was extended to nearly the entire population by 1989, facilitating access to quality medical care. New medications for TB, ethambutol and rifampin, were introduced in the 1980s. The cure rate among TB patients treated in public health centers was 56% in 1983 but improved to 79% by 1994, and the treatment default rate decreased from 15% to 4% during the same period (*12*).

The Korean National Tuberculosis Association administered a nationwide survey every 5 years during 1965–1995. TB prevalence was determined on the basis of chest radiograph findings and sputum smear and culture results (Table 1). Results of the nationwide survey during this time were remarkable. From 1965 to 1995, the prevalence of active pulmonary TB, as determined on the basis of chest radiographs, was reduced from 5,065 to 1,032 cases per 100,000 population, an annual average decrease of 5.0%. The prevalence of smear-positive pulmonary TB dropped from 668 to 93 cases per 100,000 population, a decrease of 6.8% every year. The prevalence of latent infection (defined as a ≥10-mm induration reaction to 1 dose of Tuberculin PPD RT 23) in persons <30 years of age decreased from 55.9% in 1965 to 30.8% in 1995. In conjunction with this change, the annual risk for TB infection in the same period fell from 5.3% to 0.5%. Drug resistance, especially to isoniazid, dropped from 36.4% to 9.2%;

Table 1. Prevalence of pulmonary tuberculosis, South Korea, 1965–1995*

	Prevalence, by year						
Confirmed cases	1965, N = 20,117	1970, N = 26,314	1975, N = 27,090	1980, N = 23,319	1985, N = 39,704	1990, N = 48,976	1995, N = 64,713
All pulmonary cases, radiographically active							
No. cases							
Per 100,000 population	5,065	4,222	3,326	2,509	2158	1,842	1,032
Total	1,019	1,111	901	585	857	902	668
Patient sex							
M	614	650	551	374	534	577	445
F	405	461	350	211	323	325	223
Patient age, y							
5–19	223	296	215	80	103	131	30
20–34	219	203	175	130	218	153	90
35–49	266	273	203	153	203	190	134
50–64	206	193	192	134	185	243	222
≥65	105	146	116	88	148	185	192
Bacteriologically confirmed cases, smear and/or culture positive							
No. cases							
Per 100,000 population	940	741	764	545	443	241	219
Total	189	195	207	127	176	118	142
Patient sex							
M	137	130	139	94	134	84	98
F	52	65	68	33	42	34	44
Patient age, y							
5–19	26	16	26	7	9	5	2
20–34	59	43	44	24	42	15	19
35–49	65	61	58	39	54	34	34
50–64	32	52	55	31	41	37	44
≥65	7	23	24	26	30	27	43
Smear positive cases only							
No. cases							
Per 100,000 population	686	559	480	309	239	143	93
Total	138	147	130	72	95	70	60
Patient sex							
M	96	NA	92	57	77	53	39
F	42	NA	38	15	18	17	21
Patient age, y							
5–19	18	NA	10	5	3	2	1
20–34	45	NA	32	15	23	7	11
35–49	50	NA	42	25	37	26	11
50–64	18	NA	36	17	23	22	19
≥65	7	NA	10	10	9	13	18

*Data are based on a nationwide tuberculosis survey that is conducted every 5 years (*4*). NA, not available.

overall resistance to ≥1 anti-TB drugs among new patients and those with a history of TB treatment decreased from 26.2% and 55.2% to 5.8% and 25.0%, respectively, during this 30-year period (*4*).

Economic growth brought about not only greater national capacity for disease control but also changes in nutritional status of South Korea's population. During this phase of a burgeoning economy, average body mass index increased, especially among children. In 1965, body mass index was 16.9 for children 12 years of age, and by 1995, it had reached 19.0. On average during this 30-year period, 12-year-old children increased in height by >10 cm and increased in weight by 9.5 kg (*13*). Because malnutrition and being underweight are major risk factors for TB, nutritional improvement linked to economic growth has served as a major contributing factor for successful TB outcomes in South Korea.

Other socioeconomic indicators have declined. From 1965 to 1995, the fertility rate declined from 5.0 to 1.6 births per mother, and the size of households decreased from 5.6 to 3.4 persons per household (*14*,*15*). These declines resulted from federal campaigns and family planning programs. During this same period, school class sizes decreased from 65.4 to 36.4 students per classroom. Such factors have led to less crowding at home and in school and the creation of living environments that are less favorable for disease transmission (*13*).

However, despite these improvements in South Korea, shortcomings remained with respect to TB control. Although the proportion of TB patients treated in the private sector increased from the 1980s to the 1990s, cure rates among private-sector patients in 1993 were generally lower than those for public health service patients (*12*,*16*). Inappropriate anti-TB regimens also contributed to poor treatment outcomes. In a survey conducted in 1993, only 11% of general practitioners prescribed the standard 6-month anti-TB treatment regimen, and as many as 86 different regimens were prescribed to 960 TB patients (*16*).

Recent Decades (1996–Present)

A TB notification system was adopted in 1996 in South Korea, replacing the nationwide survey. According to government legislation, when a person receives a diagnosis of TB, the relevant medical staff is required to immediately notify the case to the public health center under jurisdiction. Annual reports on notified cases have been available since 2001. Meanwhile, the South Korean economy has shown steady growth, and GDP per capita has increased from US $12,250 in 1996 to US $22,590 in 2012 (*6*). By 2013, South Korea's GDP was ranked 14th in the world.

Despite the growing economy and continuous national efforts to control TB, the disease maintains a strong foothold in South Korea, afflicting tens of thousands of new patients each year. The number of notified cases per 100,000 population has remained around 90 for more than a decade; the number of notified cases per 100,000 population reached 96.3 in 2001, peaked at 100.8 in 2011, and then decreased to 84.9 in 2014 (Table 2) (*17*). Some experts on TB in South Korea argue that TB incidence has, in fact, been stagnant; they base this argument on the possible undernotification of cases. According to a cross-sectional study of 37,820 TB patients identified from national health insurance claim data in 2008, only 21,611 (57.1%) cases were reported to the Korean Tuberculosis Surveillance System (*18*). To determine whether TB incidence is decreasing, more data are needed on the number of notified TB patients and the number of TB patients who received national health insurance benefits.

Notwithstanding such speculation, South Korea has a disproportionately high burden of TB compared with most high-income countries (Figure 2) (*19*). According to the World Health Organization (WHO), the incidence of TB in South Korea was 108 cases per 100,000 population in 2012, which is >7 times higher than the average for member countries of the Organisation for Economic Co-operation and Development. For example, the annual incidence of TB in the United States and Japan in 2012 was 3.6 and 19 cases per 100,000 population, respectively.

Part II. Reasons for the High Burden of TB in South Korea

Recent data indicate that, among high-income countries, South Korea stands out in terms of TB burden, despite its astonishing rate of economic development. In this

Table 2. Number of notified tuberculosis cases, South Korea, 2001–2014*

Type of notification	No. notified cases, by year													
	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
Cases per 100,000 population	96.3	89.4	83.8	86.1	96.5	94.7	92.8	89.4	95.3	96.4	100.8	98.4	89.6	84.9
Cases in public sector	15,728	13,003	11,810	10,851	9,680	9,018	7,558	7,315	7,079	5,463	4,461	3,779	3,269	2,994
Cases in private sector	18,395	19,007	18,877	20,652	25,589	26,343	27,152	26,842	28,766	30,842	35,096	35,766	32,820	31,875

*Source: (*17*).

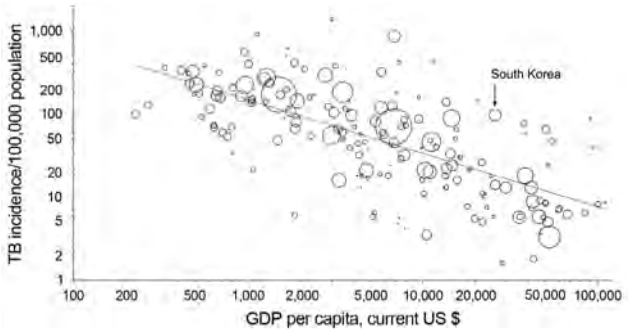

Figure 2. Relationship between per capita gross domestic product (GDP) and incidence of tuberculosis (TB), 2013. Each dot represents 1 country; South Korea is indicated. The third root of the population was used to determine the size of the circles, and the figure is drawn on a logarithmic scale. The line indicates the regression on the logarithm. The figure was adapted from (*19*) with permission from The European Respiratory Society. Updated data was derived from (*1*).

section, we attempt to delineate some of the most frequently discussed factors attributed to the tenacious character of TB in South Korea.

High Prevalence of Latent TB Infection in the Elderly Population

An older population is at higher risk for TB because host immunity against *M. tuberculosis* wanes with aging (*20*). The elderly population in South Korea has steadily increased over the last 3 decades. Such demographic transition is reflected in the increased number and proportion of TB patients >65 years of age, rising from 9,322 (20.2%) in 2001 to 15,227 (33.6%) in 2013 (Figure 3) (*17*,*21*). Among South Korea residents >60 years of age without radiographic evidence of prior TB, 67.2% were found to have latent TB (*22*). TB was highly prevalent during the youth of most persons born during 1955–1963 in South Korea. This population group, with a possibly large number of latent infections, comprises 15% of the entire population of South Korea today. When this group officially becomes part of the elderly population in 2020, a resurgence of TB is possible, and this possibility casts a bleak outlook for TB control in South Korea.

An Increasing Population with Diabetes

The increasing number of persons in South Korea with various concurrent conditions puts an additional burden on the country's TB control program. The epidemiologic trend during the past few decades in South Korea generally moved away from infectious diseases and toward chronic diseases, such as diabetes, which puts patients at greater risk for developing TB. Diabetes mellitus is a common concurrent condition that shows a strong association with TB, particularly in TB-endemic developing countries with a rising prevalence of diabetes mellitus (*23*). TB is thought to induce glucose intolerance and make it more difficult for persons with diabetes to control blood glucose. This in turn causes them to be more susceptible to new infection, reactivation of latent infection, and aggravation of active disease (*24*). The prevalence of diabetes mellitus in South Korea has increased from 1.5% to 9.9% in the past 40 years (*25*), and a positive relationship between diabetes and TB has been documented in South Korea's population (*26*).

High Smoking Rate

Although the proportion of smokers in most industrialized countries is decreasing, many TB patients in South Korea still use tobacco. In 2012, the percentage of daily smokers among men and women >15 years of age was 37.6% and 5.8%, respectively (*27*). The number of male smokers is the highest among most industrialized countries. By comparison, the percentage of daily smokers among men >15 years of age in the United States and United Kingdom during 2012 was 15.9% and 20.3%, respectively. Smoking has been documented as a risk factor in terms of TB infection, disease, and death (*28*). In a prospective cohort study involving more than 1 million South Koreans, smoking was associated with increased TB incidence, recurrence, and death (*29*). For these reasons, smoking is likely to be responsible for the relatively high burden of TB in South Korea. The current Korean Guidelines for Tuberculosis recommend that persons who smoke and who receive a diagnosis of TB should be advised to stop smoking before treatment begins (*30*).

Inadequate Patient Management

Thorough patient management during standard TB treatment has been difficult to achieve in South Korea. Directly observed therapy (DOT), short-course (DOTS), has been the hallmark of TB treatment for decades, and this program has been shown to be effective in various settings (*31*). Among the 5 elements of the DOTS strategy, DOT is an indispensable component; unfortunately, DOT is not practiced in South Korea. Furthermore, until recently, South Korea had no mandate for isolating smear-positive TB patients, even those who were noncompliant or who had multidrug-resistant disease, and most patients were treated in the outpatient setting. This lack of monitoring could have been potential barriers to the advancement of TB control.

The rationale for not adopting DOT in South Korea is that the treatment success rate in the public sector is ≈90%, which is higher than the DOTS target of 85% set by WHO (*32*). However, the proportion of TB patients receiving treatment in the private sector has increased to 90% in the past decade, and the treatment success rate for

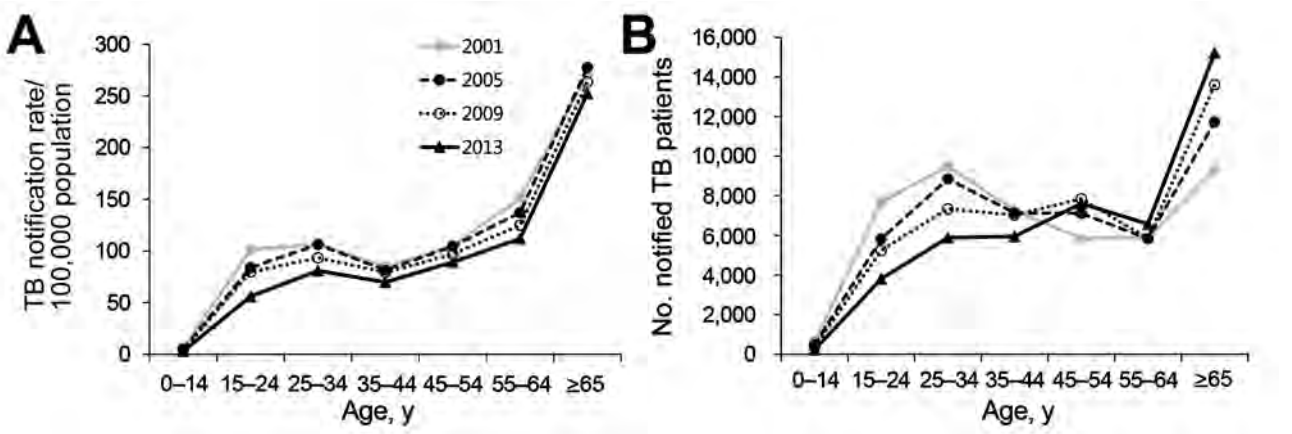

Figure 3. Rate of notified tuberculosis (TB) cases by age group and year, South Korea. A) Comparison of age-specific notified TB cases per 100,000 population between 2001 and 2013. In 2013, notification rates among all age groups were lower than those in 2001. B) Comparison of notified tuberculosis cases by patient age between 2001 and 2013. In 2013, there was a marked increase in the number of cases among persons 45–54 and ≥65 years of age. The figure was adapted from (*21*).

this sector was 75% in 2001 (10% below the WHO target). This low success rate suggests that poorly executed patient monitoring among private health care providers could have been the reason for the high treatment failure rates (*17*,*33*).

Immigrants from High-Burden Countries

South Korea has witnessed a continuous influx of immigrants in recent years. The most common countries of origin are China, Mongolia, Pakistan, Philippines, Vietnam, and North Korea (*34*). The proportion of TB patients of foreign nationality has been increasing in recent years, from 0.3% in 2001 to 3.8% in 2013 (*17*). Because this increased percentage represents mostly documented immigrants, the actual number is thought to be much higher. Many of these immigrants have inadequate access to health care because of their socioeconomic and legal status. Furthermore, the number of North Korean defectors entering South Korea has increased in the past decade, and >1% are considered to have active TB at the time of entrance (*35*). Because of the diverse backgrounds and legal circumstances among this population at high risk for TB, adoption of a more rigorous screening program is necessary.

Part III. Recent Efforts and Goals to Be Achieved

To overcome the challenges posed by TB, the South Korean National Tuberculosis Program has attempted to conduct rigorous monitoring activities in an effort to further reduce TB in the country. The "New 2020 Plan" set out by the Korean Centers for Disease Control aims to cut the incidence rate in half by 2020, preferably to <50 cases per 100,000 population. The 2 major arms of recent endeavors include expanded public–private mix collaboration and prompt outbreak investigations.

Public–Private Mix Collaboration

In 2009, the government of South Korea initiated public–private mix collaboration for TB control based on WHO recommendations (*36*). Major components of the collaboration include strict monitoring of patients, investigation of close contacts, and financial support of patients hospitalized in the private sector with multidrug-resistant TB. Preliminary results of the program were successful; patients included in the public–private mix collaboration showed higher treatment success rates than controls (91.6% vs. 71.8%). The treatment default rate for patients in the collaboration program (6.6%) was lower than that for controls (22.9%), resulting in a better treatment outcome (*33*). In 2011, public–private mix collaboration was expanded to 97 health centers, in which 31,050 (92.4%) of 33,587 total patients were reported to have been treated under rigorous monitoring by nurses trained exclusively for TB patient management (*37*).

Public–private mix collaboration has a set of stipulations that must be fulfilled to meet the intended results. Key elements of successful collaboration are decentralization; transparency; mutual respect; working through consensus; private provider involvement at all levels, including the highest-level policymaking; continual dialogue; and quality assurance (*38*). To guarantee the success of public–private mix collaboration in South Korea, policymakers should regard frontline clinicians working in the private sector as partners in every aspect of the collaboration.

Reinforcement of Outbreak Investigations

The Korean Centers for Disease Control strives to detect TB cases by screening anyone who is in frequent contact with a newly identified patient in various settings. If a notified patient with TB is a preschool child, school student,

teacher, or member of the military or is living in an institutional facility, outbreak investigation must be enforced in accordance with the South Korean National Tuberculosis Program. When a school student is determined to have infectious TB, all students sharing the same classroom must be examined. In addition, if 2 school students in a class have had known TB in the previous 6 months, every student in the class is examined. If ≥3 students in a school have infectious TB, everyone in the school, including all students and teaching staff, should be examined. Chest radiographs and sputum microscopy are used to determine active TB. If results of these tests are normal, a tuberculin skin test is administered to screen for *M. tuberculosis* infection (≥10 mm induration). If the skin test is reactive, an interferon-g release assay is used to rule out false-reactive results, and treatment is offered to those determined to have TB (*39*). These guidelines have not been strictly followed but are now being strongly enforced. In 2013 alone, a total of 3,824 patients were notified from 3,265 institutional facilities and a total of 1,194 investigations were carried out, during which 1,476 possible contacts were tested for *M. tuberculosis* infection. Of these 1,476 persons, 939 were from schools, 142 were from military bases, 274 were from health care facilities, 64 were from occupational settings, and 57 were from other settings (*40*).

Conclusion

The experiences of South Korea during the 20th century have been similar to those of many developing countries: independence from colonization, warfare, economic growth, and health conditions shifting toward chronic diseases as the population ages. Today, South Korea is a developed country, but it still has emerging problems with TB control, which largely stem from demographic transitions within the country. Despite the emerging challenges, South Korea's National Tuberculosis Program can be seen as a successful model that can be applicable to other developing countries. The history of TB in South Korea illustrates that epidemiologic changes that occur along with the development of new drugs and diagnostic tools must be accompanied by changes in policymaking. Policymakers should pursue improved national TB programs to help meet the challenges of TB control in light of the continuously evolving global disease burden.

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Ebola Virus Outbreak Investigation, Sierra Leone, September 28–November 11, 2014

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During 2014–2015, an outbreak of Ebola virus disease (EVD) swept across parts of West Africa. The China Mobile Laboratory Testing Team was dispatched to support response efforts; during September 28–November 11, 2014, they conducted PCR testing on samples from 1,635 suspected EVD patients. Of those patients, 50.4% were positive, of whom 84.6% lived within a 3-km zone along main roads connecting rural towns and densely populated cities. The median time from symptom onset to testing was 5 days. At testing, 75.7% of the confirmed patients had fever, and 94.1% reported at least 1 gastrointestinal symptom; all symptoms, except rash and hemorrhage, were more frequent in confirmed than nonconfirmed patients. Virus loads were significantly higher in EVD patients with fever, diarrhea, fatigue, or headache. The case-fatality rate was lower among patients 15–44 years of age and with virus loads of <100,000 RNA copies/mL. These findings are key for optimizing EVD control and treatment measures.

Ebola virus disease (EVD) is a severe, frequently fatal illness. In March 2014, the largest EVD outbreak in history began spreading through parts of West Africa. As of June 21, 2015, a total of 27,443 cases, including 11,207 deaths, had been reported, of which 13,059 cases and 3,928 deaths were in Sierra Leone (*1*). Case numbers are believed to be underreported because they do not include many persons with clinically confirmed EVD who evaded laboratory confirmation and persons with suspected EVD who died and were buried without a confirmed diagnosis (*2*). This epidemic became an international public health emergency, and teams of public health experts continue to be deployed to affected areas to help with disease control efforts.

To support Sierra Leone and to respond to the World Health Organization (WHO) and United Nations' appeals to help western Africa control the EVD epidemic, the China Mobile Laboratory Testing Team (CMLTT) was dispatched in September 2014 at the request of the Sierra Leone government (*3*). The team, equipped with medical experts who specialize in laboratory testing, epidemiology, clinical medicine, and nursing, worked at the Sierra Leone–China Friendship Hospital in Jui, a town in Western Area, Sierra Leone, ≈30 km southeast of Freetown (Figure 1; online Technical Appendix Figure 1, http://wwwnc.cdc.gov/EID/article/21/11/15-0582-Techapp1.pdf). The CMLTT was tasked with testing clinical samples for EVD; the samples were mainly collected from suspected EVD patients receiving care in Sierra Leone's Western Area and Northern Province. All CMLTT activities were coordinated by an emergency operations center jointly established by the Sierra Leone Ministry of Health and Sanitation (MoHS) and WHO. We report the epidemiologic and clinical characteristics of a geographically distinct case series of live and deceased suspected EVD patients, from whom samples were collected and tested by the CMLTT.

Material and Methods

Study Design and Patients

The study included all suspected EVD patients (also called persons under investigation [PUI], per the WHO case definition at the time) from whom blood or oral swab samples

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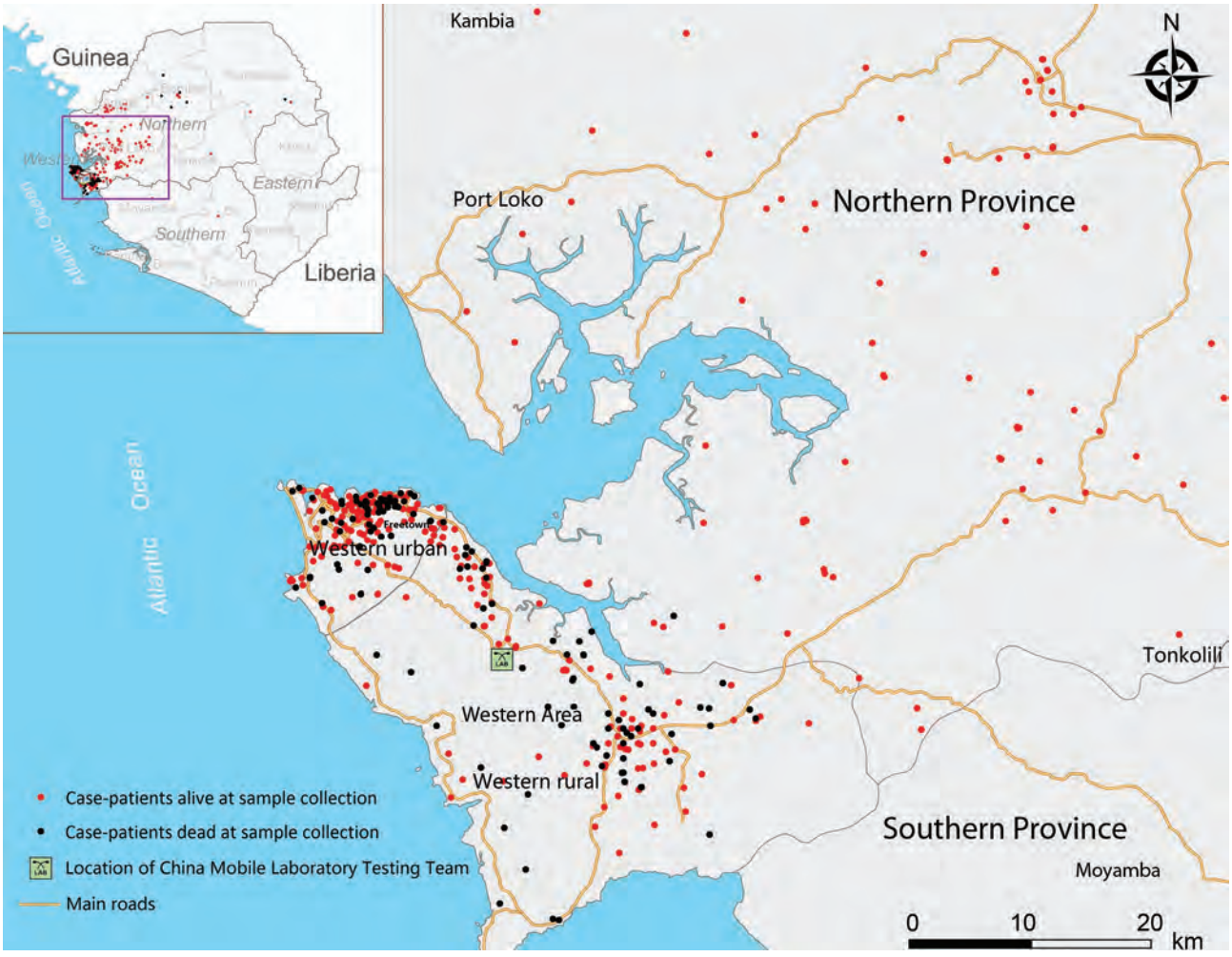

Figure 1. Geographic distribution of Ebola virus disease cases confirmed by the China Mobile Laboratory Testing Team (CMLTT), Sierra Leone, September 28–November 11, 2014. Inset shows the location of areas shown in the enlarged map. Western Area and parts of Northern Province and Southern Province are indicated on the enlarged map, as are rural and urban sections of Western Area.

were collected and sent to CMLTT for Ebola virus testing during September 28–November 11, 2014. A standardized WHO case investigation form was completed for each PUI by health care workers at the time of sample collection; the forms contained demographic information and information regarding signs and symptoms of disease, hospitalization, epidemiologic risk factors, and possible or known exposures to Ebola virus. For retrospective diagnosis of Ebola virus infection, we collected oral swab samples from deceased suspected EVD patients; information on age, sex, and address of previous residence were obtained from simple burial records for these persons. Using the case definition for disease surveillance developed by WHO, we defined confirmed EVD case-patients as persons (alive or dead) with suspected EVD whose samples were confirmed to be Ebola virus–positive by laboratory testing (*1*,*2*). For case-patients who were alive at sample collection, the definitive clinical outcomes were obtained at the end of December 2014 from a viral hemorrhagic fever database managed by the Sierra Leone MoHS.

Laboratory Testing

Before use, blood and oral swab samples from PUIs were inactivated (62°C for 60 min) within the mobile Biosafety Level 3 laboratory as previously described (*4*). RNA was extracted from samples by using the QIAamp Viral RNA Mini Kit (QIAGEN, Germantown, MD, USA) according to the manufacturer's instructions. Quantitative reverse transcription PCR targeting the glycoprotein gene of Ebola virus subtype Zaire was performed by using primer pairs 5′-TGGGCTGAAAAYTGCTACAATC-3′ and 5′-CTTTGTGMACATASCGGCAC-3′ and probe FAM-5′-CTACCAGCAGCGCCAGACGG-3′-TAMR as previously described (*5*). The cycle threshold cutoff value was 36. For quantification, virus loads were estimated as Ebola virus RNA copies per milliliter (online Technical Appendix).

Ethical Considerations

This work was conducted as part of the surveillance and public health response to contain the EVD outbreak in Sierra Leone. Activities were coordinated by the emergency operations center established by the Sierra Leone MoHS and WHO. All data obtained from this work belong to the Sierra Leone MoHS and were shared with CMLTT for reporting. The data were submitted to the Sierra Leone National Ethics and Scientific Research Committee. All information regarding individual persons has been anonymized in this report.

Data Analyses

Each confirmed case was georeferenced and linked to a digital map of Sierra Leone (http://www.mapmakerdata.co.uk.s3-website-eu-west-1.amazonaws.com/library/stacks/Africa/Sierra%20Leone/) according to the residential address of the case-patient by using ArcGIS 9.2 software (Esri, Redlands, CA, USA). We then conducted a proximity analysis of confirmed cases in relation to the main transportation routes. We identified epidemiologic and clinical data for each case-patient by extracting the necessary information from the case investigation form. The case-fatality rate was calculated as the percentage of persons who died among the confirmed EVD case-patients with a known definitive clinical outcome; outcome information was attained from the viral hemorrhagic fever database that was updated by Sierra Leone MoHS and WHO. Descriptive statistics to do with measures of central tendency and dispersion, such as mean, mode, and median, were calculated for all variables. Continuous variables were summarized as median, mean ± SD, and range; categorical variables were summarized as frequencies and proportions. To estimate the differences between groups, we used Student *t* test, χ^2 test, or Fisher exact test, as appropriate. A 2-sided $p<0.05$ was considered statistically significant. All statistical analyses were conducted by using SAS software version 9.3 (SAS Institute, Inc., Cary, NC, USA).

Results

Patients

During September 28–November 11, 2014, a total of 1,635 samples from PUIs were sent to CMLTT at the Sierra Leone–China Friendship Hospital in Jui for EVD testing. A total of 824 (50.4%) samples were Ebola virus–positive; details regarding the samples, results, and case-patients are presented in online Technical Appendix Figure 2. These 824 confirmed cases represented 33.3% of 2,471 total confirmed cases reported in Sierra Leone during the study period (online Technical Appendix Figure 3).

Epidemiologic Characteristics

The numbers of samples received by CMLTT and the rate of positive samples varied from day to day (online Technical Appendix Figure 4); however, the average percentage of positive samples during the last 10 days of testing (November 1–11, 2014) was significantly lower than that during September 28–October 31, 2014 (41.2% vs. 57.0%, respectively; $p<0.001$). A comparison of the weekly numbers of tested samples and positivity rates for case-patients who were alive and those who were deceased showed similar temporal variations (online Technical Appendix Figure 5).

The median age of confirmed EVD case-patients was 26 years (range 2 days to 99 years); 7.1% of the patients were <5 years of age (online Technical Appendix Table 1). Cases occurred in 9 districts of Sierra Leone, mainly in Western Area around the mobile Biosafety Level 3 laboratory catchment area. Most (84.6%) confirmed cases were distributed within a 3-km zone along the main roads that connect rural and urban areas (Figure 1).

The sex distribution for live and deceased case-patients (as defined as the outcome at time of testing) was similar ($p = 0.52$), and deceased case-patients were significantly older than live case-patients ($p = 0.004$) (online Technical Appendix Table 1). Oral swab samples were tested for 404 deceased persons (391 from Western Area, 12 from Northern Province, and 1 from Eastern Province); however, they could not be included in further analyses because only simple demographic information on age, sex, and address of residence was available in the patients' burial records.

Clinical Characteristics

Of 666 confirmed EVD patients who were alive when samples were collected, 606 had provided information on clinical manifestations of the disease on their case investigation forms, and 563 had a known clinical outcome (online Technical Appendix Figure 2). The most commonly reported symptoms were fatigue, anorexia, fever, headache, vomiting or nausea, diarrhea, abdominal pain, joint pain, and muscle pain (Table). Of these 563 case-patients, 530 (94.1%) reported as least 1 gastrointestinal symptom (anorexia, nausea, vomiting, diarrhea, abdominal pain, or hiccups), and 426 (75.7%) had fever. Hemorrhage (i.e., hemoptysis, bleeding from the gums and nose, hematochezia, hematuria, bleeding at injection sites, and vaginal bleeding) was observed in 6 (1.1%) patients. All signs and symptoms, except skin rash and hemorrhage, were more frequently observed in patients with confirmed EVD than in those with negative test results ($p<0.05$) (Table 1). The median time from symptom onset to seeking care at an Ebola health facility (i.e., holding or treatment center) for EVD testing was 5.0 days (interquartile range 3.0–7.0 days) (online Technical Appendix Figure 6).

Among the 563 case-patients, the overall case-fatality rate was 67.4%. Case-fatality rates for persons ≥45 years of age (72.0%) and persons <15 years of age (63.7%) were significantly higher than that for persons 15–44 years of age

(52.5%) (p = 0.001 and p = 0.026, respectively) (Figure 2, panel A). The case-fatality rate for case-patients with fever was significantly higher than that for case-patients without fever (61.0% [260/426 patients]) vs. 49.6% [68/137 patients]; p = 0.019).

Virus Loads

For comparison, we quantified and log-transformed the Ebola virus load (RNA copies/mL) for each patient with confirmed EVD. The mean virus load for EVD patients at admission to an Ebola health facility (i.e., the day of testing) varied depending on the time between the onset of signs and symptoms and admission. Mean virus load continued to increase for patients tested 1–3 days after symptom onset; values peaked at 3–7 days, began decreasing at 7–14 days, and continued decreasing thereafter (online Technical Appendix Figure 7). Virus loads for case-patients with fever, diarrhea, fatigue, and headache were significantly higher than those for case-patients without these symptoms (Figure 3). Case-patients with 10^5–10^7 or $>10^7$ viral RNA copies/mL had higher case-fatality rates than did case-patients with $<10^5$ viral RNA copies/mL (p = 0.036 and p = 0.027, respectively) (Figure 2, panel B).

Discussion

During September 28–November 11, 2014, we confirmed that a total of 824 persons in Sierra Leone were positive for EVD; this number represents one third of the reported cases in the country during this period (*6*). Most (84.6%) case-patients identified in this study resided within a 3-km zone along the main roads of Sierra Leone, which are vital connections between rural towns and densely populated cities. This finding suggests that epidemic dispersal of Ebola virus is promoted when infectious persons live in close proximity to main roads. These roads provide a convenient source of transportation for persons traveling to Ebola health facilities, which may have enabled the rapid and extensive spread of Ebola virus infection in Sierra Leone through person-to-person contact. In contrast, the simultaneous EVD outbreak in the Democratic Republic of Congo was much smaller, probably because it occurred in remote forested areas where person-to-person contact outside the local population may be more limited because access to transportation is limited (*7*).

Of note, 39.1% of swab samples collected from deceased persons were positive for Ebola virus RNA. The prompt confirmation of Ebola virus infection in dead persons can contribute to a reduction of virus transmission during funerals because safe burial practices are required in affected areas once a diagnosis of EVD is made (*1*). To decrease the transmission of Ebola virus through unsafe burial practices, samples should be collected from and a diagnosis should be determined for persons who die from unknown causes (*8*).

In our study, the most common symptoms for persons with confirmed EVD were fatigue, anorexia, fever, vomiting or nausea, headache, diarrhea, joint pain, abdominal pain, and muscle pain; these findings are comparable to those from other studies in Sierra Leone (*9*–*11*). Of the patients in our study, 94.1% had at least 1 gastrointestinal symptom; nausea, vomiting, and diarrhea were common and caused severe dehydration and electrolyte abnormalities that subsequently led to circulatory collapse and death. The high frequency of gastrointestinal symptoms further supports the proposal for administration of intravenous fluids and electrolytes in the treatment of EVD (*12*). Signs and symptoms, including the low frequency of hemorrhagic signs, for patients in our study were similar to those for contemporary case-patients in studies in other affected countries (Table 1; online Technical Appendix Table 2) (*2*,*11*,*13*). However, our results indicate that persons infected during this outbreak showed a lower

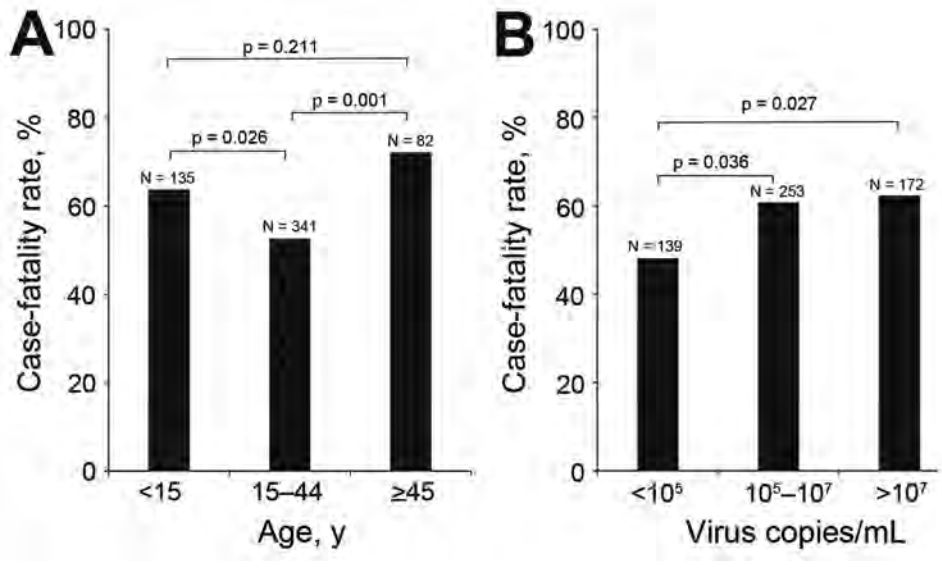

Figure 2. Case-fatality rates among patients with Ebola virus disease, Sierra Leone, September 28–November 11, 2014. A) Rates among different age groups. B) Rates among persons with different virus loads. The total number of patients in each group is shown at the top of the respective bar.

Table 1. Demographic and clinical characteristics for suspected Ebola virus disease patients, Sierra Leone, September 28–November 11, 2014*

Characteristics	No. (%) patients with positive RT-PCR results			No. (%) patients with negative RT-PCR results, n = 451
	All patients, n = 563	Patients who died, n = 328	Patients who recovered, n = 235	
Demographic characteristic				
Sex				
Female	266 (47.2)	156 (47.6)	110 (46.8)	219 (48.6)
Male	297(52.8)	172(52.4)	125(53.2)	232(51.4)
Age, y, group†				
0–5	30 (5.3)	18 (5.6)	12 (5.1)	53 (12.0)
6–14	105 (18.7)	68 (21.0)	37 (15.8)	59 (13.4)
15–44	341 (61.1)	179 (55.2)	162 (69.2)	261 (59.3)
≥45	82 (14.7)	59 (18.2)	23 (9.8)	67 (15.2)
Location of residence				
Western Area				
Rural areas	198 (35.2)	107 (32.6)	91 (38.7)	137 (30.4)
Urban areas	140 (24.9)	71 (21.6)	69 (29.4)	177 (39.2)
Northern Province				
Port Loko District	191 (33.9)	131 (39.9)	60 (25.5)	71 (15.7)
Kambia District	21 (3.7)	13 (4.0)	8 (3.4)	27 (6.0)
Bombali District	10 (1.8)	5 (1.5)	5 (2.1)	23 (5.1)
Koinadugu District	2 (0.4)	1 (0.3)	1 (0.4)	5 (1.1)
Tonkolili District	0	0	0	9 (1.2)
Southern Province				
Bo Town	1 (0.2)	0	1 (0.4)	0
Bonthe District	0	0	0	1 (0.1)
Signs and symptoms				
Fatigue	464 (84.4)	272 (82.9)	192 (81.7)	196 (43.5)
Anorexia	467 (82.9)	278 (84.8)	189 (80.4)	208 (46.1)
Fever	426 (75.7)	260 (79.3)	166 (70.6)	210 (46.6)
Vomiting or nausea	354 (62.9)	202 (61.6)	152 (64.7)	112 (24.8)
Headache	354 (62.9)	209 (63.7)	145 (61.7)	195 (43.2)
Diarrhea	349 (62.0)	207 (63.1)	142 (60.4)	103 (22.8)
Joint pain	319 (56.7)	186 (56.7)	133 (56.6)	174 (38.6)
Abdominal pain	317 (56.3)	184 (56.1)	133 (56.6)	130 (28.8)
Muscle pain	305 (54.2)	183 (55.8)	122 (51.9)	137 (30.4)
Chest pain	226 (40.1)	123 (37.5)	103 (43.8)	96 (21.3)
Cough	212 (37.7)	113 (34.5)	99 (42.1)	101 (22.4)
Difficulty breathing	191 (33.9)	110 (33.5)	81 (35.4)	88 (19.5)
Difficulty swallowing	164 (29.1)	95 (29.0)	69 (29.4)	56 (12.4)
Conjunctivitis	161 (28.6)	95 (29.0)	66 (28.1)	34 (7.5)
Confused	151 (26.8)	88 (26.8)	63 (26.8)	50 (11.1)
Sore throat	117 (20.8)	66 (20.1)	51 (21.7)	39 (8.6)
Jaundice	102 (18.1)	63 (19.2)	39 (16.6)	42 (9.3)
Hiccups	95 (16.9)	56 (17.1)	39 (16.6)	20 (4.4)
Pain behind eyes	55 (9.8)	34 (10.4)	21 (8.9)	13 (2.9)
Rash	45 (8.0)	25 (7.6)	20 (8.5)	23 (5.1)
Coma	27 (4.8)	15 (4.6)	12 (5.1)	10 (2.2)
Hemorrhage	6 (1.1)	6 (1.8)	0	7 (1.6)
Virus load, mean ± SD‡	363,078 ± 28	436,515 ± 26	288,403 ± 30	NA

*NA, not applicable; RT-PCR, reverse transcription PCR.
†Age was not known for 5 patients with confirmed disease and 11 patients with negative test results.
‡Virus loads represent RNA copies.

frequency of the primary clinical symptoms than did persons infected with a different Ebola virus strain (Bundibugyo) during an outbreak in Uganda in 2007 (online Technical Appendix Table 2) (*13*).

In our study, 75.7% (426/563) of the confirmed case-patients had a fever when their specimens were collected and tested for Ebola virus. These findings are similar to those of our colleagues, Qin et al. (*14*), who found that 18.0% (11/61) of patients in the Sierra Leone–China Friendship Hospital did not have a fever on the day of admission. During these studies, the field case definition for fever was temperature >38.0°C at the time of assessment or a history of fever. This finding implies that persons with suspected EVD but without fever may still be infective.

Our analysis showed that, except skin rash and hemorrhage, all clinical symptoms that were surveyed in the case investigation form were more frequently observed in patients with than those without confirmed EVD. This finding suggests that the case definition in use at the time was appropriate for this outbreak. In our study, the case-fatality rate

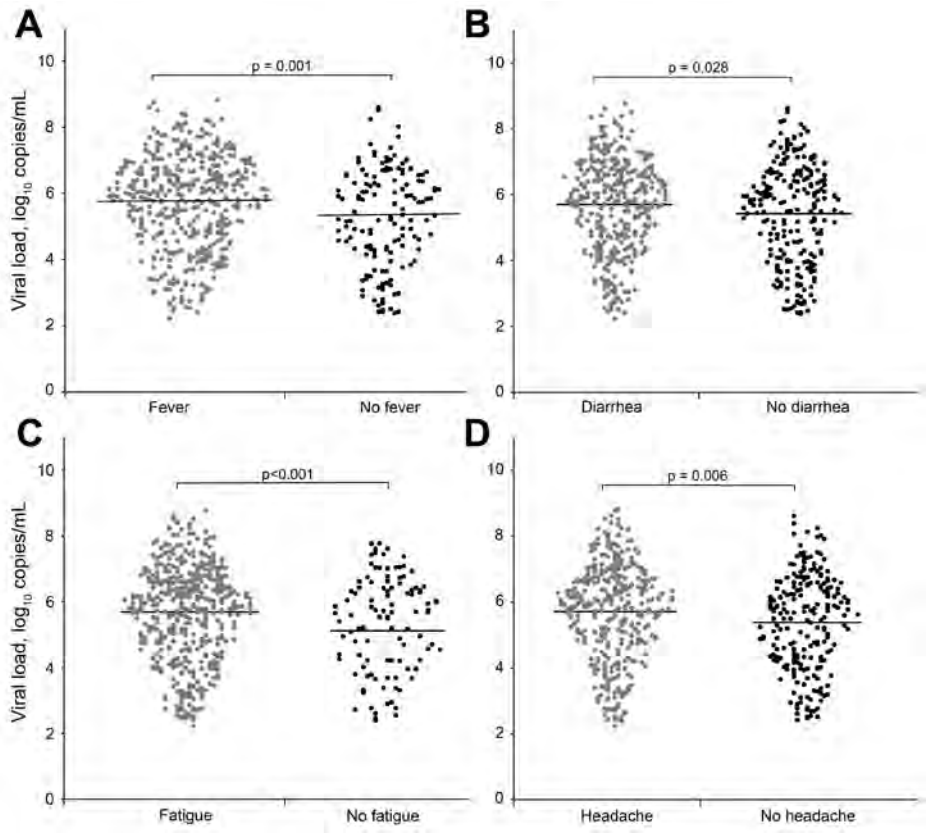

Figure 3. Comparison of virus loads for patients with Ebola virus disease with and without fever (A), diarrhea (B), fatigue (C), or headache (D). Dots represent the log-transformed virus loads in patients with and without each symptom. The horizontal line in each panel indicates the mean value of log-transformed virus loads for each group.

was 67.4% among confirmed EVD case-patients who were alive when samples were obtained; this rate is comparable to those reported for Sierra Leone by the WHO (*2*). Other studies on EVD in Sierra Leone reported a 73.6% (64/87) case-fatality rate for cases during May 25–June 18, 2014, in Eastern Province (*9*); a 31.5% (183/581) case-fatality rate during September 20–December 7, 2014, at Hastings Treatment Center in Western Area (*10*); and a 24.6% case-fatality rate (1,169 confirmed cumulative deaths among 4,744 confirmed cases) reported by the Sierra Leone MoHS during May 23–November 11, 2014 (*6*). Patients 15–44 years of age had a lower case-fatality rate than older and younger patients. This association of age with the death rate was similar to that observed in the early stage of the EVD outbreak in West Africa (*2*). Of note, in our study, the case-fatality rate for patients <15 years of age was relatively high compared with that reported in Eastern Province (*10*). These findings indicate that older patients and children <15 years of age should receive more medical attention to reduce their higher case-fatality rate and that investigations are needed to determine why EVD case-fatality rates differ by patient age.

In agreement with findings by Schieffelin et al. (*9*), we found that a low virus load at admission to a treatment facility was associated with a better outcome. However, those results might have been different had we used a cutoff value of 10^5 in 3 categories, similar to the cycle threshold value of 25 that was described in a recent article by Fitzpatrick et al. (*15*). We also found that patients with fever, diarrhea, fatigue, or headache had virus loads that were significantly higher than those for patients without these symptoms; this finding is consistent with those from other studies (*9*,*15*).

The number of confirmed cases in our analysis was quite large, accounting for one third of the cases reported in Sierra Leone during the study period. Nevertheless, our study had several limitations. First, the inclusion of confirmed EVD patients whose samples were sent to CMLTT for Ebola virus testing was subject to selection bias because the samples collected from PUIs were delivered to laboratories in a haphazard manner. Second, the purpose of our testing was to intensify the outbreak response efforts, not to conduct surveillance or accurately ascertain the prevalence of disease. Last, information on many case investigation forms was incomplete because the data were collected in the context of response operations and used for clinical care, contact tracing, and transmission prevention rather than for a rigorous epidemiologic survey. Because of these limitations, our results should be interpreted with discretion.

These findings provide key information for informing public health decision-making during Ebola virus outbreaks. EVD control measures and treatment methods should be optimized according to the transmission, clinical, and viral features specific to each outbreak.

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Neurologic Disorders in Immunocompetent Patients with Autochthonous Acute Hepatitis E

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Learning Objectives

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

- Distinguish overall aspects of neurologic disorders occurring in immunocompetent patients infected with hepatitis E virus, based on a retrospective case series
- Discuss mononeuritis multiplex occurring in immunocompetent patients infected with hepatitis E virus
- Discuss Parsonage-Turner syndrome occurring in immunocompetent patients infected with hepatitis E virus.

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Neurologic disorders, mainly Guillain-Barré syndrome and Parsonage–Turner syndrome (PTS), have been described in patients with hepatitis E virus (HEV) infection in industrialized and developing countries. We report a wider range of neurologic disorders in nonimmunocompromised patients with acute HEV infection. Data from 15 French immunocompetent patients with acute HEV infection and neurologic disorders were retrospectively recorded from January 2006 through June 2013. The disorders could be divided into 4 main entities: mononeuritis multiplex, PTS, meningoradiculitis, and acute demyelinating neuropathy. HEV infection was treated with ribavirin in 3 patients (for PTS or mononeuritis multiplex). One patient was treated with corticosteroids (for mononeuropathy multiplex), and 5 others received intravenous immunoglobulin (for PTS, meningoradiculitis, Guillain-Barré syndrome, or Miller Fisher syndrome). We conclude that pleiotropic neurologic disorders are seen in HEV-infected immunocompetent patients. Patients with acute neurologic manifestations and aminotransferase abnormalities should be screened for HEV infection.

Hepatitis E virus (HEV) infection is an emerging autochthonous disease in industrialized countries (1). Locally acquired (autochthonous) HEV 3 or HEV 4 infections are believed to be a porcine zoonosis. The virus typically affects middle-aged or elderly men and can cause severe hepatitis, particularly in patients with an underlying liver disease (2). Chronic HEV infection occurs in recipients of a solid-organ transplant, in patients with hematologic malignancies, and in patients with HIV infection (3,4). Neurologic symptoms have been reported in up to 5% of patients with an HEV infection, indicating that HEV could have a specific neurotropism (5); most patients in this preliminary study were immunosuppressed. In developing countries, cases describing neurologic involvement during acute HEV infection have also been reported (6,7): most concerned Guillain-Barré syndrome (GBS) and Parsonage-Turner syndrome (PTS). Peripheral neuropathy, small-fiber neuropathy, and myositis have also been described (8,9). Peripheral nervous system tropism is not exclusive, and rare cases of myelitis and encephalitis have also been reported (10). Recently, studies of 2 national cohorts (in the United Kingdom and the Netherlands) have found that GBS and PTS were associated with acute HEV infection in 5% and 10% of cases, respectively (11,12). Here we describe an additional range of neurologic manifestations that were found during acute autochthonous HEV infection in immunocompetent patients.

Patients and Methods

This retrospective multicenter study was conducted in France from January 2006 through June 2013. All members of the French Liver Association (Association Française pour l'Etude du Foie, 500 members) were sent a newsletter inviting them to report cases of HEV infection in which neurologic symptoms were experienced. Data were recorded from patients with neurologic disorders, regardless of the symptoms exhibited during the course of acute HEV infection. This retrospective sampling was likely to capture most patients in France who had neurologic symptoms during acute HEV infection. A second newsletter was sent 6 months later to maximize the completeness of reporting. HEV infection was diagnosed by detecting anti-HEV IgM, HEV RNA, or both in serum or fecal samples from patients with elevated aminotransferase levels. All cases occurred in France, and all patients gave their informed consent.

The EIAgen (Adaltis, Eurobio, Bologna, Italy) and Wantai (Wantai Biologic Pharmacy Enterprise Co., Beijing, China) kits were used to detect antibodies (*13*). Both tests are highly specific and sensitive for detecting IgM against HEV. HEV RNA from serum, fecal samples, or cerebrospinal fluid (CSF) was detected by using a real-time PCR as described (*3,14*). All other causes of acute hepatitis were excluded, and patients had no co-infections. All patients were screened for other causes of disease with immunoassays (antinuclear antibodies, antipolynuclear neutrophil cytoplasmic antibodies) and underwent viral serologic testing (hepatitis B virus, hepatitis C virus, HIV, Epstein-Barr virus, cytomegalovirus). *Campylobacter* was also screened for. The cases of patients 5 and 12 have been reported (*14*).

Phylogenetic analyses were performed by sequencing a 347-nt fragment within the open reading frame 2 region as described (*15*). Nucleotide identity was analyzed with BioEdit software version 7.0.9.0 (http://www.mbio.ncsu.edu/BioEdit/bioedit.html).

Statistical Analyses

Data are presented as their medians (ranges) or their means (SDs). Student *t*-test was used to compare the quantitative variables. A p value <0.05 was considered significant.

Results

Patient Characteristics and Clinical Outcomes

Fifteen patients (7 women, 8 men) from 7 different hospitals were included in this study. The clinical characteristics of the patients are reported in Table 1. The median age was 55 years (range 25–77 years). The median follow-up period was 42 weeks (range 4–161 weeks). All patients were immunocompetent. All but 1 lived in southern France: 11 lived in the southwest, 3 in the southeast, and 1 in central France.

No patient had underlying chronic liver disease; 2 patients had jaundice, 10 had asthenia, 5 had arthromyalgia, and 3 had fever. Three patients had neurologic symptoms only. Their biological characteristics are reported in Table 2. All patients had elevated liver enzyme levels, and none

Table 1. Clinical characteristics of 15 patients with hepatitis E virus–linked neurologic disorders, France, January 2006–June 2013*

Patient no.	Neurologic pathology	Age, y/sex	Treatment	Other symptoms	Neurologic symptoms at last follow-up	Follow up times, wk
1	MM	59/M	None	Asthenia	No	10
2	MM	44/F	None	Fever, asthenia, arthromyalgia	Yes	4
3	MM	65/M	Corticosteroids	Asthenia	No	157
4	MM	25/F	None	None	No	33
5	MM	49/M	None	None	No	NA
6	MM	77/F	RBV	None	No	44
7	PTS	51/M	RBV + IVIg	Arthromyalgia, asthenia	No	100
8	PTS	55/F	None	Asthenia, arthromyalgia	Yes	126
9	PTS	56/M	None	Asthenia	Yes	42
10	PTS	56/M	RBV + IVIg	Asthenia	Yes	16
11	MR	74/M	IV Ig	Arthromyalgia, asthenia	No	77
12	MR	54/F	None	Fever, nausea	No	NA
13	MR	33/F	None	Fever, arthromyalgia	No	161
14	GBS	60/F	IV Ig	Asthenia, acute low back pain	Yes	5
15	MFS	54/M	IV Ig	Asthenia, anorexia	Yes	13

*MM, mononeuritis multiplex; PTS, Parsonnage-Turner syndrome; MR, meningoradiculitis; GBS, Guillain–Barré syndrome; MFS, Miller-Fisher syndrome; RBV, ribavirin; IVIg, intravenous immunoglobulin; NA, not available.

experienced liver failure. Median enzyme levels at diagnosis were the following: alanine aminotransferase level at diagnosis was 495 IU/L (range 49–3,641 IU/L; reference range 10–41 IU/l), aspartate aminotransferase 124 IU/L (range 37–1,742 IU/L; reference range 15–41 IU/l), bilirubin 15 μmol/L (range 4–101 μmol/L; reference range 3–21 μmol/l), alkaline phosphatase 254 IU/L (range 88–704 IU/L; reference range 36–126 IU/l), g-glutamyl transferase 185 IU/L (range 33–783 IU/L; reference range 7-64 IU/l). Median prothrombin time was 96% (range 76%–100%).

All patients had IgM against HEV; 13 had IgG against HEV and 2 did not. Serum specimens of 14 patients underwent HEV PCR, and specimens from 11 patients were positive for HEV (Table 2). The median plasma HEV RNA concentration was 4.40 log-copies/mL (range 2.12–5.83).

We were able to sequence 7 virus strains from the patients: all belonged to genotype 3f, the main genotype identified in France. Phylogenetic analyses were conducted with these strains and HEV strains obtained by the French National Reference Center from patients who did not exhibit a neurologic disorder (Figure). The strains were scattered across the phylogenetic tree and were only 87.0%–93.5% identical.

Two patients had eaten game (wild boar and deer). All other patients had eaten pork. All patients had liver enzyme levels within the reference range at the last follow-up.

Neurologic Symptoms

Fourteen patients had been hospitalized. Neurologic symptoms were classified into 4 categories: mononeuritis multiplex, PTS, meningoradiculitis, and acute inflammatory demyelinating polyradiculoneuropathy (Table 3).

Mononeuritis Multiplex

Six patients (3 men and 3 women, patients 1–6) had mononeuritis multiplex; their median age was 54 years (range 25–77 years). Mononeuritis multiplex was defined by asymmetric, asynchronous involvement of the noncontiguous nerve trunks. All patients had experienced neuropathic pain and paresthesia in ≥1 nerve segments with hyporeflexia or areflexia. For 3 patiens, an electromyogram showed asymmetric axonal neuropathy, and various patterns of nerve involvement were observed. Patients 1 and 6 had confluent but asymmetric (>50%) lower-limb neuropathy (musculocutaneous and external saphenous). Patients 3 and 4 had multiple radicular or proximal troncular neuropathies, and patient 5 had troncular median and internal brachial cutaneous-nerve involvement. Three patients had asthenia and 1 had jaundice; HEV RNA was detected by PCR in serum samples from 4 patients (patients 1, 2, 5, 6).

One patient (patient 6) received specific antiviral treatment. He was given ribavirin, initially at a dose of 400 mg for 7 days (5.5 mg/kg/d) and was then given 600 mg/day (8.5 mg/kg/d) for 3 months. A serum specimen was negative for HEV after 10 days of treatment. Patient 3 was given corticosteroids for 10 weeks. Five patients had no sequelae at the last follow-up (median 33 weeks [range 4–157 weeks]). One patient (patient 2), at the last follow-up at 4 weeks, had ongoing paresthesia in a nerve segment in the lower limbs.

PTS

Four patients (3 men and 1 woman, patients 7–10) exhibited PTS, also known as neuralgic amyotrophy. Their median age was 55.5 years (range 51–56 years). These patients sought treatment at the hospital for asthenia and acute neuropathic pain in the shoulder. PTS was bilateral but asymmetric in 3 patients. Asymmetric paresis and amyotrophia appeared within a few days, with the concomitant decrease in pain. Tendon reflexes were reduced or eliminated in 2 patients (patients 9 and 10). For 3 patients, an electromyogram

Table 2. Liver test results and virologic characteristics of 15 patients with hepatitis E virus–linked neurologic disorders, France, January 2006–June 2013*

Patient no.	Diagnosis	AST, IU/L	ALT, IU/L	Bilirubin, µmol/L	ALKP, IU/L	GGT, IU/L	PT, %	GT	Serology	Serum viral load, log copies/mL	PCR stools	PCR CSF
1	MM	69	256	8.6	254	323	92	3f	IgG+/IgM+	4.92	NA	NA
2	MM	507	756	17	540	187	90	3f	IgG+/IgM+	5	Pos	NA
3	MM	NA	3,641	90	NA	NA	80	NA	IgG+/IgM+	Neg	Neg	NA
4	MM	54	120	10	NA	95	100	NA	IgG+/IgM+	Neg	Neg	NA
5	MM	50	118	14	90	95	95	NA	IgG+/IgM+	Pos	NA	Neg
6	MM	71	119	14	430	131	100	3f	IgG–/IgM+	5.57	Pos	NA
7	PTS	897	1,834	42	336	383	88	NA	IgG+/IgM+	2.12	NA	NA
8	PTS	1.330	1,900	15	194	182	95	NA	IgG+/IgM+	2.81	NA	NA
9	PTS	601	1,376	15	231	601	95	NA	IgG+/IgM+	NA	NA	NA
10	PTS	135	495	17	659	740	100	3f	IgG+/IgM+	3.89	Pos	NA
11	MR	1.742	822	101	704	528	76	3f	IgG+/IgM+	5.83	Pos	Pos
12	MR	221	566	11	124	184	100	NA	IgG+/IgM+	Pos	NA	Pos
13	MR	100	246	4.7	248	33	98	3f	IgG–/IgM+	2.9	Pos	Neg
14	AIDP	113	384	34.2	474	783	100	3f	IgG+/IgM+	Pos	Pos	Neg
15	AIDP	37	49	7	88	48	94	NA	IgG+/IgM+	Neg	Neg	Neg

*AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALKP, alkaline phosphatase; GGT, γ-glutamyl transpeptidase; PT, prothrombin time; GT, genotype; CSF, cerebrospinal fluid; MM: mononeuritis multiplex, NA, not available; Pos, positive; Neg, negative; PTS: Parsonage-Turner syndrome; MR: meningoradiculitis; AIDP, acute inflammatory demyelinating polyradiculoneuropathy.

confirmed bilateral patchy denervation, predominantly of the upper trunk of the brachial plexus. For 2 patients (patients 7 and 10), analysis of CSF showed high levels of proteins, levels of cells in reference ranges, and glucose level within reference range. HEV PCR of a CSF specimen was not done.

HEV was diagnosed after IgM against HEV was detected in 1 patient (patient 9) and after HEV RNA was detected in the serum of 3 patients (patients 7, 8, 10). Patients 7 and 10 were given ribavirin (for, respectively, 3 weeks and 2 months) at a dose of 800 mg/day. This treatment was well tolerated. They also received intravenous immunoglobulin (IVIg) at a dose of 25 g/day (patient 7) or 35 g/day (patient 10) for 5 days. All patients but 1 had persistent weakness at the last follow-up (median 71 weeks [range 16–126 weeks]); none had received a control electromyogram. Assessment of patients through disability scales was not possible because this study was retrospective and involved many centers. Notably, the only asymptomatic patient at follow-up was the patient that received both ribavirin and IVIg.

Meningoradiculitis

Three patients (2 women and 1 man, patients 11–13) had meningoradiculitis. They were, respectively, 74, 54, and 33 years of age. These patients sought treatment for meningitis symptoms (headache, photophobia) and radiculitis with pain and paresthesia restricted to 1 or a few radicular topographies. CSF was clear and showed lymphocytic meningitis with >90% lymphocytes, a high level of protein (0.79–1.37 g/L, reference range 0.28–0.53 g/L), and glucose levels within reference ranges. Patient 11 had jaundice. PCR of all serum samples from all patients with meningoradiculitis showed HEV RNA. For 2 patients, PCR of CSF was positive for HEV (patients 11 and 12). One woman was breast feeding (patient 13); HEV was detected in her serum but not in breast milk. PCR detected HEV in fecal samples from 2 patients (patients 11 and 13). Patient 11 was treated with IVIg at 0.5 g/kg/day for 4 days. No patient was symptomatic at the last follow-up visit. The median time to follow-up was 119 weeks (range 77–161 weeks).

Acute Inflammatory Demyelinating Polyradiculoneuropathy

Two patients exhibited acute inflammatory demyelinating polyradiculoneuropathy: 1 had GBS, and 1 had Miller-Fisher syndrome. Patient 14, a 60-year-old woman, was hospitalized for GBS. Initially, she had asthenia and acute low back pain. She had a positive viral load for HEV IgG and IgM. A neurologic examination revealed generalized areflexia and weakness in the lower limbs. GBS was confirmed in nerve-conduction studies. She had no hepatic symptoms. CSF analysis showed a high level of protein (2 g/L, reference range 0.28–0.53 g/L) and no HEV RNA. She was treated with IVIg for 5 days at a dose of 400 mg/day, and the symptoms partially regressed. By 5 weeks later, weakness of the lower limbs had improved, but she still experienced persistent areflexia.

Patient 15, a 54-year-old man, was hospitalized for weight loss. A neurologic examination revealed quadridistal hypoesthesia with ataxia, areflexia, and diplopia due to paresia of the right VI nerve. CSF protein concentration was elevated (1.46 g/L, reference range 0.28–0.53 g/L), and albumino-cytologic dissociation was found. Nerve-conduction studies confirmed demyelinating neuropathy. These findings were conclusive for Miller-Fisher syndrome. He had no hepatic symptoms. Serum was positive for IgM against HEV. Serum and fecal samples were negative for HEV by PCR. He received IVIg for 5 days at a dosage of 2 g/kg and was hospitalized for 1 week. His

Table 3. Average biological values of the 4 groups of patients with hepatitis E virus–linked neurological disorders*

Disorder	Age, y (range)	Sex ratio	AST, IU/L (± SD)	ALT, IU/L (± SD)	PT, % (± SD)	Bilirubin, μmol/L (± SD)	ALKP, IU/L (± SD)	GGT, IU/L (± SD)
MM	54 (25–77)	1	150 (± 200)†	835 (± 1,396)	92.8 (± 7.5)	25.6 (± 32)	328.5 (± 198)	166.2 (± 95)†
PTS	55 (51–56)	6	740.7 (± 503)	1,401.25 (± 647)	94.5 (± 4.9)	22.2 (± 13)	355 (± 211)	476.5 (± 245)
MR	54 (33–74)	2	687.7 (± 915)	544.67 (±288)	91.3 (± 13.3)	38.9 (± 54)	358.67 (± 305)	248.3 (± 253)
AIDP	57 (54–60)	1	75 (± 54)	216 (± 236)	97 (± 4)	20.6 (± 19)	281 (± 272)	415 (± 519)

*AST, aspartate aminotransferase; ALT, alanine aminotransferase; PT, prothrombin time; ALKP, alkaline phosphatase; GGT, γ-glutamyl transpeptidase; MM, mononeuritis multiplex; PTS, Parsonage-Turner syndrome; MR, meningoradiculitis; AIDP, acute inflammatory demyelinating polyradiculoneuropathy.
†$p<0.05$ when compared with PTS.

neurologic symptoms improved but did not totally disappear until >13 weeks of follow-up (persistent ataxia).

Discussion

This study shows the wide spectrum of neurologic injuries associated with patients with an acute HEV autochthonous infection. Because the study was retrospective, it was not possible to identify the prevalence of HEV infection in patients with neurologic symptoms or the prevalence of neurologic symptoms in patients with HEV infection.

Neurologic symptoms could be divided into 4 entities. Notably, the first dominant finding was mononeuritis multiplex, observed in 6 of the 15 patients, with asymmetric, asynchronous, and painful segmental nerve involvement. This condition was also the main entity in the original report, which also included immunocompromised patients with chronic HEV-3 infection (*5*). In previous reports in immunocompetent patients, painful peripheral neuropathy has rarely been reported (*8*). However, mononeuritis multiplex is a common complication in immune and viral diseases. A vasculitis process can be hypothesized, but none of the patients in this study underwent a nerve biopsy. Additional investigations, including anatomopathologic exploration, would be needed to further explore the physiopathologic (antiviral and/or immunosuppressive) pathways.

The next most frequent manifestation was PTS, also called brachial neuritis or neuralgic amyotrophy, which was found in 4 patients. PTS is a rare pathologic condition, defined by sudden, acute, and unbearable pain across the top of the shoulder, followed by severe amyotrophy. As was exhibited by the patients described here, PTS can be unilateral or bilateral but is asymmetric. A recent study reported cases of acute HEV infection in a cohort of 47 patients from Cornwall (UK) and the Netherlands (*12*); 5 cases (10.6%) of acute HEV infection were identified, indicating that HEV may be a major cause of PTS in industrialized countries with a high prevalence of HEV. That study also suggested that HEV-associated brachial neuritis more commonly produces bilateral symptoms and signs than brachial neuritis produced by other causes. In our study, 3 of 4 patients had bilateral symptoms. Three of the 4 patients with PTS had neurologic sequelae at the last follow-up (71 weeks), which suggests that concomitant HEV infection may represent a poor prognostic factor. Van Eijk et al. (*12*) found that all 5 of their HEV-positive patients exhibited persistent weakness at 6 months.

There is no specific treatment identified for PTS, but it is known to be immune mediated. However, we cannot exclude direct infection of the brachial plexus. Three of the 4 patients in our study had serum samples positive for HEV by PCR, indicating active replication. Two patients were treated with ribavirin as an antiviral therapy. This antiviral therapy is recommended to treat chronic hepatitis E in organ-transplant recipients (*16*,*17*). Case reports on the treatment of acute HEV infection in immunocompetent patients have been published, with promising results (*18*–*21*). We found that the only patient who had no persisting symptoms at the last follow-up had been treated with a combination of ribavirin and IVIg.

The third neurologic entity we found was meningoradiculitis in 3 patients. HEV was detected in the CSF of 1 case-patient, indicating neurotropism and the direct effect of HEV. This condition is probably the only one in which HEV may be directly responsible for the neurologic manifestations. No neurologic sequelae were seen in this group. A previous study found quasispecies compartmentalization in an immunocompromised patient with neurologic manifestations, and its temporal association suggests that neurologic symptoms could be linked to the emergence of neurotropic variants (*22*). The factors associated with HEV neurotropism need to be investigated further.

The last entity was acute inflammatory demyelinating polyradiculoneuropathy, which was seen in 2 patients. This condition can be classically triggered by many viruses, including hepatotropic viruses (*23*). An immune response that cross-reacts with axonemal or Schwann cell antigens is elicited and results in damage to the peripheral nerves. Both patients had persisting neurologic symptoms, commonly seen in this disease (*11*). Although several studies have demonstrated that HEV infection can induce GBS, this condition was found in only 2 of our patients. In a cohort of GBS case-patients from Bangladesh, seroprevalence of IgM against HEV was 11%, compared with 2% in a control group of patients with other

neurologic disease (*7*). The frequency of HEV infection was recently determined in a cohort of 201 patients with GBS in the Netherlands and was found to have occurred in 5% (*11*).

The number of patients in this study is too small to draw definitive conclusions on the sex ratio for these conditions, but the ratio was highest in the PTS group. We also found that neurologic disorders occurred only in those with a HEV 3f genotype, although this is the most common genotype in France (*24*), so other HEV subtypes and genotypes may induce neurologic symptoms (*5*). Moreover, because only patients with elevated liver enzyme levels were included, neurologic complications were probably underestimated in this study, in particular, for GBS and PTS, for which conditions the neurologic symptoms are often delayed after the initial event is triggered.

Patients with PTS had significantly higher values in liver enzyme tests (aspartate aminotransferase, g-glutamyl transpeptidase) than patients with mononeuritis multiplex. We have no definitive explanation, but this finding could be due to the earlier appearance of PTS symptoms during the course of HEV infection.

Three patients were treated with ribavirin with the aim of shortening the disease's duration: the 2 PTS patients and 1 patient with mononeuritis multiplex. One needed blood transfusions and erythropoietin. Whether ribavirin shortened the evolution of neurologic symptoms, particularly for patients with PTS, needs to be studied further.

One limitation of our study is the retrospective sampling by emailing through the French Liver Association. Although this method provides the advantage of reaching most practitioners who diagnose HEV infection throughout France, it may have induced an ascertainment bias, that is, the true frequency of the neurologic symptoms during HEV infection is difficult to assess. In addition, patients with neurologic symptoms who were not referred to a hepatologist obviously were not accounted for.

In summary, HEV-3 infection can induce a wide range of neurologic symptoms, including mononeuritis multiplex, PTS, meningoradiculitis, and inflammatory demyelinating polyradiculoneuropathy. Sequelae were seen with mononeuropathy multiplex, PTS, and inflammatory demyelinating polyradiculoneuropathy. Only 2 patients had overt hepatitis with jaundice. Therefore, we recommend screening patients with elevated liver enzyme levels and neurologic symptoms for HEV infection, regardless of other symptoms. Treatment with ribavirin needs to be assessed further.

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Mycotic Infections Acquired outside Areas of Known Endemicity, United States

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In the United States, endemic mycoses—blastomycosis, coccidioidomycosis, and histoplasmosis—pose considerable clinical and public health challenges. Although the causative fungi typically exist within broadly defined geographic areas or ecologic niches, some evidence suggests that cases have occurred in humans and animals not exposed to these areas. We describe cases acquired outside regions of traditionally defined endemicity. These patients often have severe disease, but diagnosis may be delayed because of a low index of suspicion for mycotic disease, and many more cases probably go entirely undetected. Increased awareness of these diseases, with a specific focus on their potential occurrence in unusual areas, is needed. Continued interdisciplinary efforts to reevaluate and better describe areas of true endemicity are warranted, along with a more nuanced view of the notion of endemicity. The term "nonendemic" should be used with care; mycoses in such regions might more accurately be considered "not known to be endemic."

Invasive fungal diseases are a growing public health problem. The endemic mycoses found in North America, namely, blastomycosis, coccidioidomycosis, and histoplasmosis, are caused by thermally dimorphic fungi and can infect immunocompetent or immunocompromised hosts, often resulting in severe illness and death (*1*). Infection is typically acquired via inhalation of fungal spores and usually results in a respiratory illness, although the clinical spectrum can range from asymptomatic to life-threatening disseminated disease (*1*–*4*). Most infections with *Blastomyces*, *Coccidioides,* and *Histoplasma* spp. occur sporadically in areas to which these fungi are geographically restricted, also referred to as mycosis-endemic areas (*1*). Cases outside these regions often result from travel, infection reactivation, latent infection in immunosuppressed hosts, or (less commonly) exposure to fomites from mycosis-endemic areas (*1*,*3*). However, a growing body of evidence suggests that some cases occur in patients with no known exposures to areas in which these diseases are most common. Because prevention of these infections is challenging, increased awareness that they can be acquired in unusual geographic locations is needed to promote early diagnosis and treatment.

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Search Strategy and Selection Criteria

To identify infections outside the known mycosis-endemic areas in the United States, we searched PubMed and Google Scholar without date or language restrictions by using combinations of the following terms: "non-endemic," "outside endemic area," "blastomycosis," "histoplasmosis," "coccidioidomycosis," and "fungal infection." We included publications in which the authors describe infections believed to be acquired from the local environment outside the traditionally defined mycosis-endemic areas in the United States. We excluded cases consistent with fomite transmission. We also reviewed relevant references in selected articles.

Blastomycosis

Blastomycosis is considered endemic to the south-central, southeastern, and midwestern US states, particularly those bordering the Ohio and Mississippi Rivers and in parts of the United States and Canada surrounding the Great Lakes and the Saint Lawrence River (*2*,*5*). Hyperendemic foci exist in north-central Wisconsin and western Ontario. Areas of blastomycosis endemicity are based primarily on reports of symptomatic disease and was first described in the late 1930s (*5*). Although the epidemiology of blastomycosis is not as well understood as that of the other mycoses endemic to North America, the incidence seems to be increasing in some states, including Illinois, Indiana, and Wisconsin (*2*).

Cases of canine blastomycosis are often recognized as sentinels for human disease, presumably because dogs' outdoor exposures are similar to, but potentially more extensive than, those of their human counterparts (*6*). A study in Illinois estimated blastomycosis incidence among dogs to be >8 times that among humans, and during 2001–2007, the annual incidence among dogs increased 50-fold (*7*). In addition to these apparent increases, blastomycosis has also

been documented far outside the known disease-endemic areas. One report describes 2 cases probably acquired in the Pacific Northwest: 1 case of ocular blastomycosis in a golden retriever that had never left Washington state and 1 case of disseminated disease in a Rottweiler mix with no history of travel outside of British Columbia (Table 1) (*8*).

Similarly, several cases of blastomycosis in humans have also occurred far outside the traditionally defined disease-endemic area. A report from 1951 describes disseminated blastomycosis in an agricultural worker in Oregon (*9*). More recently, an immunosuppressed 6-year-old girl was thought to have contracted primary cutaneous blastomycosis in north-central Florida (*10*). Two unrelated cases of osseous blastomycosis of the knee without apparent pulmonary involvement were probably acquired in Nebraska (*11*): the first case was in a previously healthy man from western Nebraska who sustained a knee injury involving farm equipment ≈1 year before diagnosis, and the second case was in a man from central Nebraska with a 1-month history of knee pain but no acute knee injury (*11*). These cases highlight the difficulties associated with diagnosing blastomycosis in patients with no exposures to disease-endemic areas and whose disease manifestations are unusual (*11*). Similarly, 2 other cases in previously healthy adults occurred in Colorado (*12*); each patient had pulmonary disease and had been extensively exposed to soil while excavating prairie dog burrows on the eastern slope of the Rocky Mountains (*12*). De Groote et al. suggest that higher-than-normal rainfall in that region could have contributed to conditions favorable for *Blastomyces* growth and sporulation (*12*).

Table 1. Cases of blastomycosis, coccidioidomycosis, and histoplasmosis acquired outside the traditionally defined mycosis-endemic areas, United States*

Infection and reference	No. cases, species	Location	Type of infection	Method of diagnosis
Blastomycosis				
(*8*)	1, dog	Central Washington	Ocular	Histopathology and culture
(*9*)	1, human	Oregon	NS	Culture
(*10*)	1, human	North-central Florida	Primary cutaneous	Histopathology
(*11*)	2, human	Western (1 case) and central Nebraska (1 case)	Bone	Culture plus confirmation by DNA probe
(*12*)	2, human	Central Colorado	Pulmonary	Histopathology and culture (1 case), histopathology and culture plus confirmation by DNA probe (1 case)
Coccidioidomycosis				
(*13*)	61, human	Butte County, California	Pulmonary, complications NS	Clinical findings (61 cases), skin test or serology (27 cases)
(*14*)	17, human	Tehama County, California	Pulmonary, complications NS	Clinical findings (17 cases), skin test or serology (10 cases)
(*15*)	10, human	Northeastern Utah	Pulmonary, complications NS	Clinical findings (10 cases), serology (9 cases)
(*16*)	3, human	South-central Washington	1 pulmonary, 1 primary cutaneous, 1 pulmonary → meningitis	Culture and serology
Histoplasmosis				
(*8*)	1, dog	Western Idaho	Disseminated	Histopathology and PCR
(*17*)	1, otter	Kodiak Island, Alaska	Disseminated	Histopathology and PCR
(*18*)	5, cats	California (2 cases), Colorado (2 cases), and New Mexico (1 case)	Disseminated (4 cases), localized (1 case)	Histopathology and PCR (4 cases), culture and PCR (1 cases)
(*19*)	2, cats	San Joaquin Valley, California	Pulmonary (1 case), cutaneous/ocular (1 case)	Cytopathology (1 case), cytopathology and culture plus confirmation by DNA probe (1 case)
(*20*)	2, raccoons	San Francisco, California	Disseminated	Histopathology, culture, and PCR
(*21*)	2, human	California	NS	Skin test and chest x-ray
(*22*)	1, human	California	Endocarditis	Histopathology, PCR, and urine EIA
(*23*)	1, human	Arizona	Gastrointestinal → central nervous system	Histopathology and culture
(*24*)	6, human	Southwestern (3 cases) and eastern (2 cases) Montana; southwestern Idaho (1 case)	Disseminated (3 cases), pulmonary (1 case), unknown (2 cases)	Histopathology (2 cases), culture (2 cases), urine EIA (2 cases)
(*25*)	5, human	Southern Florida	Disseminated	Histopathology
(*26*)	1, human	Northern Florida	Pulmonary	Skin test and serology
(*27*)	15, human	Central New York		Histopathology (10 cases), serology (9 cases)
(*28*)	1, human	Staten Island, New York	Pulmonary	Skin test and serology
(*29*)	5, human	South Bronx, New York	Disseminated	Culture

*EIA, enzyme immunoassay; NS, not specified.

Moisture is believed to be an influential factor in the growth and dispersal of *Blastomyces*, although the precise ecology of the organism is not well understood, partly because of the difficulties associated with its recovery from the environment (*2*). Previously, blastomycosis was believed to be caused by 1 species, *B. dermatitidis*, but phylogenetic analysis indicates that *B. dermatitidis* is probably 2 species, *B. dermatitidis* and *B. gilchristii* (*30*). Furthermore, *B. gilchristii* is hypothesized to inhabit a specific ecologic niche in areas of hyperendemicity, whereas *B. dermatitidis* may be adapted to a wider range of environmental conditions and distribution throughout North America may be scattered (*30*). Additional research into the genetic, geographic, and clinical differences between these 2 species may contribute to a better understanding of blastomycosis epidemiology, both inside and outside the traditionally defined areas of endemicity.

Coccidioidomycosis

Coccidioidomycosis is caused by *Coccidioides* spp. and is endemic to the southwestern United States and parts of Mexico and Central and South America. An estimated 60% of *Coccidioides* infections are asymptomatic (*3*,*31*). The remaining 40% of infections most commonly cause an influenza-like illness also known as Valley fever, which is often self-limiting but can result in serious illness, particularly because some cases progress to severe pulmonary or disseminated disease. Prior *Coccidioides* infection usually provides immunity against reinfection, which can be assessed by use of a skin test antigen.

The first complete description of the geographic distribution of coccidioidomycosis was accomplished through large-scale evaluations of coccidioidin sensitivity prevalence. During the mid-1940s to early 1950s, coccidioidin skin tests were performed on ≈110,000 lifetime residents of a single county, most of whom were white men and women 17–21 years of age (*32*). The study identified Arizona, California, Nevada, New Mexico, Utah, and Texas as *Coccicioides*-endemic states; the highest rates of skin test positivity (50%–70%) were in California's southern San Joaquin Valley and Arizona's Sonoran Desert (*32*). Point-source coccidioidomycosis outbreaks near the borders of the areas identified by Edwards and Palmer further contributed to what is known about the geographic distribution of *Coccidioides* spp. (*32*). For example, in an outbreak that occurred in 1970, at least 61 archeology students were affected after excavating Native American ruins near Chico, Butte County, California, ≈70 miles north of the recognized disease-endemic area at the time (*13*). In 1972, at least 17 persons were infected during a similar excavation near Red Bluff, Tehama County, California, 20 miles north of the previous outbreak near Chico (*14*). In 2001, another outbreak occurred among 10 workers at an archeological site in Dinosaur National Monument in northeastern Utah, ≈200 miles north of the previously defined disease-endemic area (*15*). These outbreaks support the idea that foci of *Coccidioides* exist outside of the traditional areas of endemicity, yet they are not always represented on maps depicting these areas.

During 2010–2011, three unrelated coccidioidomycosis cases were identified in south-central Washington, far north of the area of known *Coccidioides* endemicity (*16*). Whole-genome sequencing of 1 clinical isolate from the patient and soil isolates recovered from the patient's location of exposure revealed that the isolates were identical, providing direct evidence that the infection was acquired in Washington (*33*).

Evidence of coccidioidomycosis far outside the areas of known endemicity has also been seen in fossil records. *Coccidioides* spherules were morphologically identified in 2 fossilized, 8,500-year-old bison mandibles recovered from a flood plain in central Nebraska, suggesting that bison had migrated from disease-endemic areas or that *Coccidioides* previously inhabited a different or broader geographic range (*34*).

Histoplasmosis

Histoplasmosis-endemic areas were also established by using nationwide skin testing to evaluate histoplasmin sensitivity among ≈70,000 white persons, 17–21 years of age, who were lifetime residents of a single county (*35*). The highest proportion of positive reactors (60%–90%) occurred in states bordering the Ohio and Mississippi River valleys; a zone of moderate prevalence (30%–60%) extended outward around the central area for up to 300 miles (*35*). *Histoplasma* spp. seems to be less geographically restricted than *Blastomyces* or *Coccidioides* and is also endemic to parts of central America and various other locations worldwide, such as Africa and Asia. Because *Histoplasma* grows well in soil containing bird or bat droppings, the organism probably exists in microfoci outside of these broadly defined regions (*4*).

Histoplasma can infect many animal species and has been found in domestic and wild animals far outside the traditional disease-endemic areas, including disseminated disease in a dog with no history of travel outside of western Idaho (*8*) and in a northern sea otter found in Alaska (*17*). Otters do not migrate, so the authors of that report hypothesized that the infection was acquired from windborne spores or spores carried on the wings, feet, or beaks of migratory birds (*17*). Spores carried in seawater may be another possible explanation (*36*).

Histoplasmosis has also occurred in cats in the putatively non–disease-endemic states of Colorado, New Mexico, and California (*18*,*19*). Multilocus sequence typing of cat tissue samples indicated that the infecting strains of *H. capsulatum* were closely related to but clustered separately

from the North American-1 clade (1 of 2 clades common to North America), suggesting that the genetic differences represent either geographic variation among *H. capsulatum* or differences in the strains capable of infecting animals and humans (*18*). Histoplasmosis in other cats near Vacaville, California (G. Thompson, unpub. data), and in two 6-month-old raccoons rescued near San Francisco (*20*) provides further evidence that *Histoplasma* may be established in California.

Among humans, histoplasmosis potentially acquired in California was first described in 1949; in a series of 5 cases of histoplasmosis in children in California, 1 patient was a girl who had never traveled >100 miles from San Francisco and another was a girl who had lived only in California and Wyoming, which is also not a traditional histoplasmosis-endemic area (*21*). Notably, these histoplasmosis diagnoses were based on chest radiograph findings and positive results for histoplasmin skin tests, which can cross-react with *Coccidioides*. A more recent case occurred in the Central Valley of California, in an immunocompetent 87-year-old man who seroconverted during a febrile illness and in whom *Histoplasma* endocarditis later developed (*22*). Another case provides further evidence of histoplasmosis far west of the traditional disease-endemic area; in an immunocompetent woman from Arizona with no history of travel to histoplasmosis-endemic areas, an initial gastrointestinal infection was later followed by development of an intramedullary spinal cord abscess (*23*). Another 6 unrelated cases were reported from Montana (5 cases) and Idaho (1 case); of these 6 patients, 5 had immunocompromising conditions and 3 experienced substantial diagnostic delays, probably because of the low index of suspicion for histoplasmosis in an unusual location (*24*).

Histoplasmosis has also been observed both south and north of the known disease-endemic areas. In a series of 7 cases of disseminated histoplasmosis in HIV/AIDS patients from south Florida, 5 patients had no relevant travel history (*25*). In northern Florida, acute pulmonary histoplasmosis developed in a college student after he had explored a bat-infested cave (*26*). New York is repeatedly described as a non–histoplasmosis-endemic area and is typically not represented on maps depicting histoplasmosis-endemic areas, probably because the skin test surveys by Manos et al. estimated <10% positive reactors for the entire state and <2% positive reactors in certain counties (*35*). However, cases reported from New York date back several decades, including an outbreak beginning in 1978 at a prison in rural central New York; this outbreak was suspected to have been related to removal of accumulations of bird droppings and trees that served as bird roosting sites (*27*). Evidence also exists of histoplasmosis acquisition in urban areas of New York, such as in a previously healthy child from Staten Island with extensive exposure to birds (*28*) and in a series of 5 cases of disseminated histoplasmosis in HIV/AIDS patients from the south Bronx who had no exposures to birds or bats (*29*).

Discussion

Reasons for the observed occurrences of blastomycosis, coccidioidomycosis, and histoplasmosis outside areas to which they are traditionally classified as endemic are unclear but are probably multifaceted. Because weather and climate affect the growth and distribution of these fungi in these regions, these factors might also contribute to environmental conditions that could support these fungi elsewhere. The role of animal vectors in the life cycles and geographic distribution of these fungi is unknown; rodents have been suggested as possible reservoirs for *Coccidioides*, and birds and bats can carry *Histoplasma*, indicating that they may be capable of introducing microfoci in geographic regions not considered to be endemic (*37*). In contrast, areas in which endemicity seems to be emerging might represent areas of previously unrecognized disease. Despite potential increased clinician awareness, these diseases probably remain underdiagnosed. Documented cases also probably represent the most severe or symptomatic cases, although most cases are unrecognized, and self-resolution is the norm. Fungal infections outside the traditional mycosis-endemic areas clearly represent an emerging public health issue; however, the full scope of the problem remains largely unknown.

Maps of the mycosis-endemic areas are based on outdated and incomplete data and are often reprinted without substantial revisions, even as new data regarding suspected endemicity become available. Although sufficient evidence exists that the original descriptions of these diseases' distributions are probably not entirely representative of their true range, no comprehensive attempts have been made to systematically reevaluate these estimates. Several potential strategies exist to clarify the true areas of endemicity for these diseases (Table 2).

First, the distributions of histoplasmosis and coccidioidomycosis were historically defined by large-scale skin test surveys, and it is reasonable to assume that a similar method could be used to further refine our understanding of these areas. Skin test reagents to detect prior exposure to *Histoplasma* and *Coccidioides* have been unavailable in the United States for more than a decade, but a reformulated spherule-derived skin test antigen (Spherusol; AllerMed, San Diego, CA, USA) to detect delayed-type hypersensitivity to *Coccidioides* was recently approved by the Food and Drug Administration. Although access to skin testing may enable reduction of potential exposure for nonimmune patients, widespread skin test surveys to reevaluate areas of endemicity may be difficult to implement because of cost and concerns about reagent specificities.

Table 2. Advantages and disadvantages of potential strategies to refine areas of blastomycosis, coccidioidomycosis, and histoplasmosis endemicity

Strategy	Advantages	Disadvantages
Skin testing	Could cover large geographic areas; is likely to yield results that could be easily compared with early studies of skin test reactivity distribution	Availability, specificity, and cost of reagents may be limiting; may be difficult to identify persons who have no relevant travel history
Expand surveillance for fungal diseases in humans	Provides foundation for more comprehensive surveillance already in place in some states; would provide valuable information about the overall epidemiology of these diseases	Disease reporting can be time- and resource-intensive for state and local health departments; yield for areas of low or no endemicity is potentially low; not likely to capture information on asymptomatic infections; may be difficult to pinpoint location of exposure or rule out reactivation disease in persons who have extensive travel histories
Surveillance for fungal diseases in animals	Animals can be good sentinels for human disease because of potentially more extensive environmental exposures and limited travel	No comprehensive surveillance systems are currently in place; would be time and resource intensive to establish
Improved environmental detection	Detection of fungi in the environment can be a more direct measure of endemicity than disease data; positive results can provide a more definitive link between infection and the environment	Culture-based methods are insensitive; new technologies still in development; is challenging for large geographic areas
Additional ecologic niche modeling	Leads to increased understanding of the fundamental niche for these fungi and locations where human or animal exposures could occur	Model validity relies on the quality of reported locations of human and animal diseases, environmental sampling results, or both

Second, improved disease surveillance and mandatory case reporting could improve detection of infections in persons not exposed to areas of known endemicity and would contribute to a more comprehensive epidemiologic understanding of these infections in general. One of the current challenges with state-based reporting methods is that these diseases are typically reportable only in states within the traditionally defined areas of endemicity (and are sometimes not reportable even in states with known endemicity); thus, infections acquired in unusual geographic locations probably go undocumented. In addition, identifying the location of exposure for persons with histories of travel to places where these diseases are most common may be difficult. Because exposures of animals are theoretically similar to those of humans but animals travel less, animals can be good sentinels for human disease; however, no comprehensive surveillance methods exist to monitor fungal diseases in animals. Epidemiologic surveillance for blastomycosis, coccidioidomycosis, and histoplasmosis in humans and animals fundamentally relies on accurate diagnosis. The challenges associated with diagnosing these infections are well recognized; sensitivity of histopathology and cytology is low, and cross-reactions between the endemic mycoses are a particular concern with serologic testing (*2*–*4*). Our review is subject to the same limitations as those of the original reports regarding diagnostic methods for non–culture-confirmed cases.

Third, because detection of pathogenic fungi in environmental samples is a more direct measure of whether a given location can support the fungus than are data about the occurrence of disease, environmental studies could be helpful for further characterizing the areas of endemicity. Testing of environmental samples for *Coccidioides*, *Histoplasma*, or *Blastomyces* has traditionally relied on culture methods and animal inoculation, which are associated with low sensitivity and which are labor and resource intensive; however, molecular methods such as PCR are promising (*33*). As these technologies advance and become more widely used, they may serve as valuable tools to help determine sources of infection in environmental material and air.

Last, ecologic niche modeling has been beneficial for understanding the probable distribution and environmental conditions favorable for *Blastomyces* (*38*) and *Coccidioides* (*39*). Better environmental detection methods could help refine the inputs for these types of models, thereby improving their predictive abilities.

The concept of endemicity of fungal diseases is well established and has proven useful, but it may, in some instances, be potentially misleading. An "endemic disease" is one "occurring frequently in a particular region or population" (*40*). The dichotomy of endemic and nonendemic diseases or endemicity or nonendemicitiy in geographic areas may not be fully adequate to capture the nuances of fungal disease epidemiology; the seemingly increasing frequency of acquisition of fungal infections in areas well removed from those known to be endemic suggests that consideration should be given to discarding the term "nonendemic" in favor of "not known to be endemic" (Figure). Furthermore, even within a region of known endemicity, there may be areas in which the pathogen seems to be absent, as reflected in a lack of recognition of locally acquired infections. In contrast, some areas within a region of endemicity may be regions of hyperendemicity, contributing large numbers of cases, and others may be regions of hypoendemicity. These conceptual issues pertain not only to space, but also to time; hyperendemicity in some areas may be seasonal or endemicity only transient. Approaching the

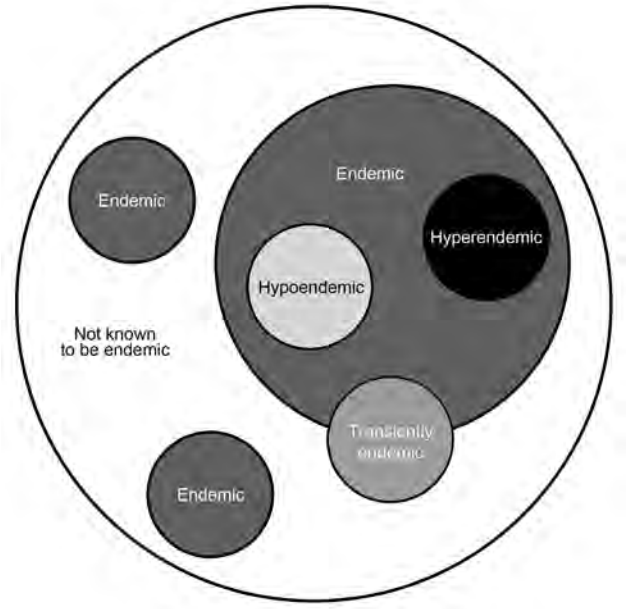

Figure. Proposed classification for endemicity of fungal infections. This schematic depicts the range of endemicity of fungal infections and discards the notion of "nonendemic," replacing it with "not known to be endemic," accounting for new areas of infection acquisition. It also accounts for the variability in the intensity of endemicity and indicates that the presence of a fungus in the environment may be transient as the result of environmental influences.

notion of endemicity for fungal diseases with a more nuanced and dynamic view has both epidemiologic and clinical value. Future work defining fungal disease endemicity should use a classification such as this.

Because primary prevention of these infections is extremely difficult, early diagnosis and treatment are particularly beneficial and may contribute to improved outcomes. The nonspecific symptoms of blastomycosis, coccidioidomycosis, and histoplasmosis are often clinically indistinguishable from those of other community-acquired respiratory illnesses. Mild or self-resolving cases frequently go undetected, and diagnoses may be missed or delayed, especially in settings where these diseases are uncommon. As a result, diagnoses in unexpected geographic locations probably represent the most severe cases. Therefore, clinicians should not exclude the possibility of these infections in patients who have not been exposed to known areas of endemicity.

The clinical and public health challenges associated with these diseases are not limited to the United States; the distribution of *Blastomyces* and *Histoplasma* extends into Canada, and histoplasmosis and coccidioidomycosis comprise a substantial burden of disease in parts of Central and South America. Further ecologic and epidemiologic studies, including revision of the geographic distribution, are needed to provide a better understanding the public health implications of these fungal diseases in the United States and elsewhere around the world.

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Uncommon *Candida* Species Fungemia among Cancer Patients, Houston, Texas, USA

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Release date: October 15, 2015; Expiration date: October 15, 2016

Learning Objectives

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

- Distinguish the most prevalent uncommon *Candida* infection in the current study
- Evaluate trends in bloodstream infections with uncommon *Candida* infections
- Describe the phenomenon of breakthrough fungemia
- Determine variables associated with a higher risk for mortality among patients with candidemia

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Many uncommon *Candida* species that cause bloodstream infections (BSIs) are not well-characterized. We investigated the epidemiology, antifungal use, susceptibility patterns, and factors associated with all-cause death among cancer patients in whom uncommon *Candida* spp.

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BSIs were diagnosed at a cancer treatment center during January 1998–September 2013. Of 1,395 *Candida* bloodstream isolates, 79 from 68 patients were uncommon *Candida* spp. The incidence density of uncommon *Candida* spp. BSIs and their proportion to all candidemia episodes substantively increased during the study period, and the rise was associated with increasing use of echinocandin antifungal drugs. Thirty-seven patients had breakthrough infections during therapy or prophylaxis with various systemic antifungal drugs for ≥7 consecutive days; 21 were receiving an echinocandin. *C. kefyr* (82%), and *C. lusitaniae* (21%) isolates frequently showed caspofungin MICs above the epidemiologic cutoff values. These findings support the need for institutional surveillance for uncommon *Candida* spp. among cancer patients.

Despite the widespread use of antifungal prophylaxis and the introduction of new antifungal agents, the incidence of candidemia and associated mortality rates among patients with cancer remain relatively unchanged (*1*). In previous studies (*1*–*3*), >90% of all *Candida*-associated invasive fungal infections were caused by 1 of 5 *Candida* spp.: *C. albicans, C. glabrata, C. parapsilosis, C. tropicalis,* or *C. krusei*. However, the use of antifungal drugs such as azoles for prophylaxis and echinocandins that are being used more frequently among high-risk populations have been associated with a continuous shift from *C. albicans* to various non-*albicans Candida* spp. during the past 2 decades (*1*,*4*–*9*). Moreover, uncommon *Candida* spp. have emerged as causes of nosocomial bloodstream infections (BSIs) in studies of specific *Candida* spp. Those isolates commonly exhibit decreased in vitro susceptibility to antifungal agents (*10*–*15*).

The epidemiology and clinical features of many uncommon *Candida* spp. BSIs have not been well characterized. To that end, we evaluated the epidemiologic characteristics, susceptibility patterns, and factors associated with all-cause death among cancer patients who had uncommon *Candida* spp. BSIs. We also determined whether the increasing frequency of uncommon *Candida* spp. BSIs in the study cohort correlated with the increased use of specific antifungal agents.

Patients and Methods

Isolates

In this retrospective study, we examined the clinical microbiology database at the University of Texas MD Anderson Cancer Center (Houston, Texas, USA) to identify blood cultures that were positive for *Candida* spp. from patients ≥18 years of age during January 1998–September 2013. *Candida* isolates were grown on Sabouraud dextrose medium (37°C/48 h/200 rpm) and then phenotypically identified by using CHROMagar Candida medium (CHROMagar Company, Paris, France) and VITEK-2 YST (bioMérieux, Marcy l'Etoile, France). The identification methods were not changed during the study period. We excluded unidentified *Candida* spp. For our analyses, we selected only the first isolate recovered from blood if a patient had several blood cultures drawn that were positive for the same uncommon *Candida* spp. Antifungal susceptibility was tested by using the Clinical Laboratory Standards Institute broth microdilution reference method (*16*). The MIC for caspofungin was tested after March 2005 in the center. For uncommon *Candida* spp., other than for *C. guilliermondii*, clinical breakpoints are undefined; therefore, isolates that showed MICs higher than the epidemiologic cutoff value (ECV) were considered potentially resistant (*17*). There was no ECV for *C. famata*; therefore, those isolates were excluded from susceptibility comparisons.

Data Collection

We retrospectively reviewed the electronic medical records of patients to obtain demographic, clinical, and laboratory data on the day of blood culture collection (Table 1); we also determined 28-day, all-cause mortality rates using a standardized electronic data collection form. Only first episodes of uncommon *Candida* spp. BSIs per patient were included in survival analyses. The study and a waiver of informed consent for anonymous data collection were approved by the Institutional Review Board of the MD Anderson Cancer Center.

Definitions

An episode of candidemia was defined as signs or symptoms of infection and ≥1 blood culture that was positive for *Candida* spp. Episodes were considered to be separate if they occurred ≥1 month apart. Breakthrough candidemia was defined as candidemia in a patient who had undergone therapy or prophylaxis with any systemic antifungal drug for ≥7 consecutive days before the index blood culture (*18*).

Neutropenia was defined as an absolute neutrophil count (ANC) of <500/mL, with further stratification at <100. Persistent neutropenia was defined as an ANC of <500 for ≥7 days. Neutrophil recovery was defined as restoration of the ANC to ≥500 for ≥3 consecutive days (*18*,*19*). The source of candidemia was considered to be intraabdominal if the patient had undergone abdominal surgery or had gastrointestinal graft-versus-host disease, peritonitis, cholecystitis, or cholangitis.

Catheter-related bloodstream infections were defined as described by Raad et al. (*20*) as 1) a colony count of blood obtained through the catheter hub that was ≥5-fold higher than that in blood obtained from a peripheral vein or 2) a catheter tip culture that was positive for *Candida* spp. The department of pharmacy provided defined daily doses

Table 1. Characteristics of 68 cancer patients with candidemia caused by uncommon *Candida* species, Houston, Texas, USA*

Parameter	Result
Median age, y (range)	54 (19–82)
Male sex, no. (%)	39 (57)
Malignancy, no. (%)	
Leukemia	42 (62)
Lymphoma/multiple myeloma	9 (13)
Solid tumor	17 (25)
Charlson Comorbidity Index, median (range)	5 (2–10)
APACHE II score, median (range)	18 (3–39)
≥19, no. (%)	27 (40)
<19, no (%)	41 (60)
Intraabdominal source,† no. (%)	37 (54)
Central venous catheter, no. (%)	65 (96)
Corticosteroid-based treatment within 30 d before the day of blood culture collection, no. (%)	29 (43)
Chemotherapy within 30 d before the day of blood culture collection, no. (%)	51 (75)
HSCT, no. (%)	18 (27)
GVHD, no. (%)	10 (15)
TPN, no. (%)	12 (18)
Hemodialysis, no. (%)	10 (15)
ICU stay, no. (%)	35 (52)
Intubation, no. (%)	11 (16)
Neutropenia at onset, no. (%)	
ANC <500/μL	44 (65)
ANC <100/μL	40 (59)
Duration of neutropenia (<500/μL) before the day of blood culture collection, no. (%)	
1–14 d	22/44 (50)
15–28 d	8/44 (18)
>28 d	14/44 (32)

*Characteristics were recorded on the day of blood culture collection, unless otherwise specified. APACHE II, Acute Physiology and Chronic Health Evaluation II; HSCT, hematopoietic stem cell transplant; GVHD, graft-versus-host diseases; TPN, total parenteral nutrition; ICU, intensive care unit; ANC, absolute neutrophil count.
†Intraabdominal source was defined as cases of abdominal surgery, gastrointestinal GVHD, peritonitis, cholecystitis, and cholangitis.

according to the World Health Organization Anatomical Therapeutic Chemical classification system definition (http://www.whocc.no) for echinocandins, azoles, and amphotericin B (ampB) per 1,000 adult inpatient-days during the study period.

Statistical Analysis

We used descriptive statistics to summarize the demographic, clinical, and outcome variables and the in vitro susceptibility data. We compared percentages with the χ^2 test or Fisher exact test if the expected numbers were <5 in >20% of all cells. Poisson regression and the Cochran-Armitage test were used for the trend analysis of the annual BSI incidence densities and the proportions of candidemia caused by uncommon *Candida* spp., respectively. We also compared BSI incidence densities for 2 time periods—1998–2005 and 2006–2013—using Poisson distribution and test-based methods. The correlation between the annual use of antifungals and time was evaluated by using the Spearman correlation. The associations between the incidence densities of uncommon *Candida* spp. BSIs and the annual use of antifungals (defined as daily doses per 1,000 patient-days) were evaluated by using Poisson regression.

We used Cox regression analysis to identify factors that were significantly associated with death. Clinically relevant parameters in the univariate analyses ($p<0.1$) were included at model entry. The full model was reduced to a final model by using a stepwise elimination procedure. The proportional hazards assumption was tested graphically and by building time-dependent variables. Two-tailed p values <0.05 were considered statistically significant. All analyses were done by using SPSS statistical software version 21 (SPSS IBM, Armonk, NY, USA).

Results

Incidence Trends and Antifungal Use

We identified 1,395 blood cultures that were positive for *Candida* over the 16-year study period. We excluded 14 cultures that grew unspecified *Candida* spp. A total of 79 episodes of illness among 68 patients were caused by 5 uncommon *Candida* spp.: *C. guilliermondii* (n = 28, 41%), *C. lusitaniae* (n = 19, 28%), *C. kefyr* (n = 13, 19%), *C. famata* (n = 7, 10%), and *C. dublinensis* (n = 1, 1%). Patient demographic and clinical characteristics are shown in Table 1. Most patients had hematologic malignancies (n = 51, 75%). Of 44 patients who had low neutrophil counts, 40 were severely neutropenic (91%, ANC <100/mL).

The overall incidence of uncommon *Candida* spp. BSIs and their proportion relative to all episodes of candidemia increased significantly during 1998–2013 (incidence density $p<0.0001$; proportion $p = 0.001$) (Figure 1). The overall incidence density of uncommon *Candida* spp. BSIs was 3.17 episodes per 100,000 inpatient days, which increased from 1.89 (1998–2005) to 4.2 (2006–2013; $p = 0.0001$). The overall proportion of uncommon *Candida* spp. relative to all episodes of candidemia was 5.7% and increased from 3.6% (1998–2005) to 7.2% (2006–2013; $p = 0.0004$). During 2006–2013, *C. lusitaniae* had the highest incidence density (1.45 episodes/100,000 inpatient days), followed by *C. guilliermondii* (1.16), *C. kefyr* (1.01), and *C. famata* (0.51). The incidence density of candidemia caused by *C. lusitaniae* ($p = 0.013$) and *C. kefyr* ($p = 0.01$) increased significantly during 2006–2013 compared with that during 1998–2005; the incidence density of *C. guilliermondii* BSIs did not increase, and *C. famata* BSIs showed a trend for increase ($p = 0.068$) (Figure 1).

Echinocandins became available at the cancer center in 2001. The annual use of echinocandins increased significantly during 2001–2013 (Spearman $r = 0.98$; $p<0.0001$) (Figure 2), whereas annual azole and ampB use did not (data not shown). The increase in incidence density of uncommon *Candida* spp. BSIs was associated with the continuous increase in echinocandin use ($p = 0.0062$).

Breakthrough Fungemia

Fungemia was detected in samples from 37 of 68 patients (54%) while they were being treated with antifungal agents, specifically with echinocandins (n = 21, 57%), ampB (n = 9, 24%), azoles (n = 6, 16%), or antifungal combinations (n = 1, 3%) (Table 2). Among 6 patients who experienced breakthrough fungemia during treatment with caspofungin, susceptibility data was available for 5 isolates; none were susceptible to caspofungin (MICs 4, 8, 8, 8, and 16 μg/mL). The most common species that caused breakthrough fungemia were *C. guilliermondii* (16/37 patients, 43%), *C. kefyr* (8/37 patients, 22%), *C. lusitaniae* (7/37 patients, 19%), and *C. famata* (6/37 patients, 16%). Most patients with breakthrough infections had underlying leukemia (33/37, 89%), compared with 9/31 patients (29%) who had no breakthrough infections (p<0.001), and neutropenia (31/37, 84%), compared with 13/31 (42%) who had no breakthrough infections (p<0.001). In addition, more patients who had breakthrough candidemia (26/37, 74%) than de novo candidemia (9/31, 29%) were admitted to the intensive care unit (ICU) (p = 0.001). The crude 28-day mortality rate among patients with breakthrough fungemia was 76% (28/37) (Table 2) and was significantly higher than that for patients with de novo candidemia (12/29, 41%; p = 0.005); Information regarding 28-day survival was available for 29 of 31 patients with de novo candidemia.

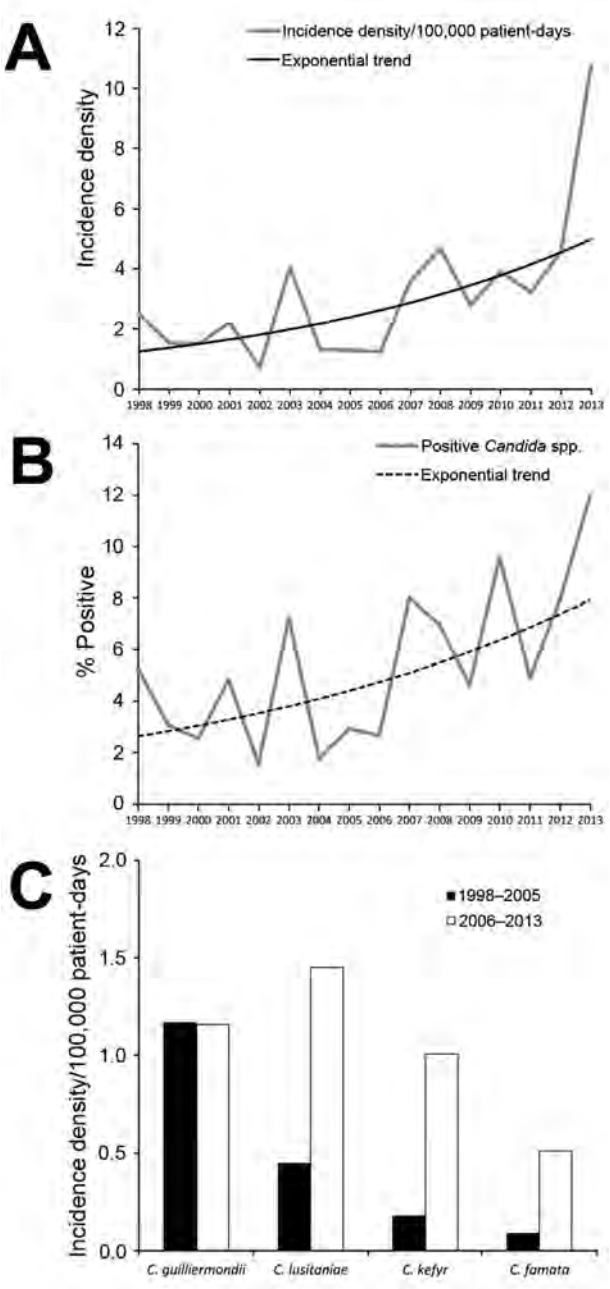

Figure 1. Increasing A) incidence density and B) proportion relative to all episodes of candidemia for bloodstream infections caused by uncommon *Candida* species at the University of Texas MD Anderson Cancer Center, Houston, Texas, USA, January 1998–September 2013. A) p<0.0001 and B) p = 0.001 for trend analyses. C) Incidence density of fungemia caused by uncommon *Candida* spp. during 1998–2005 compared with 2006–2013. There was a significant increase for *C. lusitaniae* (p<0.0001) and *C. kefyr* (p<0.0001) and a trend for increase for *C. famata* infections (p=0.068). *C. guilliermondii* infections remained stable.

In Vitro Susceptibility

In vitro susceptibility results were available for 57 isolates (Table 3). *C. guilliermondii* strains exhibited high rates of azole MICs above ECVs (fluconazole, 17%; voriconazole and posaconazole, 24%; Table 3). The 2 species that commonly were positive for caspofungin MICs above ECVs were *C. kefyr* (82% vs. 17% among other species; p<0.001) and *C. lusitaniae* (21%) (Table 3). Caspofungin

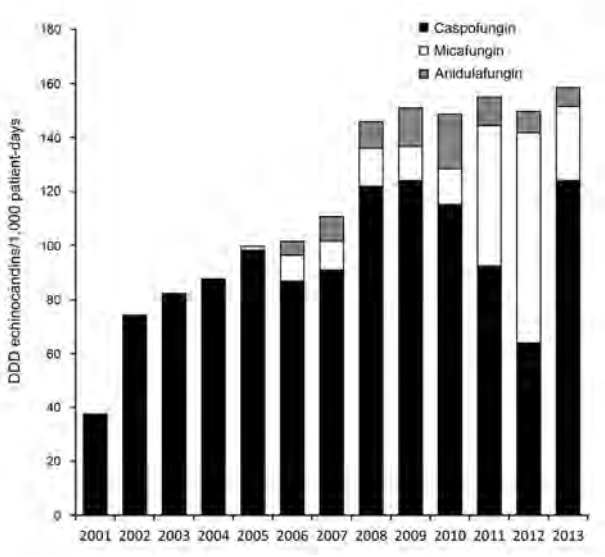

Figure 2. Increasing annual use of echinocandin antifungal drugs at the University of Texas MD Anderson Cancer Center, Houston, Texas, USA, January 2001–September 2013. Spearman correlation coefficient r = 0.98, p<0.0001. DDD, defined daily doses.

Table 2. Characteristics of 37 cancer patients with breakthrough candidemia caused by uncommon *Candida* species, Houston, Texas, USA

Parameter	Patients, no. (%)
Patients with leukemia	33 (89)
Patients with lymphoma or multiple myeloma	3 (8)
Breakthrough infection while receiving	
Amphotericin B	9 (24)
Echinocandin	21 (57)
Caspofungin	6 (29)
Micafungin	10 (48)
Anidulafungin	5 (24)
Azole	6 (16)
Fluconazole	2 (33)
Voriconazole	1 (17)
Itraconazole	3 (50)
Combination*	1 (3)
Species causing breakthrough fungemia	
C. guilliermondii	16 (43)
C. kefyr	8 (22)
C. lusitaniae	7 (19)
C. famata	6 (16)
Outcome of breakthrough fungemia	
Dissemination†	5 (14)
28-day crude mortality rate	28 (76)

*Caspofungin and liposomal amphotericin B.
†Site of dissemination on autopsy: heart, lungs, and liver.

MIC clinical breakpoints have been proposed only for *C. guilliermondii* (*17*); consequently, 13 *C. guilliermondii* isolates (87%) were susceptible to caspofungin (MIC ≤2 μg/mL), 1 was intermediate (MIC = 4 μg/mL), and 1 was resistant (MIC ≥8 μg/mL). One *C. famata* isolate had high caspofungin and fluconazole MICs (16 μg/mL for each). Even though ECVs for that species have not been defined, on the basis of ECV and clinical breakpoints for other *Candida* spp., that isolate could be considered azole/candin-nonsusceptible, making it multidrug resistant.

All-Cause Mortality

The all-cause 28-day mortality rate among this study cohort was 61% (40/66) (Table 4) and was positively associated with underlying leukemia, steroid exposure, ICU stay on the day candidemia was suspected and tested for, intubation, persistent neutropenia, high APACHE II scores (≥19), hypoalbuminemia, and breakthrough fungemia (Table 5). We found no statistically significant association between all-cause deaths and specific *Candida* spp. or central venous catheter removal. In the multivariate Cox regression analysis, an ICU stay (adjusted hazard ratio [aHR] 4, 95% CI 1.8–9.05), persistent neutropenia (aHR 3, 95% CI 1.52–6.05), and a high APACHE II score (≥19; aHR 2.8, 95% CI 1.39–5.78) were independently associated with the 28-day all-cause mortality rate (Table 5).

Discussion

Comprehensive population-based registries of candidemia surveillance have documented the shift from human infections with *C. albicans* to non-*albicans* species over the past 2 decades (*4*,*21*,*22*). However, institutional surveillance is equally essential. For example, higher rates of echinocandin resistance are reported from oncology and transplantation centers in the United States (*23*–*25*) compared with population-based cohorts (*4*). At the MD Anderson Cancer Center hospital, the incidence of BSIs caused by uncommon *Candida* spp. and the proportion of those cases relative to all candidemia cases more than doubled during the past 16 years. Uncommon *Candida* spp. were frequently nonsusceptible to azoles and echinocandins and were commonly associated with breakthrough infections and high mortality rates. Notably, the incidence density for BSIs caused by uncommon *Candida* spp. was positively associated with the annual use of echinocandins.

Uncommon *Candida* spp. distributions vary by geographic region, patient population, and antifungal practices. In general, reported frequencies have been <10% among all *Candida* isolates (*21*,*22*,*26*,*27*), which is similar to the proportion of uncommon *Candida* spp. among all *Candida* BSIs (3.6%) during the first period of our study (1998–2005) and to that (3.3%) found in another study of cancer patients during 2009–2012 (*28*). Nevertheless, the proportion of uncommon *Candida* spp. BSIs relative to all episodes of candidemia in the MD Anderson Cancer Center hospital increased over the years, accounting for 12% of all *Candida* BSIs reported during 2013 (Figure 1), which is among the highest proportions reported to date. This striking difference reflects a severely immunocompromised patient population: 75% had hematologic malignancies, compared with 10.7% in the study by Tang et al (*28*). However, the most crucial determinant of this marked increase in uncommon *Candida* BSIs is likely the broad use of echinocandins. For example, in the study by Tang et al. (*28*), 88.8% of cancer patients with candidemia had previously received fluconazole and <2% had received an echinocandin; the opposite was true in our cohort, where almost one third of patients with uncommon *Candida* spp. fungemia had breakthrough infections while being treated with an echinocandin. Moreover, the incidence density of the uncommon *Candida* spp. BSIs in our study was positively associated with the increase in treatment with echinocandins.

In previous reports, *C. guilliermondii* was one of the most commonly isolated uncommon *Candida* spp. among patients with cancer (*9*,*13*,*29*); *C. dubliniensis* was common in the outpatient setting (*27*). Nevertheless, in our study, during the years 2006–2013, *C. guilliermondii* was not the most common isolate, and the incidence of *C. guilliermondii* fungemia did not increase substantially over the study period (Figure 2). This finding is in agreement with another study, wherein the increased use of echinocandins was not associated with an increase in the incidence of *C. guilliermondii* fungemia (*30*). The increase in the

Table 3. Available susceptibility data for uncommon *Candida* isolates associated with fungemia among cancer patients, Houston, Texas, USA*

Medication	No. (%) cases; ECV, μg/mL				
	C. guilliermondii, n = 24 (41%)†	*C. lusitaniae*, n = 19 (28%)†	*C. kefyr*, n = 13 (19%)†	*C. famata*, n = 0 (10%)†	*C. dubliniensis*, n = 1 (1%)†
Amphotericin B					
No.‡	24	19	13	7	1
Wild type	24 (100); ≤2	19 (100); ≤2	NE	NE	0; ≤2
Non–wild type	0; >2	0; >2	NE	NE	1 (100); >2
Fluconazole					
No.	24	19	13	7	1
Wild type	20 (83); ≤8	16 (84); ≤2	12 (92); ≤1	NE	1 (100); ≤0.5
Non–wild type	4 (17); >8	3 (16); >2	1 (8); >1	NE	0; >0.5
Voriconazole					
No.	17	14	12	7	1
Wild type	13 (76); ≤0.25	13 (93); ≤0.03	10 (83); ≤0.015	NE	1 (100); ≤0.03
Non–wild type	4 (24); >0.25	1 (7); >0.03	2 (17); >0.015	NE	0; >0.03
Itraconazole					
No.	24	19	13	7	1
Wild type	21 (88); ≤1	19 (100); ≤0.5	NE	NE	1 (100); ≤0.25
Non–wild type	3 (12); >1	0; >0.5	NE	NE	0; >0.25
Posaconazole					
No.	17	14	12	7	1
Wild type	13 (76); ≤0.5	12 (86); ≤0.12	11 (92); ≤0.25	NE	1 (100); ≤0.12
Non–wild type	4 (24); >0.5	2 (14); >0.12	1 (8); >0.25	NE	0; >0.12
Caspofungin					
No.	15	14	11	7	1
Wild type	13 (87); ≤2	11 (79); ≤1	2 (18); ≤0.03	NE	1 (100); ≤0.12
Non–wild type	2 (13); >2‡	3 (21); >1	9 (82); >0.03	NE	0; >0.12

*Data were available for 57 of 68 isolates (24/28 *C. guilliermondii*, 0/7 *C. famata*). ECV, epidemiologic cutoff value (*17*); NE, not evaluable for susceptibility isolates because there are no defined ECVs for that species.
†Numbers shown are number of isolates evaluable for susceptibility; percentages are percentage of isolates among all isolates.
‡Results were the same by using a clinical breakpoint (MIC >1) or ECV.

incidence of *C. kefyr*, predominantly among patients with hematologic malignancies, is in agreement with the results of another recent report (*12*), in which the increase was also attributed to the increasing use of the echinocandin drug micafungin. Taken together, those findings highlight the need, at an institutional level, to systematically monitor changes in *Candida* spp. distribution and the association with the selective pressure from antifungals.

The clinical features and outcomes of breakthrough candidemia with uncommon *Candida* spp. have not been well described. In our study, more than half of all patients with fungemia caused by uncommon *Candida* spp., and 36 of 51 patients who had hematologic malignancies (70%), had breakthrough infections. On the contrary, in a 1993–1998 candidemia study at our institution in which uncommon *Candida* spp. were excluded, ≈25% of all patients, and 46% of those with hematologic malignancies, had breakthrough infections (*31*). Nevertheless, the percentage of breakthrough infections among all *Candida* spp. BSIs (53%) in a more recent report (*32*) was almost identical to that in this study of fungemia caused by uncommon *Candida* spp. (54%). Those differences are further reflective of the changing epidemiologic characteristics of candidemia and the unique features of uncommon *Candida* spp. breakthrough infections, which seem to affect a more compromised patient population.

A direct comparison between common and uncommon *Candida* spp. was beyond the scope of this study, but in another report, among candidemic patients with acute leukemia, we observed a trend for higher mortality rates with the same uncommon *Candida* spp. infections on univariate analysis, but not on multivariate analysis (*25*). The only independent predictors of death in the study described here were ICU stay, persistent neutropenia, and high APACHE II score (Table 5), confirming that host characteristics are the most powerful predictors of response and should be adequately adjusted for in studies of candidemia outcomes.

We used the ECV to characterize uncommon *Candida* spp. bloodstream isolates as susceptible or potentially resistant, according to the updated Clinical and Laboratory Standards Institute/EUCAST definitions (*17*). *C. guilliermondii* strains exhibited high rates of azole resistance (Table 3), in agreement with the results of previous reports (*13*,*33*,*34*). However, echinocandin resistance among *C. guilliermondii* bloodstream isolates in our study was uncommon (a MIC >1 mg/L was observed for only 13% of isolates); in contrast, Girmenia et al. reported that a caspofungin MIC >1 mg/L was observed for 67% of *C. guilliermondii* strains (*13*). Moreover, the incidence of *C. guilliermondii* BSIs remained stable during the 16 years of our study (Figure 1) and was not substantially associated with echinocandin use. On the contrary, the most common species with caspofungin

Table 4. Treatment and outcome of 68 cancer patients with candidemia caused by uncommon *Candida* species, Houston, Texas, USA*

Parameter	Value
Antifungal treatment	66 (97)
Amphotericin B–based regimen	5 (8)
Echinocandin-based regimen	13 (20)
Azole-based monotherapy	12 (18)
Combination antifungal treatment	36 (55)
Median duration of treatment, d (range)	16 (0–76)
Catheter-related infection	17 (25)
C. guilliermondii	6 (35)
C. lusitaniae	7 (41)
C. famata	2 (12)
C. kefyr	2 (12)
Central venous catheter removal, no. patients/no. in category (%)	41/65 (63)
Median time to central venous catheter removal, d (range)	3 (0–21)
Resolution of neutropenia, no. patients/no. in category (%)	15/44 (34)
Persistent neutropenia†	24 (35)
Growth factors	42 (62)
Leukocyte transfusion	5 (7)
Abscess drainage	3 (4)
Crude mortality rate at 28 d	40 (61)
Mortality rate by *Candida* spp.,‡ no. patients/no. in category (%)	
C. guilliermondii	16/27 (59)
C. lusitaniae	10/19 (53)
C. kefyr	10/13 (77)
C. famata	4/7 (57)

*Values are no. (%) patients except as indicated.
†Persistent neutropenia was defined as an absolute neutrophil count of <500/mcL for ≥7days.
‡No available data for *C. dubliniensis*.

MICs above ECV was *C. kefyr* (82%); the incidence density for the species increased substantially over time (Figure 1) and was positively associated with the annual use of echinocandins (p = 0.004), but not azoles or ampB. Dufresne et al. (*12*) recently reported a similar rate (88%) of micafungin resistance (MIC >0.12 mg/L) in *C. kefyr* bloodstream isolates in patients with hematologic malignancies, possibly associated with institutional use of micafungin.

Our study has limitations that should be taken into consideration. First, it was a retrospective study from a single cancer center with a small number of episodes caused by individual uncommon *Candida* spp.; therefore, our observations might not be applicable to different patient groups at risk for uncommon *Candida* spp. BSIs. Second, uncommon *Candida* spp. were identified phenotypically, and it is possible that during the study period, some *C. dublinensis* isolates were identified as *C. albicans*, underestimating the frequency of that species. It should also be noted that with the introduction of molecular identification, the taxonomy of the *Candida* genus is in a state of change (*35*). The recent implementation of internal transcriber section sequencing (http://www.cbs.knaw.nl/databases, http://www.ncbi.nlm.nih.gov/genbank) and matrix-assisted laser desorption/ionization in mass spectrometry for *Candida* spp. identification are expected to further advance understanding of the epidemiology and clinical course of serious infections with uncommon *Candida* spp.

Third, we used in vitro caspofungin MIC alone to define echinocandin resistance, using no data on DNA mutations. However, there is evidence that caspofungin MIC interlaboratory variability may lead to incorrect categorization of susceptibility results (*36*), and micafungin and

Table 5. Factors associated with 28-day crude mortality rate among cancer patients with candidemia caused by uncommon *Candida* species, Houston, Texas, USA*

	Univariate analysis		Multivariate analysis	
Variable	Hazard ratio (95% CI)	p value	Adjusted hazard ratio (95% CI)	p value
Underlying leukemia	7.6 (2.47–23.14)	<0.001	NR	
Steroid exposure	3.0 (1.03–8.71)	0.040	NR	
ICU admission	26.4 (6.42–108.55)	<0.001	4.0 (1.8–9.05)	0.001
Intubation	8.3 (1–69.64)	0.040	NR	
Total parenteral nutrition	4.0 (0.80–20.02	0.105	NR	
Persistent neutropenia†	30.6 (3.77–247.93)	<0.001	3.0 (1.52–6.05)	0.002
APACHE II score ≥19	12.8 (3.27–49.93)	<0.001	2.8 (1.39–5.78)	0.004
Hypoalbuminemia‡	3.5 (1.10–11.45)	0.030	NR	
Breakthrough fungemia	4.4 (1.53–12.64)	0.005	NR	

*NR, not retained in the multivariate analysis model; APACHE II, Acute Physiology and Chronic Health Evaluation II.
†Persistent neutropenia was defined as an absolute neutrophil count of <500/μL for ≥7 days.
‡Serum albumin level <3.0 g/dL.

anidulafungin MICs correlate better with the presence of FKS mutations and clinical outcomes (*37*). Resistance to echinocandins emerges as a result of treatment and has been associated with mutations in FKS 1/2 genes, which encode the target enzyme for this specific class of antifungals, b-D-glucan synthase (*24*,*38*,*39*). In agreement with what we know about more common *Candida* spp., investigators have recently identified novel and established FKS1 gene mutations in *C. kefyr* clinical isolates that are associated with in vitro echinocandin resistance (*35*,*39*). Still, the spectrum of mutations that predispose patients to antifungal resistance, the role of epigenetic mechanisms, and the virulence of nonsusceptible, uncommon *Candida* strains (compared with wild-type) remain unknown at present. Therefore, some experts propose the concept of "clinical resistance," which is a composite of factors related to the host, pathogen, and specific antifungal agent (*38*).

In summary, we observed a marked increase in the frequency of BSIs caused by uncommon *Candida* spp. in a contemporary series of patients with malignancies; those species were often associated with breakthrough infections and high mortality rates. The positive correlation between the increasing incidence of uncommon, potentially resistant *Candida* bloodstream isolates and the increasing use of echinocandins underscores the need for institutional surveillance and the rational use of antifungal drugs in cancer patients.

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Maternal Effects of Respiratory Syncytial Virus Infection during Pregnancy

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Given the illness and deaths caused by respiratory syncytial virus (RSV) infection during the first year of life, preventing infant RSV infections through maternal vaccination is intriguing. However, little is known about the extent and maternal effects of RSV infection during pregnancy. We describe 3 cases of maternal RSV infection diagnosed at a US center during winter 2014. Case-patient 1 (26 years old, week 33 of gestation) received a diagnosis of RSV infection and required mechanical ventilation. Case-patient 2 (27 years old, week 34 of gestation) received a diagnosis of infection with influenza A(H1N1) virus and RSV and required mechanical ventilation. Case-patient 3 (21 years old, week 32 of gestation) received a diagnosis of group A streptococcus pharyngitis and RSV infection and was monitored as an outpatient. Clarifying the effects of maternal RSV infection could yield valuable insights into potential maternal and fetal benefits of an effective RSV vaccination program.

Respiratory syncytial virus (RSV) is a major cause of serious, potentially fatal, respiratory infection in infants, but no preventive vaccine is available. In addition to ongoing research and development of an RSV vaccine for infants and children, the strategy of maternal vaccination, leading to passive transplacental transfer of anti-RSV antibodies, has been proposed. A recent animal study demonstrated passive transfer of RSV antibodies that controlled RSV pulmonary virus load in the offspring after maternal vaccination with a formalin-inactivated RSV vaccine (*1*). Although active research that examines an RSV vaccine in pregnancy is underway (*2*,*3*), the potential for maternal benefit that may be derived from such a vaccination program is unexplored.

Because of physiologic changes in cardiorespiratory function and obligatory immunologic alterations to allow for fetal tolerance, pregnant women are vulnerable to viral infections, such as influenza. In addition to maternal complications, including hospitalization, cardiorespiratory complications, and death, maternal influenza infection is associated with increased incidence of spontaneous abortion, fetal death, preterm birth, and low-birthweight infants (*4*). Consequently, the Centers for Disease Control and Prevention's Advisory Committee on Immunization Practice recommends vaccination with inactivated influenza virus for all women who will be pregnant or postpartum during influenza season. However, little has been documented on the incidence and effects of maternal RSV infection on mother or infant. Only recently was RSV recognized as a key pathogen in respiratory infections afflicting elderly and immunocompromised adults; the estimated disease incidence was similar to that of nonpandemic influenza (*1*). It is likely that RSV is an important, yet unrecognized, pathogen in pregnancy. We describe 3 cases of antepartum RSV infection treated at a single tertiary care facility from January to March of 2014. These cases highlight the variability of clinical features, maternal and neonatal disease, and current lack of effective therapy for maternal RSV infection.

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Case-Patients

Case-Patient 1

The first case occurred in a 26-year-old primagravida at week 33 of gestation who initially sought care at an urgent care facility for a 5-day history of malaise, cough, and wheezing. She reported recent exposure to a young child with an upper respiratory infection. Her medical history was notable for asthma, hypothyroidism, and gastresophageal reflux. Her social history was remarkable for smoking half of a pack of cigarettes per day and using marijuana daily. She had declined influenza vaccination during pregnancy.

The patient received a diagnosis of bronchitis and was treated with a β-agonist inhaler and amoxicillin. The next day she went to her local hospital when her symptoms worsened with new-onset fever. A chest radiograph showed no notable findings, and oxygen saturation was 94% on room air. With a diagnosis of worsening acute bronchitis, she was admitted to the hospital and administered ceftriaxone. Over the next few days, an oxygen requirement developed, and bilateral opacities were shown on chest x-ray, which evoked concern for possible acute respiratory distress syndrome. Azithromycin, vancomycin, and oseltamivir were empirically added to her treatment regimen, and imaging demonstrated no evidence of pulmonary embolism. Although she received broad-spectrum antibiotic drugs, her

condition continued to deteriorate, and she required 100% fraction of inspired oxygen on a nonrebreather mask. She was therefore intubated and transferred to a tertiary care facility on hospital day (HD) 4.

On arrival at our institution, the patient was taken to the medical intensive care unit (ICU) where she remained intubated on assist control with 100% fraction of inspired oxygen, positive end–expiratory pressure of 16 cm H_2O, and inspiratory pressure of 16 cm H_2O. She continued to receive the previously prescribed antibiotic regimen, and a bronchoscopy was performed with collection of bronchial washings. The washings were analyzed by using the eSensor Respiratory Panel (GenMarkDx, Carlsbad, CA, USA), a multiplex, reverse transcription nucleic acid amplification test designed to detect influenza viruses A and B, RSV, and rhinovirus. PCR of respiratory specimens showed positive results for RSV. Additional tests for acid-fast bacilli, *Legionella* spp., *Streptococcus pneumoniae*, *Pneumocystis* spp., and parainfluenza virus were all negative. All antibiotic drugs were discontinued at this time. Ribavarin was considered but was not administered because it is likely to be most effective when started early in the disease course, and its potential benefit in adults with RSV infection appears to be limited to immunocompromised adults (*5*–*8*).

The patient's respiratory status improved with supportive therapy, and she was extubated on HD5. However, stridor and oxygen desaturation later developed, which required re-intubation. On HD6, a fever developed, and she was started on a course of vancomycin and piperacillin-tazobactam for presumed hospital-acquired pneumonia. Blood cultures were negative, and her respiratory status remained stable while she was intubated. Because her respiratory status did not improve enough to permit extubation, the decision was made to proceed with cesarean delivery to potentially accelerate her recovery by decreasing overall cardiorespiratory demand. On HD8, at 34 weeks and 1 day of gestation, the patient remained intubated and was transferred to the operating room, where she underwent an uncomplicated cesarean delivery of a male infant weighing 2,007 g and with 1- and 5-minute Apgar scores of 5 and 7, respectively. The infant required blow-by oxygen at birth and was admitted to the neonatal ICU. He was quickly weaned to room air. There was no clinical indication that the infant was infected with RSV, and therefore no virus testing was done. The infant was discharged to his home on day of life 8 without complication.

Two days after delivery, the patient's respiratory status improved and she was extubated. She received oxygen by nasal cannula and was transferred to the medicine floor. Antibiotic drugs were discontinued, and she was given daily beclomethasone and albuterol, which substantially improved wheezing. She was discharged on HD14 with normal oxygen saturation levels on room air.

Case-Patient 2

The second case-patient was a 27-year-old gravida 2, para 0, at week 34 and day 4 of gestation who sought treatment with worsening cough, congestion, fevers, and chills. Her pregnancy was complicated by fetal hydrocephalus consistent with aqueductal stenosis, which was diagnosed during the second trimester and was therefore unrelated to her pulmonary complications. Her fetus was noted to be in the breech presentation. Her prior obstetric history was notable for a spontaneous abortion at 8 weeks, and her medical history was notable for childhood asthma and smoking one half to 1 pack of cigarettes per day before pregnancy. She received an influenza vaccine during pregnancy.

Five days before seeking treatment at our institution, she was seen in a local emergency room for worsening cough and dyspnea; a rapid influenza test was negative, and she was treated with a 5-day course of prednisone and albuterol for exacerbation of asthma. Three days later, fever developed, and her cough was productive for green sputum; at that time she was given azithromycin. When she was admitted to our institution after no improvement in her illness, leukocyte count was 21×10^9 cells/L (normal range $5.9–16.3 \times 10^9$ cells/L) and chest radiograph showed a nonspecific opacity in the right middle lobe, which evoked concern for pneumonia. A nasopharyngeal aspirate was sent to be tested by viral respiratory PCR panel (eSensor; GenMarkDx). The panel was positive for influenza A(H1N1) virus and RSV. The patient was given ceftriaxone, vancomycin, azithromycin, and oseltamavir because of concern for secondary bacterial infection. Her oxygen saturation was stable (91%–94% on room air). On the afternoon of HD2, her membranes ruptured. Because of her pulmonary status, the decision was made to defer delivery because there were no signs of active labor. On HD3 at week 34 and day 5 of gestation, she reported contractions, and cervical dilation was noted. Given the fetal hydrocephalus (biparietal diameter 11.6 cm) and breech presentation, she underwent a classical cesarean delivery of a female infant weighing 3,640 g and with 1- and 5-minute Apgar scores of 3 and 7, respectively.

During the cesarean closure, the patient required intubation and conversion to general anesthesia owing to increased secretions, hypotension, and tachycardia. She remained intubated and was transported to the surgical ICU after delivery. She was extubated without difficulty within an hour of ICU admission and transferred to the postpartum unit 12 hours after delivery. While recovering in the postpartum unit, she had an episode of pulmonary edema that was successfully treated with furosemide. The remainder of her postpartum course was uncomplicated, and she completed a regimen of 10 days of azithromycin and 5 days of oseltamivir. She was discharged to her home on HD10 with percentages of oxygen saturation in the mid-90s on room

air. The infant did well from a respiratory standpoint and was discharged on day of life 20 after an uncomplicated shunt placement due to hydrocephalus; to date, the infant is doing well.

Case-Patient 3

The third case-patient was a 21-year-old primagravida who sought treatment at our outpatient clinic at week 32 of gestation with a 3-day history of sore throat, congestion, and fever. Her medical history was complicated by mild aortic coarctation and a cognitive delay. She had no history of asthma or smoking and had been vaccinated against influenza during pregnancy. At her initial visit, she was afebrile with stable vital signs and was therefore discharged to her home with empiric oseltamivir. A throat culture specimen was positive for group A streptococcus, and a nasopharyngeal aspirate (eSensor; GenMarkDx) was positive for RSV by viral PCR. The patient was treated with penicillin (500 mg, twice a day, for 10 days) for the group A streptococcus infection without any further complications. At 39 weeks of gestation, she underwent an uncomplicated primary cesarean delivery of a 2,855-g female infant with Apgar scores of 9 at 1 and 5 minutes. The infant was admitted to the newborn nursery and discharged home with the mother on postoperative day 4.

Discussion

The 3 case-patients described highlight the underrecognized effects of maternal RSV infection on maternal and neonatal outcomes. It is well documented that despite nearly universal exposure to the virus by age 2, previous RSV infection does not confer long-term immunity; reinfection has even been documented within the same season (*9*). For most immunocompetent adults these reinfections are milder than the original infection and produce a cold-like illness that is self-limited. However, RSV infection–related complications and sequelae may be more serious in the setting of pregnancy. The patients in our study with the first and second cases required ICU level care and intubation, which suggests that maternal RSV infection can lead to clinically severe maternal disease. Although the infection in the third case-patient was self-limited, and she was treated as an outpatient, it indicates that maternal RSV may be a critical factor in mild respiratory illness that leads to physician visits, lost work time, and possible inappropriate administration of antibiotic drugs.

Notably, all 3 cases were complicated by preexisting lung conditions, such as asthma in case-patient 1, or a coexisting infection, such as influenza A(H1N1) in case-patient 2 and group A streptococcus in case-patient 3. Although the severity of these cases may have been influenced by these concomitant conditions, many virus infections are known to have a more severe course during pregnancy. Perhaps the most compelling evidence to suggest that RSV might be more virulent during pregnancy is that infections with many viruses (e.g., varicella-zoster, hepatitis, and most notably, influenza) are known to have a more severe course in pregnant women (*10*–*12*). The prevalence of influenza in pregnancy is similar to that in the general population, yet influenza carries a 5-fold increased risk of death in pregnant women (*13*). The pathophysiology underlying the increased risk for illness and death caused by influenza during pregnancy remains poorly understood. The immunologic changes that allow a genetically distinct fetus to inhabit a mother with a functioning immune system likely contribute greatly to the increased illness and death caused by influenza in pregnancy. Cytotoxic adaptive immunity, which is involved in defense against virus infection, is diminished during pregnancy, whereas the regulatory adaptive immune system, the basis for immune memory and vaccine immunology, is heightened (*14*–*17*).

Despite the potential for devastating consequences, RSV infection in pregnant women has likely been unrecognized, as this infection once was in the elderly population. Falsey et al. (*1*) reported RSV infection in 4% to 10% of high risk adults annually, among whom >85% were symptomatic, 16%% required hospitalization, and 4% died. They also estimated that the disease prevalence of RSV infection in immunocompromised and elderly adults is similar to that of nonpandemic influenza (*1*).

A major barrier to the effective diagnosis of RSV is the inability to make the diagnosis solely on the basis of clinical features, which are similar to those of infections caused by adenovirus, rhinovirus, parainfluenza virus, or influenza virus. The reference standard for diagnosis (i.e., isolation of the virus in cell culture and identification of plaque morphologic features with syncytium formation) is time consuming and expensive. More recently, PCR-based testing has become more available and allows for a more rapid and specific diagnosis. Laboratory testing has only recently been adopted in academic hospital centers and is still underused in the community setting. Although RSV subtype A typically causes more severe disease than RSV subtype B (*18*), the PCR-based test used in our institution does not allow for RSV subtyping. As was evident from the cases presented, co-infection with other viruses or bacteria can also add to diagnostic challenges and severity of clinical features.

Although the cases presented occurred at a single institution during a single RSV season, no overall increase in RSV incidence or severity occurred among the general population of children and adults that would explain an increase in incidence among the pregnant population. With the increased availability of viral PCR panels that detect RSV, we suspect that we are now recognizing an infection that has long been present. Once RSV infection is suspected and diagnosed in pregnancy, treatment is also more

complicated. Although the cornerstone of RSV treatment is supportive therapy, disease management can include administration of ribavirin, a nucleoside analog that has been shown to improve outcomes in severely immunocompromised RSV-infected adults (*19*). However, ribavirin is contraindicated in pregnancy because of its teratogenic effects, which have been documented in multiple animal studies up to 7 months after cessation of treatment (*20*), but these findings are unsupported by human data. The Ribavirin Pregnancy Registry reported 6 cases of birth defects in 49 live births (over a 5-year surveillance period) in which the mother received ribavirin. The birth defects included polydactyly, glucose 6-phosphate dehydrogenase deficiency, hypospadias, ventricular septal defect, cyst of the fourth ventricle of the brain, and torticollis (*21*). Although the evidence remains limited, ribavirin is currently classified by the US Food and Drug Administration as pregnancy category X drug. Nonetheless, despite safety concerns, in the setting of maternal life-threatening illness, antiviral agents should be considered, given that fetal well-being is ultimately maximized by maternal well-being.

No data have documented vertical RSV transmission in humans, and in other viral illnesses, such as influenza, this phenomenon is rare (*22*,*23*). Fortunately, none of the infants of the case-patients described herein had any signs of RSV infection.

Vaccine trials are currently underway, and questions like the following will inevitably arise: Who should get the vaccine? At what gestational age should it be administered? Is the vaccine cost effective? For pregnant women, RSV infection may pose a substantial risk for hospitalization and further complications, and the infection is likely worsened in the setting of baseline pulmonary disease, such as asthma, and tobacco use (*24*). The cases presented here demonstrate the wide spectrum of maternal disease and emphasize the potential for maternal benefit of an RSV vaccine administered during pregnancy. However, studies that can characterize the maternal effects of RSV infection at the population-level are necessary to answer these questions and enable any effective RSV vaccine to be used to its full potential to protect mother and infant.

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RESEARCH

Serotype Changes and Drug Resistance in Invasive Pneumococcal Diseases in Adults after Vaccinations in Children, Japan, 2010–2013

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After 7-valent pneumococcal conjugate vaccine (PCV) for children was introduced in Japan in November 2010, we examined changes in *Streptococcus pneumoniae* serotypes and in genetic antimicrobial drug resistance of isolates from adults with invasive pneumococcal diseases. During April 2010–March 2013, a total of 715 isolates were collected from adults with invasive pneumococcal diseases. Seven-valent PCV serotypes in adults decreased from 43.3% to 23.8%, most noticeably for serotype 6B. Concomitantly, 23-valent pneumococcal polysaccharide vaccine (PPSV23) serotypes decreased from 82.2% to 72.2%; non-PPSV23 serotypes increased from 13.8% to 25.1%. Parallel with serotype changes, genotypic penicillin-resistant *S. pneumoniae* decreased from 32.4% to 21.1%, and 6 non-PPSV23 serotypes emerged (6D, 15A, 15C, 16F, 23A, and 35B). Respective vaccine coverage rates for 13-valent PCV and PPSV23 differed by disease: 73.9% and 84.3% for patients with pneumonia, 56.4% and 69.2% for patients with bacteremia and sepsis, and 45.7% and 69.3% for patients with meningitis.

Streptococcus pneumoniae can cause invasive pneumococcal diseases (IPDs), which can be treated with generally effective antimicrobial drugs. In adults, well-known risk factors for IPDs include underlying conditions such as splenectomy, functional asplenia, immunodeficiency, and HIV infection. Risk is also increased with age >65 years, diabetes, renal dialysis, and chronic hepatic dysfunction (*1*,*2*).

In 2010, the US Advisory Committee on Immunization Practices (ACIP) issued recommendations that all persons ≥65 years of age should be vaccinated with 23-valent pneumococcal polysaccharide vaccine (PPSV23) to prevent pneumococcal diseases (*3*). In 2012, the ACIP also recommended that 13-valent pneumococcal conjugate vaccine (PCV13), which the US Food and Drug Administration approved in 2011, be given to adults ≥19 years of age if they have immunocompromising conditions (*4*). Recently, the ACIP recommended routine administration of both PCV13 and PPSV23 to all adults ≥65 years of age (*5*).

In the United States, 7-valent pneumococcal conjugate vaccine (PCV7) has been used to vaccinate children since 2000, resulting in individual and herd immunity and a decline in pneumococcal infection in children (*6*–*9*). After PCV7 was introduced, serotype 19A pneumococcal strains with penicillin resistance increased (*8*–*10*); the change in 2012 to PCV13 covers serotype 19A (*11*).

Presently, pneumococcal conjugate vaccines (PCVs) are incorporated into pediatric vaccination schedules in >120 countries, and PCV13 has been approved for adults in >100 countries. Pneumococcal infections in adults have decreased as an indirect effect of widespread use of PCVs for children (*2*,*6*,*8*,*9*,*12*). However, in countries where PCV7 or PCV13 was introduced, overall coverage of serotypes by the vaccine gradually decreased because of pneumococcal serotype replacement from vaccine type to nonvaccine type. In particular, increases of nonvaccine serotypes, such as 6C, 15A, 23A, and 35B, have been reported in the United States (*13*,*14*) and other countries (*15*–*18*).

In Japan, PCV7 vaccination was introduced for children <5 years of age in November 2010 by the Provisional Fund for the Urgent Promotion of Vaccination. PCV7 was incorporated into routine vaccination schedules in April 2013 and replaced by PCV13 in November 2013. PCV7 rapidly decreased IPD infections in children. However, pneumococcal infections caused by non-PCV7 serotypes (19A, 15A, 15B, 15C, and 24) increased in children during 2012 (*19*).

Meanwhile, Japan became the first "super-aging" society in the world in 2013, when 25.1% of the nation's

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population was ≥65 years of age (Statistics Bureau, Ministry of Internal Affairs and Communications, http://www.stat.go.jp/english/data/handbook/c0117.htm). Lifestyle-related and other diseases that affect response to infection have increased in older adults, and pneumonia has again become the third leading cause of death in Japan. Nevertheless, the voluntary PPSV23 immunization rate among older persons long remained <18%. In November 2014, the Japanese Ministry of Health, Labour and Welfare began promoting routine vaccination with PPSV23 for adults ≥65 years of age.

Because of these developments, we sought to clarify changes in serotypes and in genetic antimicrobial resistance in isolates from adults with IPDs after PCV7 for children was introduced in Japan. We describe capsular serotypes, drug-resistance genotypes, and multilocus sequence typing in isolates from adults with IPDs, and we examine the relationships of these factors to specific IPDs.

Methods

Patients and Pneumococcal Strains

Our study included adult patients (≥19 years of age) with IPD. Pneumococcal isolates from sterile clinical samples such as blood, cerebrospinal fluid, pleural effusion, and joint fluid were collected from clinical laboratories at 341 hospitals participating in this IPD surveillance study. Each hospital had a microbiology laboratory, and the hospitals were distributed nearly uniformly throughout Japan. The hospitals participated in the surveillance project after written permission was obtained from laboratory or hospital directors.

From April 2010 through March 2013, a total of 715 pneumococcal isolates were collected for the study: 275 during April 2010–March 2011, the first surveillance period; 213 during April 2011–March 2012, the second period; and 227 during April 2012–March 2013, the third period. This large-scale IPD surveillance for adults was performed in conjunction with IPD surveillance for children (*19*). During the first surveillance period, the rate of voluntary PCV7 vaccination for children was ≈10%. The second period occurred simultaneously with the Urgent Promotion of Vaccination incentive for children, and the immunization rate was ≈50%–60%. The third period occurred just before the transition from PCV7 to PCV13 for children, and the PCV7 immunization rate was ≈80%–90%. However, the rate of voluntary vaccination with PPSV23 in adults remained <18% throughout the surveillance periods.

The collected pneumococcal isolates were sent promptly from each clinical laboratory to our laboratory (Molecular Epidemiology for Infectious Agents, Kitasato Institute for Life Sciences, Kitasato University, Tokyo, Japan), accompanied by survey forms completed by attending physicians but not identifying patients in compliance with ethical guidelines for epidemiology in Japan. The surveys collected information on patient's age at illness onset, sex, clinical manifestations, blood test results at hospitalization, and outcome. Clinical manifestations and diagnoses were verified in all patients, according to diagnostic criteria and guidelines for sepsis and related conditions (*20*).

Serotypes and Antimicrobial Drug–Resistant Genotypes

Serotypes were determined by the capsular quellung reaction by using antiserum purchased from the Statens Serum Institute (Copenhagen, Denmark). By using real-time PCR methods described previously (*21*), alterations were identified in 3 penicillin-binding protein (PBP) genes mediating β-lactam resistance in *S. pneumoniae*: *pbp1a* (encoding enzyme PBP1A), *pbp2x* (encoding PBP2X), and *pbp2b* (encoding PBP2B). Amino acid substitutions within or near each PBP's conserved amino acid motifs, such as serine-threonine (Thr)–methionine-lysine (STMK), were detected by using real-time PCR. The *mef* (A) and *erm* (B) genes mediating macrolide resistance were also identified by real-time PCR (*21*). Quinolone resistance was analyzed by sequencing the quinolone-resistance–determining region in the genes *gyrA*, *gyrB*, *parC*, and *parE* in strains with MICs of levofloxacin of >4 μg/mL (*22*).

Gene analysis identified genotypes (g) on the basis of their responses to β-lactam antimicrobial drugs: penicillin-susceptible *S. pneumoniae* (gPSSP) possessing 3 normal *pbp* genes; penicillin-intermediate *S. pneumoniae* (gPISP), also classified as gPISP (*pbp2x*), gPISP (*pbp2b*), gPISP (*pbp1a*+*pbp2x*), or gPISP (*pbp2x*+*pbp2b*); and penicillin-resistant *S. pneumoniae* (gPRSP), which possessed all 3 abnormal *pbp* genes (*19*,*21*). Macrolide-resistant genotypes were also represented variously: macrolide-susceptible *S. pneumoniae* that possessed no resistance genes; macrolide resistance mediated by the *mef*(A) gene, MLR-*mef*(A); macrolide resistance mediated by the *erm*(B) gene, MLR-*erm*(B); and macrolide resistance involving both genes, MLR-*mef*(A)+*erm*(B).

Identification of capsular type by the quellung reaction and resistance genotyping by real-time PCR was performed within 1 day of sample collection for each strain. Results were reported immediately to the medical staff at the referring hospital.

Antimicrobial Susceptibility Testing

MICs of 7 intravenous antimicrobial agents (penicillin, ampicillin, cefotaxime, ceftriaxone, meropenem, panipenem, and vancomycin) were determined for all pneumococcal isolates by agar-dilution methods (*23*). We obtained each agent from the respective manufacturer.

Multilocus Sequence Typing

Multilocus sequence typing (MLST; http://www.MLST.net) was performed for nonvaccine serotype strains, except for PPSV23 serotypes, according to previously described methods with minor modifications (*24*). Primers used were based on sequences listed by the US Centers for Disease Control and Prevention (http://www.cdc.gov/ncidod/biotech/strep/alt-MLST-primers.htm). Analyses by using MLST and eBURST (Department of Infectious Disease Epidemiology, Imperial College of London, UK) were performed, as described by the MLST website (http://spneumoniae.mlst.net).

Statistical Analysis

We assessed differences in serotypes by age groups, clinical symptoms and signs, resistance types, and surveillance periods. To determine whether differences were statistically significant, we performed χ^2 tests or the Fisher exact test by using Ekuseru-Toukei 2012 software for statistics (Social Survey Research Information, Tokyo, Japan).

Results

Yearly Changes in Pneumococcal Serotypes

During each annual surveillance period (April–March) during 2010–2013, pneumococcal serotypes from IPD isolates varied (Table 1). All strains were isolated from sterile samples, such as blood, cerebrospinal fluid, pleural effusion, or joint fluid.

During all surveillance periods, the estimated voluntary inoculation rate for PPSV23 in adults was ≤18% in Japan. In February 2010, the vaccination incentive for PCV7 in children began on a voluntary basis. In November 2010, the Special Fund for the Urgent Promotion of Vaccination was initiated, and PCV7 vaccination of children <5 years of age became an official priority throughout

Table 1. Pneumococcal serotypes in isolates from adult patients with invasive pneumococcal diseases, Japan, April 2010–March 2013*

Vaccine serotype	2010–2011, no. (%), n = 275	2011–2012, no. (%), n = 213	2012–2013, no. (%), n = 227	p value†
PCV7				
4	14 (5.1)	17 (8.0)	7 (3.1)	0.075
6B	41 (14.9)	24 (11.3)	12 (5.3)	0.003
9V	7 (2.5)	5 (2.3)	1 (0.4)	0.168
14	21 (7.6)	16 (7.5)	17 (7.5)	0.998
18C	1 (0.4)	1 (0.5)	1 (0.4)	ND
19F	14 (5.1)	8 (3.8)	7 (3.1)	0.511
23F	21 (7.6)	13 (6.1)	9 (4.0)	0.232
Total	119 (43.3)	84 (39.4)	54 (23.8)	<0.001
PCV13				
1	1 (0.4)	1 (0.5)	0	ND
3	45 (16.4)	27 (12.7)	42 (18.5)	0.226
5	0	0	1 (0.4)	ND
6A	11 (4.0)	4 (1.9)	6 (2.6)	0.367
7F	9 (3.3)	2 (0.9)	3 (1.3)	0.128
19A	18 (6.5)	11 (5.2)	17 (7.5)	0.593
Total	203 (73.8)	129 (60.6)	123 (54.2)	<0.001
PPSV23				
10A	10 (3.6)	8 (3.8)	11 (4.8)	0.754
11A	3 (1.1)	8 (3.8)	5 (2.2)	0.142
15B	3 (1.1)	4 (1.9)	5 (2.2)	0.605
22F	10 (3.6)	19 (8.9)	18 (7.9)	0.040
Other‡	8 (2.9)	1 (0.5)	8 (3.5)	0.082
Total	226 (82.2)	165 (77.5)	164 (72.2)	0.036
Nonvaccine serotype				
6C	13 (4.7)	12 (5.6)	17 (7.5)	0.407
15A	6 (2.2)	10 (4.7)	8 (3.5)	0.310
15C	0	4 (1.9)	4 (1.8)	ND
23A	2 (0.7)	8 (3.8)	8 (3.5)	0.053
35B	7 (2.5)	6 (2.8)	9 (4.0)	0.626
37	3 (1.1)	0	1 (0.4)	ND
38	3 (1.1)	2 (0.9)	3 (1.3)	0.928
Other§	3 (1.1)	2 (0.9)	5 (2.2)	0.454
Total	37 (13.5)	44 (20.7)	55 (24.2)	0.007
Nontypeable¶	1 (0.4)	0	2 (0.9)	

*Years run from April 1 to March 31 of the following year. ND, not determined because of a low number of strains.
†p values characterize comparisons of 2010–2011, 2011–2012, and 2012–2013.
‡Serotypes 12F, 20, and 33F.
§Serotypes 6D, 7C, 16F, 18B, 24F, and 34.
¶These serotypes were excluded from the statistical analysis because they were unclassifiable.

Japan. Estimates of the PCV7 immunization rate in children was <10% in 2010, 50%–60% in 2011, and 80%–90% in 2012 (*19*).

PPSV23 coverage of serotypes found in IPD isolates decreased significantly each year, from 82.2% in 2010 to 77.5% in 2011 to 72.2% in 2012 (p = 0.036). Respective coverages with PCV7 and PCV13 also decreased significantly, from 43.3% and 73.8% in 2010 to 39.4% and 60.6% in 2011 to 23.8% and 54.2% in 2012, respectively (p<0.001). Although serotype 22F increased significantly in IPD isolates, yearly decreases of serotype 6B contributed markedly to decreases in PPSV23 coverage. Nonvaccine serotypes (i.e., non-PPSV23) increased significantly during the surveillance periods from 13.5% in 2010 to 20.7% in 2011 to 24.2% in 2012 (p = 0.007).

Introduction of PCV7 in children indirectly affected adults with IPD, shown by changes in each serotype during 2010–2012 (online Technical Appendix Figure 1, http://wwwnc.cdc.gov/EID/article/21/11/14-2029-Techapp.pdf). Decreases occurred in PCV7 serotypes and serotype 6A, the latter of which exhibits cross-immunity with 6B, but almost all other serotypes except those in PCV13 increased, especially serotypes 22F, 6C, 23A, 15C, 15A, and 35B.

Yearly Changes in Genotypes Resistant to Penicillin, Macrolides, and Quinolones

Genotypes resistant to penicillin, macrolides, and quinolones varied during the surveillance periods (Table 2). Among genotypes resistant to penicillin, 40% were gPISP (*pbp2x*), 26% were gPRSP, 10.2% were gPISP (*pbp1a+pbp2x*), and 5.9% were gPISP (*pbp2x+pbp2b*). Only 16.1% were gPSSP strains without any abnormal *pbp* genes. Other genotypes were gPISP (*pbp2b*; 1.3%) and gPISP (*pbp1a+2b*; 0.6%). In contrast to a gradual increase in gPISP (*pbp2x+pbp2b*; p = 0.025), gPRSP decreased significantly over the surveillance periods (p = 0.008). In addition, 89.7% of all pneumococcal strains possessed *mef*(A) or *erm*(B) genes that mediated macrolide resistance: 23.6% carried the *mef*(A) gene mediating intermediate macrolide resistance; 56.9% carried the *erm*(B) gene mediating high macrolide resistance; and 9.1% possessed both genes. Macrolide-susceptible strains accounted for only 10.3%. Furthermore, only 0.7% of strains were highly resistant to levofloxacin (MIC >16 μg/mL) and possessed mutations in both *gyrA* and *parC* genes. Intermediate-resistance strains (MIC 4 μg/mL) that possessed the *parC* mutation accounted for 1.3% of strains.

Yearly Changes in Resistance Genotypes and Serotypes

Yearly changes in genotypes and serotypes affecting β-lactam resistance also occurred during the 3 periods (Figure). Decreases closely related to a reduction of serotypes 6B, 19F, and 23F, but not of serotype 14, were found for a number of gPRSP. Emergence of gPRSP was evident among 6 non-PPSV23 serotypes (6D, 15A, 15C, 16F, 23A, and 35B), although such resistance strains were few. Almost all non-PPSV23 serotype strains showed macrolide resistance (online Technical Appendix Figure 2), which did not change significantly from year to year (Table 2).

Invasive Pneumococcal Diseases and Patient Age

To examine relationships between IPDs and patient age at illness onset, we classified IPDs into 4 categories (Table 3): pneumonia (n = 375), including cases of empyema (n = 17) and pleuritis (n = 16); bacteremia and sepsis with no obvious focus (n = 172), including sepsis (n = 51), severe

Table 2. Isolates positive for invasive pneumococcal diseases and resistant to penicillin, macrolide, and quinolone, by genotype, Japan, April 2010–March 2013*

Genotype	Total, no. (%), N = 715	2010–2011, no. (%), n = 275	2011–2012, no. (%), n = 213	2012–2013, no. (%), n = 227	p value†
Penicillin resistance					
gPSSP	115 (16.1)	42 (15.3)	40 (18.8)	33 (14.5)	0.431
gPISP (*pbp2x*)	286 (40.0)	96 (34.9)	89 (41.8)	101 (44.5)	0.076
gPISP (*pbp2b*)	9 (1.3)	5 (1.8)	0	4 (1.8)	ND
gPISP (*pbp1a+2x*)	73 (10.2)	30 (10.9)	23 (10.8)	20 (8.8)	0.701
gPISP (*pbp1a+2b*)	4 (0.6)	3 (1.1)	1 (0.5)	0	ND
gPISP (*pbp2x+2b*)	42 (5.9)	10 (3.6)	11 (5.2)	21 (9.3)	0.025
gPRSP (*pbp1a+2x+2b*)	186 (26.0)	89 (32.4)	49 (23.0)	48 (21.1)	0.008
Macrolide resistance					
Resistance gene (-)	74 (10.3)	34 (12.4)	16 (7.5)	24 (10.6)	0.216
mef(A) gene	169 (23.6)	63 (22.9)	56 (26.3)	50 (22.0)	0.538
erm(B) gene	407 (56.9)	152 (55.3)	123 (57.7)	132 (58.1)	0.777
mef(A) and *erm*(B)	65 (9.1)	26 (9.5)	18 (8.5)	21 (9.3)	0.925
Quinolone resistance‡					
gyrA+parC	5 (0.7)	2 (0.7)	3 (1.4)	0	ND
parC	9 (1.3)	5 (1.8)	1 (0.5)	3 (1.3)	ND

*Years run from April 1–March 31 of the following year. ND, not determined because of a low number of strains.
†p values characterize comparison of the 3 surveillance periods.
‡Quinolone resistance, Ser81Phe substitution in GyrA and Ser79Tyr in ParC, respectively.

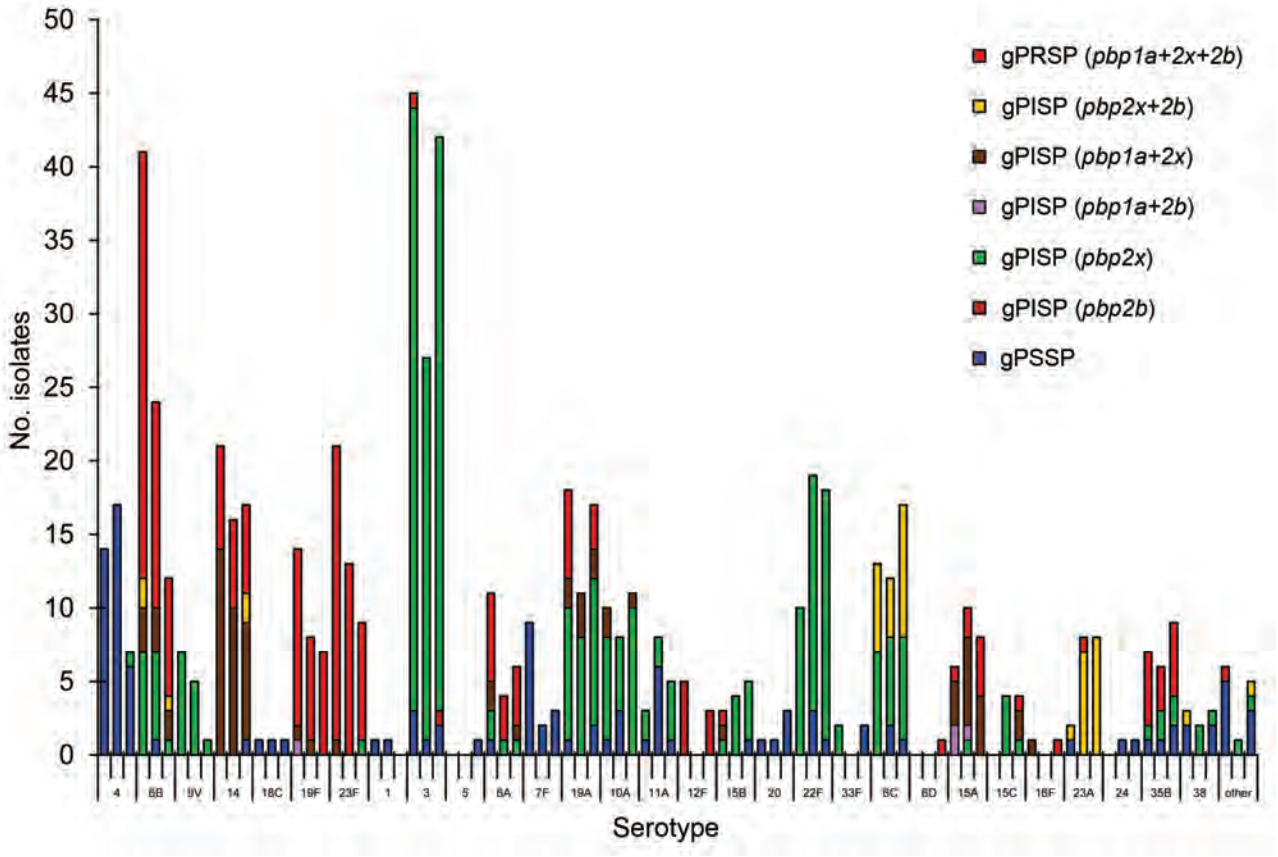

Figure. Yearly changes in number of serotypes and in penicillin resistance in genotypes found in isolates from adults with invasive pneumococcal diseases, Japan, April 2010–March 2013. Serotypes are shown for each of the 3 yearly surveillance periods: April 2010–March 2011, April 2011–March 2012, and April 2012–March 2013. Short tic marks on horizontal axis represent yearly number of isolates for specific serotypes; longer tic marks represent the 3-year surveillance period for each serotype. gPSSP, genotypic penicillin-susceptible *Streptococcus pneumoniae*; gPISP, genotypic penicillin-intermediate resistant *S. pneumoniae*; gPRSP, genotypic penicillin-resistant *S. pneumoniae*. Parentheses enclose abnormal *pbp* genes that mediate penicillin resistance.

sepsis (n = 57), and septic shock (n = 24); meningitis (n = 127); and others (n = 41), including cellulitis (n = 7), arthritis (n = 6), spondylitis (n = 4), and peritonitis (n = 3). Pneumonia (52.4%) was the most common category of IPDs, followed by bacteremia and sepsis (24.1%) and meningitis (17.8%). Mean age of patients with meningitis was 65 years, younger than those with pneumonia or bacteremia and sepsis (mean age 74 and 70 years, respectively). Meningitis occurred significantly more often among patients ≤64 years of age, in contrast to pneumonia, which occurred most often in persons ≥75 years of age (p<0.001).

Data for deaths and survival were available for 656 (91.7%) of the 715 patients. Proportions of deaths occurring within 30 days of hospitalization increased significantly with age: 6.0% at 19–49 years, 17.8% at 50–64 years, 17.9% at 65–74 years, 25.4% at 75–84 years, and 26.3% at ≥85 years (p = 0.002). Overall rate of death occurring within 30 days after hospitalization was 21.3%; overall rate for serious sequelae was 10%.

Serotype Distribution by Disease

A comparison of proportions of serotypes included in PCV7, PCV13, PPSV23, and non-PPSV23 by disease category (i.e., pneumonia, bacteremia and sepsis, meningitis, and others) showed that frequency of serotypes differed considerably by type of IPD (Table 4). Prevalence of serotypes 3, 14, and 19A was higher in isolates of patients with pneumonia than in those from patients with other illnesses (p value range <0.001–0.076). In contrast, serotypes 10A, 15A, 23A, and 23F were significantly more prevalent in isolates of patients with meningitis than in those from patients with other illnesses (p value range <0.001–0.004).

Coverages for vaccines among all isolates collected and examined during the study duration were 35.5% for PCV7, 63.6% for PCV13, and 77.6% for PPSV23. Respective coverages of PCV13 and PPSV23 differed significantly for each IPD group: 73.9% and 84.3% for pneumonia, 56.4% and 69.2% for bacteremia and sepsis, 45.7% and 69.3% for meningitis, and 56.1% and 78.0% for others (p<0.001 for

Table 3. Isolates positive for invasive pneumococcal diseases in adults, by age group, Japan, April 2010–March 2013

Disease	No. (%)						Mean age, y (±SD)	p value
	Total	19–49	50–64	65–74	75–84	≥85		
Pneumonia*	375 (52.4)	33 (39.8)	66 (42.0)	103 (49.8)	105 (60.7)	68 (71.6)	74 (14.9)	<0.001
Bacteremia and sepsis†	172 (24.1)	20 (24.1)	42 (26.8)	55 (26.6)	34 (19.7)	21 (22.1)	70 (15.0)	0.497
Meningitis	127 (17.8)	24 (28.9)	38 (24.2)	37 (17.9)	22 (12.7)	6 (6.3)	65 (14.4)	<0.001
Other‡	41 (5.7)	6 (7.2)	11 (7.0)	12 (5.8)	12 (6.9)	0	67 (14.5)	0.133
Total	715	83	157	207	173	95	70 (14.9)	

*Includes empyema (n = 17) and pleuritis (n = 16).
†Bacteremia (n = 39), sepsis (n = 51), severe sepsis (n = 58), and septic shock (n = 24).
‡Cellulitis (n = 7), arthritis (n = 6), spondylitis (n = 4), peritonitis (n = 3), and other conditions.

all). The most prevalent serotype was serotype 3 (15.9%), followed by serotypes 6B (10.8%), 14 (7.6%), 22F (6.6%), 19A (6.4%), and 23F (6.0%). Death rates did not differ significantly by pneumococcal serotype.

Antimicrobial Drug Susceptibility by Genotype

The online Technical Appendix Table shows susceptibilities (50% MIC, 90% MIC, and MIC range) of 6 parenteral β-lactam agents for *S. pneumoniae* strains (n = 710). PBP gene alterations affect MICs of penicillin, ampicillin, cefotaxime, and meropenem (online Technical Appendix Figure 3). Most strains in this study were gPISP with *pbp2x* gene alterations that reduced susceptibility to cephalosporin agents rather than to penicillin. MICs of cefotaxime and ceftriaxone were affected more by *pbp2x* alterations than by *pbp2b* alterations. In contrast, MICs of penicillin, ampicillin, and meropenem were affected more by *pbp2b* alterations than by *pbp2x* alterations. Strains showing MICs ≥4μg/mL to cefotaxime had *pbp2a* gene alteration and also had *pbp1a*, *pbp2x*, and *pbp2b* gene alterations (data not shown).

MLST in Non-PCV13 Serotype Strains

MLST results for 92 strains (non-PCV13 serotypes) collected during 2012 and results for 2 gPRSPs (serotypes 15B collected during 2010 and 23A collected during 2011) indicate that sequence types (STs), 8 of which were new, and clonal complexes (CCs) varied (Table 5). We identified 3 new STs of gPRSPs possessing macrolide-resistant genes: ST6138 (CC81) in serotype 15C, ST8351 (CC3117) in serotype 16F, and ST9619 (CC156) in serotype 23A. Also, we identified gPRSPs of ST282 (CC81) in serotype 6D, reported from South Korea in 2008 and derived from Vietnam serotype 23F in 1996; ST63 (CC63) in serotype 15A, derived from Sweden^{15A}-25 in 1992; ST83 (CC81) in serotype 15B, reported from South Korea in 2007 and derived from Taiwan serotype 23F in 1997; and ST558 (CC558) in serotype 35B, reported from the United States in 1999 (Table 5). We confirmed that all gPRSPs examined in our study had abnormal PBPs by DNA sequencing: substitutions of Thr371Serine or Thr371Alanine (Ala) within conserved amino acid motif STMK in PBP1A; Thr338Ala within STMK in PBP2X; and Thr445Ala adjacent to the serine-serine-asparagine amino acid motif in PBP2B, respectively.

Discussion

Wide use of PCV7 or PCV13 in young children has contributed to a decline in IPD (*6*–*9*) and other pneumococcal diseases, including pneumonia (*25*,*26*) and otitis media (*27*,*28*). The decline resulted from both direct vaccine effect and herd immunity.

In the United States, introduction of PCV7 led to decreased rates of IPD, resulting from vaccine-related strains in children and in unvaccinated adults because of indirect effects of pediatric use (*6*,*29*,*30*). After increased incidence of serotype 19A among causative pathogens in children, multivalent PCV13 replaced PCV7 in 2010 (*11*). PCV13 now has been introduced in >120 countries, and various pneumococcal diseases have decreased as a result. Unfortunately, serotype replacement by non-PCV13 serotypes such as 6C, 15B, 22F, 23A, and 35B has also ensued (*13*,*14*,*16*–*18*,*31*).

In Japan, incorporation of PCV7 and *Haemophilus influenzae* type b conjugate vaccine into routine vaccination schedules for children occurred later than in other countries and was spurred by the Provisional Special Fund for the Urgent Promotion of Vaccination, initiated in November 2010. The vaccination rate for children improved rapidly from <10% in 2010 to 50%–60% in 2011 and 80%–90% in 2012. Routine PCV7 for children was implemented officially in April 2013 and was replaced with PCV13 in November 2013.

Although PPSV23 was approved in 1988, few (<18% in 2012) adults voluntarily received PPSV23 vaccination. Since October 2014, vaccination with PPSV23 in adults ≥65 years of age has been supported by public government funding. However, at this time, vaccination rates are unclear because these older persons can receive 1 PPSV23 vaccination over a 5-year period.

In our large-scale surveillance, we aimed to clarify molecular epidemiology for pneumococci and β-hemolytic streptococci and to collect background data on patients with IPD throughout Japan during April 2010–March 2013. Pediatric IPD caused by PCV7 serotypes and other PCV7-related 6A serotypes decreased significantly soon after

Table 4. Pneumococcal serotypes by type of invasive pneumococcal diseases in adults, Japan, April 2010–March 2013*

Serotype	Pneumonia, no. (%), n = 375	Bacteremia and sepsis, no. (%), n = 172	Meningitis, no. (%), n = 127	Others, no. (%), n = 41	Total, no. (%), N = 715	p value†
PCV7 serotypes						
4	25 (6.7)	8 (4.7)	5 (3.9)	0	38 (5.3)	0.403
6B	42 (11.2)	20 (11.6)	11 (8.7)	4 (9.8)	77 (10.8)	0.667
9V	10 (2.7)	3 (1.7)	0	0	13 (1.8)	0.161
14	36 (9.6)	9 (5.2)	6 (4.7)	3 (7.3)	54 (7.6)	0.076
18C	1 (0.3)	1 (0.6)	1 (0.8)	0	3 (0.4)	ND
19F	14 (3.7)	9 (5.2)	4 (3.1)	2 (4.9)	29 (4.1)	0.617
23F	16 (4.3)	7 (4.1)	15 (11.8)	5 (12.2)	43 (6.0)	0.004
Total	144 (38.4)	57 (32.6)	42 (32.3)	14 (34.1)	257 (35.5)	0.325
Additional PCV13 serotypes						
3	84 (22.4)	17 (9.9)	7 (5.5)	6 (14.6)	114 (15.9)	<0.001
6A	7 (1.9)	9 (5.2)	4 (3.1)	1 (2.4)	21 (2.9)	0.101
7F	10 (2.7)	2 (1.2)	2 (1.6)	0	14 (2.0)	0.463
19A	32 (8.5)	9 (5.2)	3 (2.4)	2 (4.9)	46 (6.4)	0.035
Other‡	0	3 (1.7)	0	0	3 (0.4)	ND
Total	277 (73.9)	97 (56.4)	58 (45.7)	23 (56.1)	455 (63.6)	<0.001
Additional PPSV23 serotypes						
10A	6 (1.6)	8 (4.7)	11 (8.7)	4 (9.8)	29 (4.1)	0.001
11A	10 (2.7)	3 (1.7)	3 (2.4)	0	16 (2.2)	0.798
15B	4 (1.1)	4 (2.3)	2 (1.6)	2 (4.9)	12 (1.7)	0.533
22F	20 (5.3)	12 (7.0)	12 (9.4)	3 (7.3)	47 (6.6)	0.269
Other§	6 (1.6)	4 (2.3)	6 (4.7)	1 (2.4)	17 (2.4)	0.139
Total¶	316 (84.3)	119 (69.2)	88 (69.3)	32 (78.0)	555 (77.6)	<0.001
Nonvaccine serotypes						
6C	21 (5.6)	10 (5.8)	7 (5.5)	4 (9.8)	42 (5.9)	0.994
15A	5 (1.3)	9 (5.2)	9 (7.1)	1 (2.4)	24 (3.4)	0.003
15C	1 (0.3)	4 (2.3)	3 (2.4)	0	8 (1.1)	ND
23A	2 (0.5)	7 (4.1)	8 (6.3)	1 (2.4)	18 (2.5)	<0.001
35B	7 (1.9)	9 (5.2)	4 (3.1)	2 (4.9)	22 (3.1)	0.101
38	6 (1.6)	1 (0.6)	1 (0.8)	0	8 (1.1)	ND
Other#	7 (1.9)	4 (2.3)	3 (2.4)	0	14 (2.0)	0.918
Total	49 (13.1)	44 (25.6)	35 (27.6)	8 (19.5)	136 (19.0)	<0.001
Nontypeable**	3 (0.8)	0	0	0	3 (0.4)	

*ND, not determined because of low number of strains. PCV7, 7-valent pneumococcal conjugate vaccine; PCV13, 13-valent pneumococcal conjugate vaccine; PPSV23, 23-valent pneumococcal polysaccharide vaccine.
†Significant difference obtained by comparing pneumonia, sepsis, and meningitis.
‡Serotypes 1 and 5.
§Serotypes 12F, 20, and 33F.
¶Serotype 6A is not included in PPSV23.
#Serotypes 6D, 7C, 16F, 18B, 24F, 34, and 37.
**Serotypes that were excluded from the statistical analysis because they were unclassifiable.

vaccine implementation (*19*), but the non-PCV7 serotypes 19A, 15A, 15B, 15C, and 24 increased each year. These results reflect simultaneous replacement of PCV7 serotypes with non-PCV7 serotypes among pneumococci that colonize the nasopharynx of children (K. Ubukata et al., unpub. data). Consequently, frequency of transmission of PCV7 serotypes from children to adults would be expected to decrease.

In this study, we found epidemiologic changes among pneumococcal strains isolated from adult patients with IPD; vaccine-type strains decreased significantly (*6*-*9*). However, the prominent decrease in serotype 6B detected by our surveillance differed from findings in the United States, where serotype 14 decreased most prominently after PCV7 introduction (*14*). IPD cases in Japan resulting from serotype 14 decreased significantly for adults during our ongoing surveillance in 2014. Given Japan's high population density, effects of herd immunity were likely present in children and adults. However, non-PCV7 types, especially serotype 22F, increased.

A decrease in penicillin-nonsusceptible *S. pneumoniae* strains has been associated with serotype changes occurring after vaccine introduction (*29*,*30*,*32*). In our previous genetic studies, the gPRSP with 3 PBP alterations encoded by the *pbp1a*, *pbp2x*, and *pbp2b* genes also decreased significantly (*19*). However, our current study found increases in the gPISP with *pbp2x* gene alterations that reduce susceptibilities to cephalosporin agents (e.g., cefotaxime and ceftriaxone), rather than reducing susceptibility to penicillin. This trend likely reflects preference among medical practices in Japan for cephalosporin and macrolide agents.

We also identified some gPRSPs not previously reported as new STs. Of 7 STs identified in gPRSP and non-PCV7 serotypes, 4 STs had been posted in the pneumococcal database of the MLST website: ST558 (CC558) of serotype 35B, identified in the United States (*33*); ST63 (CC63) of serotype 15A derived from Sweden^{15A}-25; ST282 (CC81) of serotype 6D in South Korea (*34*,*35*); and ST83 (CC81)

Table 5. Serotypes, resistance genotypes, and multilocus sequence type for non-PCV13 serotype isolates from adults with invasive pneumococcal diseases, Japan, April 2010–March 2013*

Serotype (total no. isolates)	No. isolates, N = 92	Penicillin resistance genotype	Macrolide resistance gene	Clonal complex	ST	Earliest report of same ST†	
						Year	Country (city)
6C (17)	1	gPISP(*pbp2x+2b*)	*erm*(B)	315	386	1996	Portugal (Lisbon)
	4	gPISP(*pbp2x*)	*erm*(B)	2924	2924	2003	Japan (Hyogo)
	2	gPISP(*pbp2x*)	*erm*(B)	2924	6183	2008	Japan (Kanagawa)
	1	gPISP(*pbp2x*)	*erm*(B)	2924	**9336‡**	2012	Japan (Fukuoka)
	1	gPSSP	Non	473	473	1994	England (Oxford)
	3	gPISP(*pbp2x+2b*)	*erm*(B)	156	5241	2008	Japan (Kumamoto)
	2	gPISP(*pbp2x+2b*)	*mef*(A)	5832	**5025‡**	2012	Japan (Kanagawa)
	2	gPISP(*pbp2x+2b*)	*mef*(A) or *erm*(B)	5832	5832	2009	Japan (Chiba)
	1	gPISP(*pbp2x+2b*)	*mef*(A)+*erm*(B)	5832	5832	2009	Japan (Chiba)
6D (1)	1	gPRSP	*mef*(A)	81	282	1996; 2008	Vietnam [23F]; South Korea [6D]
10A (11)	1	gPISP(*pbp2x*)	Non	156	1263	1998	USA (Arizona/New Mexico)
	1	gPISP(*pbp2x*)	Non	156	**3395‡**	2012	Japan (Toyama)
	8	gPISP(*pbp2x*)	*erm*(B)	113	5236	2007	Japan (Kanagawa)
	1	gPISP(*pbp1a+2x*)	*erm*(B)	113	**3078‡**	2012	Japan (Yamaguchi)
11A (5)	1	gPSSP	*mef*(A)	99	99	2007	South Korea (Seoul)
	4	gPISP(*pbp2x*)	*mef*(A)+*erm*(B)	99	99	2007	South Korea (Seoul)
15A (8)	4	gPISP(*pbp1a+2x*)	*erm*(B)	63	63	1992	[Sweden^{15A}-25]
	4	gPRSP	*erm*(B)	63	63	1992	[Sweden^{15A}-25]
15B (6)	1	gPSSP	*mef*(A)	199	199	1987	[Netherlands^{15B}-37]
	3	gPISP(*pbp2x*)	*erm*(B)	199	199	1987	[Netherlands^{15B}-37]
	1	gPISP(*pbp2x*)	*erm*(B)	199	**5609‡**	2012	Japan (Tokyo)
	1	gPRSP	*erm*(B)	81	83	1997	Taiwan [23F]
						2007	South Korea [15]
15C (4)	1	gPISP(*pbp2x*)	*erm*(B)	199	199	1987	[Netherlands^{15B}-37]
	2	gPISP(*pbp1a+2x*)	*erm*(B)	199	199	1987	[Netherlands^{15B}-37]
	1	gPRSP	*erm*(B)	81	**6138‡**	2013	Japan (Tokyo)
16F (1)	1	gPRSP	*mef*(A)	3117	**8351‡**	2011	Japan (Mie)
20 (3)	3	gPSSP	*erm*(B)	4745	4745	2005	China (Wuhan, Hubei)
22F (18)	1	gPSSP	Non	433	433	1997	Poland (center)
	4	gPISP(*pbp2x*)	Non	433	433	1997	Poland (center)
	10	gPISP(*pbp2x*)	*mef*(A) or *erm*(B)	433	433	1997	Poland (center)
	1	gPISP(*pbp2x*)	*mef*(A)	113	5236	2007	Japan (Kanagawa)
	2	gPISP(*pbp2x*)	*erm*(B)	113	7158	2007	Japan (Aichi)
23A (9)	2	gPISP(*pbp2x+2b*)	*erm*(B)	156	338	1995	[Colombia^{23F}-26]
	4	gPISP(*pbp2x+2b*)	*erm*(B)	156	5242	2009	Japan (Kumamoto)
	2	gPISP(*pbp2x+2b*)	*erm*(B)	156	5246	2008	Japan (Niigata)
	1	gPRSP	*erm*(B)	156	**9619‡**	2011	Japan (Toyama)
35B (9)	2	gPSSP	*erm*(B)	1816	2755	2004	China (Shanghai)
	2	gPISP(*pbp2x*)	*mef*(A) or *erm*(B)	1816	2755	2004	China (Shanghai)
	1	gPRSP	Non	558	558	1999	USA (New York) [35B]
	4	gPRSP	*mef*(A) or *erm*(B)	558	558	1999	USA (New York) [35B]

*PCV13, 13-valent pneumococcal conjugate vaccine; ST, sequence type. Brackets in final column represent clones reported in the Pneumococcal Molecular Epidemiology Network Clone Collection (http://web1.sph.emory.edu/PMEN/index.html).
†Earliest year and first location are shown for the applicable ST registered in the Multilocus Sequence Typing website (http://spneumoniae.mlst.net).
‡Identified as a new ST in this study.

of serotype15B, originating in Taiwan serotype 23F. The remaining 3 STs identified in gPRSP, serotypes 15C, 16F, and 23A, appear to be novel STs arising in Japan. ST6138 of serotype 15C and ST9619 of serotype 23A were estimated to be extended from ST83 and ST338 (Pneumococcal Molecular Epidemiology Network clone Colombia^{23F}-26), respectively (*36*).

Recent pneumococcal genomic analysis (*37*,*38*) indicated occurrence of capsular switching by genetic recombination among strains of different serotypes. Recombination of regions consisting of the capsular *cps* gene locus and 2 adjacent *pbp1a* and *pbp2x* genes results in capsular switching and penicillin resistance. A pneumococcal strain arising from such an event will show high penicillin resistance from mutations in *pbp* genes because of selection pressure from antimicrobial drugs. MLST and genotypic results of gPRSPs identified in non-PCV13 strains in this study support occurrence of such genetic events.

In relation to serotypes and IPDs in adults, PPSV23 coverage has been reported to differ by disease (*39*). Our results show respective coverages of PCV13 and PPSV23 to be 73.9% and 84.3% for patients with pneumonia and 45.7% and 69.3% for patients with meningitis. This finding supports recommendations by the ACIP (*5*) for routine vaccinations with both PCV13 and PPSV23 in all adults ≥65 years of age, especially those with serious underlying

diseases. For pneumococci with a large number of capsular types, development of a novel vaccine against components other than the capsule is expected (*40*). Overuse of antimicrobial agents should be avoided worldwide to avoid selection of novel resistant strains capable of undermining vaccine efficacy.

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Role of Maternal Antibodies in Infants with Severe Diseases Related to Human Parechovirus Type 3[1]

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Human parechovirus type 3 (HPeV3) is an emerging pathogen that causes sepsis and meningoencephalitis in young infants. To test the hypothesis that maternal antibodies can protect this population, we measured neutralizing antibody titers (NATs) to HPeV3 and other genotypes (HPeV1 and HPeV6) in 175 cord blood samples in Japan. The seropositivity rate (≥1:32) for HPeV3 was 61%, similar to that for the other genotypes, but decreased significantly as maternal age increased (p<0.001). Furthermore, during the 2014 HPeV3 epidemic, prospective measurement of NATs to HPeV3 in 45 patients with severe diseases caused by HPeV3 infection showed low NATs (≤1:16) at onset and persistently high NATs (≥1:512) until age 6 months. All intravenous immunoglobulin samples tested elicited high NATs to HPeV3. Our findings indicate that maternal antibodies to HPeV3 may help protect young infants from severe diseases related to HPeV3 and that antibody supplementation may benefit these patients.

Human parechoviruses (HPeVs) are small, nonenveloped, single-stranded, positive-sense RNA viruses classified in the genus *Parechovirus* of the family *Picornaviridae* (*1*). Among the 16 genotypes identified, the most common genotypes collected from patients are HPeV types 1 (HPeV1), 3 (HPeV3), and 6 (HPeV6) (*2*). HPeV type 4 is less common (*3*), and although HPeV type 5 has not been detected in Japan (*4*), it is circulating in the United States (*5*) and Europe (*6*). Generally, HPeV1 and HPeV6 cause mild gastroenteritis and respiratory infections in infants and children (*2,7*). HPeV1 (known as echovirus 22 until 1999) has occasionally been associated with severe diseases, such as encephalitis, acute flaccid paralysis, myocarditis, and neonatal sepsis (*2*), but in Japan, it has been isolated only from patients with mild diseases, including gastroenteritis, upper respiratory tract infection, and hand, foot, and mouth disease (*3*). In contrast, HPeV3, an emerging pathogen first reported in Japan in 2004 (*8*), causes severe diseases (e.g., sepsis and meningoencephalitis) in infants <3 months of age (*9*). HPeV3 infection can be accompanied by sepsis-like syndrome (*10,11*), which can lead to neurologic sequelae (*12*) and death (*13*), and has therefore attracted pediatricians' attention (*14*). Clinical signs and symptoms and age of patients differ for various genotypes for reasons that are unclear.

We hypothesized that maternal antibodies may help protect neonates and young infants from severe diseases related to HPeV3. Seroepidemiologic data on HPeV3 have been available only from healthy persons or patients for whom viral infections other than HPeV3 have been diagnosed (*8,15*); data on perinatal antibody titers to HPeV3 have not been published. Although neutralizing antibody titers (NATs) have been reported as lower for mothers of infants with HPeV3 infection than for mothers of infants with other viral infections (*16*), little is known about patients infected with HPeV3 (*15*). Such data might help determine why severe diseases related to HPeV3 develop in some neonates and young infants. We measured NATs to HPeVs in cord blood from newborns and in serum samples from neonates and young infants with diseases related to HPeV3 infection who were admitted to hospitals in Niigata, Japan. In the latter group, samples were obtained from disease onset through convalescence. We also measured NATs in intravenous immunoglobulin (IVIG) preparations sold in Japan to determine if IVIG could be a potential treatment for HPeV3-related diseases in neonates and young infants.

Materials and Methods

Cord Blood Samples

Cord blood samples were collected during September 2013–January 2014 at Saiseikai Niigata Daini Hospital, a

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hospital affiliated with Niigata University in Niigata, Japan. Samples were collected from healthy newborns born at full term to women with uncomplicated pregnancies. Preterm infants (gestational age <37 weeks) were excluded because they have lower levels of maternally derived immunoglobulins than do full-term neonates (*17*). Also excluded were babies born to mothers with maternal conditions that could affect levels of maternal antibodies in newborns (e.g., pregnancy-induced hypertension and abnormalities in the placenta). After the umbilical cord was double-clamped and cut, an obstetrician obtained 3 mL of cord blood from the umbilical artery. The samples were centrifuged at 700 × *g* for 10 min at 4°C; plasma (≥500 μL) was frozen at −80°C; and the samples were sent to the laboratory of the Department of Pediatrics, Niigata University, where they were stored at −80°C until analysis. The study was approved by the Ethics Committees of Niigata University and Saiseikai Niigata Daini Hospital. Informed written consent was obtained from the parents of all study participants.

Patients with Severe Diseases Related to HPeV3

Since 2012, the viral causes of fever in neonates and young infants (i.e., ≤3 months of age) have been routinely evaluated by use of real-time PCR for enteroviruses (*18*), herpes simplex virus types 1 and 2 (*19*), and HPeVs (*20*) at Niigata University Hospital and its 40 affiliated hospitals in Niigata Prefecture (a population of ≈88,000 children <5 years of age). We evaluated patients with severe diseases related to HPeV3 who were admitted to Niigata University Hospital or 1 of its 11 affiliated hospitals during August 2013–September 2014. At onset, severe disease related to HPeV3 was defined as clinical symptoms and signs indicating sepsis or sepsis-like syndrome; disease status was confirmed by a positive result for HPeV3 by PCR analysis of samples of serum or cerebrospinal fluid (CSF). Information about patients' contacts who were ill was obtained from interviews with parents and caregivers. With informed consent from parents, blood samples were collected prospectively at 3 and 6 months of age. Frozen serum samples were sent to the laboratory at Niigata University and stored at -20°C until analysis. The study was approved by the Ethics Committee of Niigata University.

IVIG Preparations

Of the 5 commercially available IVIG preparations in Japan, 3 are from plasma pools of several thousand Japanese donors: polyethylene glycol (PEG)–treated IVIG (Venoglobulin IH; Mitsubishi Tanabe Pharma, Osaka, Japan), freeze-dried PEG-treated IVIG (Glovenin-I; Takeda, Osaka), and 2 batches of sulfonated IVIG (Venilon-I; Teijin Pharma, Tokyo, Japan); Another available IVIG preparation, ion-exchange resin-treated IVIG (Gammagard; Baxter, Tokyo), is from US donors; another, pH 4–treated IVIG (Sanglopor; CSL Behring, Tokyo), is from German donors. The plasma pools were collected during 2010–2012.

Cell Line and Virus Cultivation

LLC-MK2 cells from the kidney of a healthy adult rhesus monkey were used for the neutralization assay (*3*). The cells were maintained in Eagle's minimum essential medium (Sigma-Aldrich, St. Louis, MO, USA) containing 8% fetal bovine serum, 200 μg/mL gentamicin, and 2.5 μg/mL amphotericin B at 37°C, with 5% CO_2, for 1 week before passage. HPeV1 Harris (*21*), HPeV3 A308/99 (*8*), and HPeV6 NII561–2000 (*3*) were cultured in LLC-MK2 cells to obtain a sufficient amount of working seed viruses. The viruses were stored at −80°C until the neutralization test. The median tissue-culture infectious dose ($TCID_{50}$) was calculated by using the method of Reed and Muench (*22*).

Neutralization Test

All samples (i.e., cord blood samples, serum samples from patients infected with HPeV3, and IVIG preparations) were subjected to neutralization testing. Serum samples for real-time PCR were used to measure NATs at disease onset. A total of 25 μL of 100 $TCID_{50}$ viruses and 25 μL of serially diluted serum samples or IVIG preparation (started at 1:4 and then serially diluted twice until 1:2,048 in cord blood samples and IVIG preparations and until 1:512 in HPeV3-infected patients) were mixed in a 96-well plate and incubated at 37°C for 1 h. Then, 100 μL of suspended LLC-MK2 cells (10^6/mL) was added to the wells. Cell controls and virus back titration were performed. The appearance of a cytopathic effect (CPE) was evaluated daily by light microscopy from day 3 and determined at day 10, when CPE was positive in control wells containing 100 $TCID_{50}$ and was confirmed by virus back titration (*3*). CPE >50% was considered positive. The test was performed in triplicate when the IVIG preparations were evaluated.

Real-time PCR

Real-time PCR was performed on serum or CSF samples collected from patients during the acute phase of the disease (≤2 days after disease onset). CSF samples were centrifuged at 700 × *g* for 6 min at 4°C, and the supernatants were used in the analysis. Viral RNA was extracted from the serum and CSF samples by using a QIAamp MinElute Virus Spin Kit (QIAGEN, Valencia, CA, USA), according to the manufacturer's instructions. Real-time reverse transcription PCR (RT-PCR) was performed by using a PrimeScript One Step RT-PCR Kit (TaKaRa, Tokyo, Japan) and the Thermal Cycler Dice Real Time System II (TaKaRa) with virus-specific primers and a TaqMan probe that targeted the conserved 5′ untranslated region (*20*). The thermocycling settings were 42°C for 5 min for cDNA synthesis; 95°C for 3 min followed by 45 cycles at 95°C

for 5 s for denaturation; and 60°C for 40 s for annealing and extension.

Genotyping of HPeVs

A seminested RT-PCR assay with modifications was used to amplify the viral protein (VP) 1 region (*23*). After viral RNA was converted to cDNA by using SuperScript VILO MasterMix (Invitrogen, Carlsbad, CA, USA), an HPeV-VP1S forward primer (5′-GGD ARR MTK GGD VAW GAY GC-3′) and HPeV-VP1AS2 reverse primer (5′-TCY ARY TGR TAY ACA YKS TCT CC-3′) pair was used in the first PCR; and HPeV-VP1AS (5′-CCA TAR TGY TTR TAR AAA CC-3′) was used as reverse primer in the second PCR. These primer sequences were determined by using the International Union of Pure and Applied Chemistry nucleotide ambiguity codes. The 20-μL PCR mixture contained 2 μL of cDNA, 0.5 μM of each primer, and 10 μL of iQ Supermix (Bio-Rad Laboratories, Hercules, CA, USA). The cycling conditions were 95°C for 3 min, followed by 30 cycles at 95°C for 15 s, 42°C for 30 s, and 72°C for 1 min, terminating in a final extension at 72°C for 10 min. Amplicon size was 830 bp. HPeV-positive samples were typed by sequencing the VP1 PCR product. When the amount of the second PCR product was insufficient to sequence the VP1 region, a third PCR was performed with the same primers used in the second PCR. The PCR products were purified by using Illustra ExoProStar (GE Healthcare, Tokyo, Japan) and sequenced with a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) on an automated sequencer (Applied Biosystems 3130xl Genetic Analyzer). Each type was determined by comparing the nucleotide sequence of the VP1 region with available HPeV sequences from the DNA data banks of DDBJ, EMBL, and GenBank.

Serum-derived cDNA was used for VP1 sequencing. When the serum sample was unsuccessful in amplifying the VP1 region, CSF or stool samples collected during the acute phase of disease were used. Stool samples were diluted with 1× phosphate buffered saline to 10% wt/vol suspensions, followed by vortex and centrifugation at 6,250 × *g* for 20 min at 4°C. The supernatants were filtered with a Millex-GV Filter Unit (EMD Millipore, Billerica, MA, USA) with a pore size of 0.22 μm. After sample preparation, stool samples underwent RNA extraction and cDNA synthesis reactions as described for previous samples.

Statistical Analyses

All statistical analyses were performed by using SPSS Statistics 22.0 (IBM SPSS, Chicago, IL, USA). Geometric mean titers (GMTs) and seropositivity rates to HPeV1, 3, and 6 were compared by using the Kruskal-Wallis test and χ^2 test, respectively. In the calculations, an antibody titer <1:4 was regarded as 1, and a titer >1:2,048 was regarded as 2,048. A 2-tailed $p<0.05$ was used to indicate statistical significance.

Results

NATs in Cord Blood Samples

During the study period, 175 cord blood samples were collected. Median gestation age was 39.7 (range 37.1–41.9) weeks, and median maternal age was 32 (range 16–44) years. GMTs of antibodies to HPeV1, 3, and 6 were 52.0 (95% CI 40.5–66.8), 33.9 (95% CI 25.4–45.3), and 48.9 (95% CI 35.7–66.9), respectively. GMTs showed no significant differences among the 3 genotypes ($p = 0.17$) (Figure 1).

NATs in Patients with Severe Diseases Related to HPeV3

During the study period, 46 patients had a diagnosis of severe diseases related to HPeV, determined by PCR analysis of serum or CSF samples. NATs were measured in serum samples from 45 of these patients (1 patient was excluded because only a CSF sample was available on admission). Most (42/45 [93%]) were enrolled in the study during the epidemic of HPeV3 infection in Niigata in 2014. HPeV3 infection was identified in most (44/45 [98%]) HPeV-positive samples by sequencing the VP1 region of the virus by using cDNA derived from samples of serum (n = 40), CSF (n = 3), or stool cDNA (n = 1). The VP1 region could not

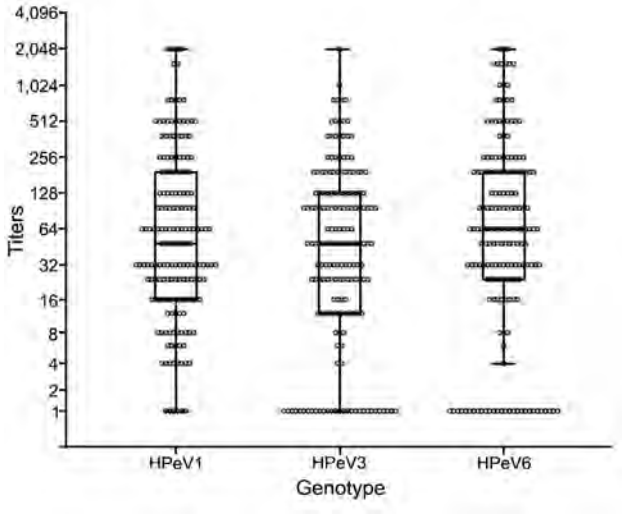

Figure 1. Distribution of neutralizing antibody titers to human parechovirus (HPeV) types 1, 3, and 6 in 175 cord blood samples from healthy neonates, Niigata, Japan, September 2013–January 2014. Titers are shown as reciprocal numbers. Boxes indicate first and third quartile values; bars within boxes indicate medians. Top and bottom bars indicate the 5th and 95th percentiles of data in a normal distribution. In the analysis, antibody titers <1:4 and >1:2,048 were regarded as 1 and 2,048, respectively.

be successfully amplified in 1 sample, so HPeV3 infection was diagnosed on the basis of a positive PCR result and a >4-fold increase in NATs to HPeV3.

The 45 patients with HPeV3-related diseases had a median age of 1 month (range 4 days–3 months, 21 days); 43 (96%) were <3 months of age, and 28 (62%) were male. For 25 (56%) patients, contact with a sick family member was identified. Clinical diagnoses at time of admission were sepsis (n = 37), sepsis-like illness (n = 7), and encephalitis with septic shock (n = 1).

All 45 serum samples at disease onset were available for neutralization testing. Most (42/45 [93%]) patients had a NAT of <1:4 to HPeV3 (HPeV3 negative). Three patients had low NATs to HPeV3: 1:4 in 1 (2%) patient and 1:16 in 2 (5%) patients.

Follow-up NATs were measured in the 34 (76%) patients whose parents agreed to participate in the follow-up study. During ongoing follow-up, 33 patients were evaluated at age 3 months and 19 patients at age 6 months. NATs to HPeV3 were ≥1:512 in all patients at age 3 months and remained ≥1:512 at age 6 months (Figure 2).

HPeV Seropositivity in Cord Blood Samples

Because the maximum antibody titer at disease onset was 1:16 in patients infected with HPeV3 and animal studies have shown high protection against enteroviral infection at titers of 1:32 (*24*), we chose a titer of ≥1:32 to indicate the level of neutralizing antibody necessary to prevent infection. At this level, seropositivity rates in cord blood samples for HPeV1, 3, and 6 were 65%, 61%, and 71%, respectively, and did not significantly differ among the 3 genotypes (p = 0.12) (Table 1). When the data were stratified by maternal age, the seropositivity rate for HPeV3 declined as maternal age increased (Table 1). When mothers were divided into age groups of 16–24 years (n = 11), 25–34 years (n = 107), and 35–44 years (n = 57), the group of youngest mothers had significantly higher GMTs (336.5, 95% CI 176.1–642.9) than those for older mothers (31.9 [95% CI 22.0–46.4] for mothers ages 25–34 and 24.4 [95% CI 15.4–38.6] for mothers ages 35–44; p<0.001). In contrast, more cord blood samples had a titer <1:4 for HPeV3 (n = 29, 17%) than for HPeV1 (n = 6, 3%), and seropositivity rates for these 2 groups differed significantly when a lower cutoff value (1:4) was considered (83% vs. 97%, respectively; p<0.001).

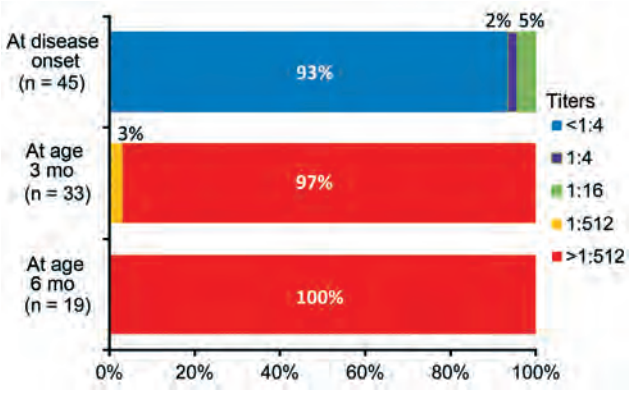

Figure 2. Change in neutralizing antibody titers to human parechovirus type 3 (HPeV3) in severe diseases related to HPeV3 in neonates and infants at disease onset and at 3 and 6 months of age, Niigata, Japan.

NATs in IVIG Preparations

All IVIG preparations we examined contained high NATs to all HPeVs (Table 2). NAT was ≥1:1,024 to HPeV1 and HPeV6 and ≥1:512 to HPeV3. No differences in antibody levels were observed among IVIG preparations originating from Japan, the United States, and Germany.

Discussion

Our findings show that NATs to HPeV3 were low (<1:32) in ≈40% of cord blood samples from healthy neonates, so this population could be at risk for developing severe diseases related to HPeV3. In addition, negative or low NATs (≤1:16) were observed in all HPeV3-infected patients at disease onset. During follow-up, these titers were elevated and remained high (≥1:512) until patients were 6 months of age. These data strongly suggest that maternal antibodies help protect neonates and young infants from severe disease related to HPeV3.

When the data were stratified by maternal age, the proportion of samples that were seropositive for HPeV3 decreased as maternal age increased. This finding suggests that neonates and young infants born to older mothers might be more likely to develop severe diseases related to HPeV3. One possibility is that NATs to HPeV3 wane if no reinfection or boosting of HPeV3 occurs in mothers. However, we found no significant differences in NATs and seropositivity rates to HPeV1, 3, and 6 at a cutoff of 1:32. Thus, for reasons yet to be identified, HPeV3

Table 1. Numbers and rates of seropositivity to HPeV types 1, 3, and 6 in cord blood samples from healthy full-term infants, by maternal age, Niigata, Japan, September 2013–January 2014*

		Maternal age, no. (%) samples		
HPeV type	All ages, n = 175†	16–24 y, n = 11	25–34 y, n = 107	35–44 y, n = 57
HPeV1	113 (65)	6 (55)	75 (70)	32 (56)
HPeV3	107 (61)	11 (100)	68 (64)	28 (49)
HPeV6	125 (71)	8 (73)	69 (64)	48 (84)

*HPeV, human parechovirus. A cutoff of 1:32 was used for HPeV1, 3, and 6.
†Antibody positivity rates compared among the 3 genotypes; p = 0.12.

Table 2. NATs to HPeV types 1, 3, and 6 in intravenous immunoglobulin preparations commercially available in Japan*

Preparation	Country†	Year obtained	IVIG treatment	NATs		
				HPeV1	HPeV3	HPeV6
Batch 1	Japan	2010, 2011	PEG	1:1,024	1:512	1:2,048
Batch 2	Japan	2010	Freeze-dried PEG	1:1,024	1:512	1:1,024
Batch 3-1	Japan	2011	Sulfonation	1:1,024	1:512	1:2,048
Batch 3-2	Japan	2011, 2012	Sulfonation	1:1,024	1:1,024	1:2,048
Batch 4	United States	2012	Ion-exchange resin	1:1,024	1:512	1:2,048
Batch 5	Germany	2012	pH4	1:2,048	1:512	1:2,048

*HPeV1, human parechovirus type 1; IVIG, intravenous immunoglobulin; NATs, neutralizing antibody titers; PEG, polyethylene glycol.
†Country where IVIG was obtained.

appears to be more pathogenic than HPeV1 and HPeV6 in neonates and young infants. When we used a cutoff of 1:4, seropositivity rates for HPeV3 and HPeV1 were significantly different ($p<0.001$). The different seropositivity rates at a lower cutoff might explain the differences in age distribution of children infected with HPeV1 compared with those infected with HPeV3, although no difference in seropositivity rates was found between HPeV3 (83%) and HPeV6 (84%) ($p = 0.885$).

We used LLC-MK2 cells for the viral assay of all HPeVs, including HPeV3, because we previously found that HPeV3 efficiently replicates in this cell line (*3*). HPeV3 seropositivity rates and the method of the neutralization assay were compared with those in previous reports (Table 3). A study in Japan's Aichi Prefecture (≈200 miles from Niigata) reported a HPeV3 seropositivity rate of 74% among 92 persons 15–39 years of age (*8*). Our results are consistent with these findings, although Vero cells were used in the previous neutralization test and the cutoff was set arbitrarily at 1:8. In contrast, a study found low seropositivity rates for HPeV3 (FI0688, a strain isolated from the stool sample of a healthy child in Finland) among adults in Finland (13%; n = 72) and the Netherlands (10%; n = 77) (*15*). Vero cells were also used in those neutralization assays, and the cutoff was set arbitrarily at 1:16. The difference found in these reports (*8,15*) might result from the different virus strains used in the neutralization tests. HPeV3–150237 (the clinical isolate in the Netherlands) was not neutralized by anti-HPeV3 A308/1999 (the first reported isolate from a fecal sample of a 1-year-old girl with transient paralysis in Japan) (*8*) antibody in vitro (*25*). Although the populations studied differ geographically, location might have no effect because HPeV3 circulates worldwide and HPeV3 strains in Japan and Europe are closely related (*26*).

The study in Finland and the Netherlands described antibody response after HPeV3 infection in 3 children whose individual responses varied: no response, a decrease after temporary elevation, and sustained elevation (*15*). In contrast, NATs were elevated and remained high after HPeV1 (*27*) and HPeV6 infection (*15*). However, the report from Finland and the Netherlands used isolation of virus from stool samples to diagnose HPeV3 infection, without confirmation of clinical manifestations. HPeV3 is detectable in stool samples of healthy children (*28*); therefore, the 3 patterns mentioned in that study might include such cases. To avoid confounding infection and virus detection, HPeV3 infection should be clinically suspected and then confirmed by PCR analysis of sterile samples (e.g., serum or CSF). A strength of our study is that HPeV3 infection was confirmed in all patients by PCR analysis of serum or CSF samples. Also, low NATs at disease onset were later elevated and remained high during convalescence of patients with HPeV3-related diseases. This pattern has also been observed in infections with viruses closely related to HPeVs, such as enteroviruses (*29*).

Our findings of high NATs to HPeV1, 3, and 6 in all IVIG preparations are consistent with the seropositivity rates in cord blood samples. In contrast, the NATs to HPeV3 contained in Dutch IVIG preparations were low (1:10–1:40), although NATs to HPeV1 were similar (1:1,280–1:2,560) (*25*). In that study, HPeV3–150237 and HPeV3 A308/1999 were used in the neutralization assay with Vero cells. However, we used HPeV3 A308/1999 with LLC-MK2 cells. The use of a different cell line in the neutralization assay might affect results.

A standard method should be established for the neutralization assay for HPeV3. Previous studies used Vero cells for HPeV3 and HT29 cells for HPeV1 and HPeV6

Table 3. Summary of rates of HPeV type 3 seropositivity and neutralization assay methods used in previous studies and in study in Niigata, Japan*

Study location	Seropositivity rate, % (no. samples)	Maternal age range, y	Cutoff	Cells used for viral culture	Virus strain
Aichi, Japan (*8*)	74 (92)	15–39	1:8	Vero	A308/1999
Finland (*15*)	13 (72)	21–40	1:16	Vero	FI0688
Netherlands (*15*)	10 (77)	16–60	1:16	Vero	FI0688
This study	61 (175)†	16–44	1:32	LLC-MK2	A308/1999

*HPeV, human parechovirus.
†Maternal antibody seropositivity rate.

(*15*,*25*) because of differences in cell tropism in the laboratory. Ideally, the same cell line should be used for assays for different HPeVs. Our findings indicate that using LLC-MK2 cells might be preferable in a neutralization assay for HPeVs because all 3 HPeVs replicate efficiently in LLC-MK2 cells and effects of using different cell lines can be excluded when interpreting results.

HPeV3 infection can cause severe diseases in neonates and young infants and may require intensive care and ventilator support. Patients with severe illness may need additional therapy because no current therapy is effective for HPeV-related diseases. One report described successful treatment with IVIG therapy for a 5-month-old boy with HPeV1 infection who developed severe myocarditis and dilated cardiomyopathy (*30*). Antibody titers increased after infection, and the IVIG contained high NATs. Currently, no data are available on the effectiveness of IVIG treatment for HPeV3 infection. In neonates with severe enteroviral infection, administration of IVIG containing NATs to the corresponding serotype resulted in rapid resolution of viremia and viruria because such patients have low NATs to the specific enteroviral serotype (*31*). Our findings suggest that IVIG treatment may be beneficial for patients with HPeV3 infection because these patients have low NATs, and IVIG preparations that are commercially available in Japan contain high NATs to HPeV3 in vitro. These high titers may help neutralize HPeV3 when used at an appropriate time during the course of infection. Future studies should investigate the efficacy of IVIG treatment for patients with HPeV3 infection, especially for patients with severe disease requiring intensive care or mechanical ventilation.

This study has some limitations. First, because cord blood samples were collected in only 1 hospital during the study period, GMT and HPeV seropositivity rates might not be generalizable to other regions of Japan or to other countries. Second, we used LLC-MK2 cells because of our previous favorable experience in isolating HPeV3 from clinical samples (*3*), and we chose HPeV3 A308/1999 because it is an HPeV3 reference strain (*2*). However, other cell lines and virus strains may need to be included in the system to evaluate differences in cell lines and viral strains. Third, we were unable to evaluate NATs of mother–infant pairs with or without HPeV3 infection. Maternal sampling with a control group may further clarify the contribution of maternally derived antibodies to severe disease related to HPeV3. Finally, follow-up samples from infants at ages 3 months and 6 months were collected from only some of the study participants because the study is ongoing.

We have shown a correlation between low titers of maternally derived antibodies to HPeV3 and development of severe diseases related to HPeV3 infection in neonates and young infants. Commercially available IVIG in Japan contains high titers to HPeV3 and thus might be an option for preventing or treating severe HPeV3-related diseases in this population.

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USA300 Methicillin-Resistant *Staphylococcus aureus*, United States, 2000–2013

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In the United States, methicillin-resistant *Staphylococcus aureus* (MRSA) with the USA300 pulsed-field gel electrophoresis type causes most community-associated MRSA infections and is an increasingly common cause of health care–associated MRSA infections. USA300 probably emerged during the early 1990s. To assess the spatiotemporal diffusion of USA300 MRSA and USA100 MRSA throughout the United States, we systematically reviewed 354 articles for data on 33,543 isolates, of which 8,092 were classified as USA300 and 2,595 as USA100. Using the biomedical literature as a proxy for USA300 prevalence among genotyped MRSA samples, we found that USA300 was isolated during 2000 in several states, including California, Texas, and midwestern states. The geographic mean center of USA300 MRSA then shifted eastward from 2000 to 2013. Analyzing genotyping studies enabled us to track the emergence of a new, successful MRSA type in space and time across the country.

Staphylococcus aureus is among the most common causes of bacterial infections in humans and probably has been a member of the human commensal flora for millennia (*1*). Chambers and Deleo identified serial "waves of resistance" in the history of 20th-century *S. aureus* epidemiology (*2*). They described the emergence of penicillin-resistant *S. aureus* in the 1940s and rapid spread during the 1950s and 1960s, initially in the health care setting and then in the community, as the first wave of resistance. With the introduction of semisynthetic antistaphylococcal penicillins, the first of which was methicillin in 1959, the second wave of resistance emerged with methicillin-resistant *S. aureus* (MRSA). Many of the early, or archaic, MRSA clones were related to the so-called "First MRSA" strain, which was later designated as sequence type (ST) 250 by multilocus sequence typing (MLST). These archaic MRSA clones caused health care–associated infections primarily in Europe until the 1980s. At that time, new strains of MRSA predominantly belonging to 5 clonal clusters (CC) (designated with MLST as CC8, CC22, CC5, CC45, and CC30) emerged worldwide, causing the third wave of resistance in *S. aureus* that continued into the 21st century (*2*).

Beginning in the late 1990s, new strain types of non–multidrug-resistant MRSA began to circulate outside the health care setting in the United States, a phenomenon seen even earlier in Australia (*3*). These community-associated MRSA infections, particularly skin and soft tissue infections, became common in the United States after 2000 (*4*). They constituted the fourth wave of resistance for *S. aureus*.

The community-associated MRSA strain USA300, which nearly always carries genes for the Panton-Valentine leukocidin (PVL) and the staphylococcal cassette chromosome *mec* (SCC*mec*) type IV, became the predominant strain type of MRSA circulating in the United States by 2011 (*5*). Community-associated MRSA infections, defined as infections in patients who lacked recent exposure to the health care setting, disproportionately affected children (*6–8*), incarcerated populations (*9–11*), underserved urban populations (*3*,*12*), and other specific groups (*13–17*). Early US reports on community-associated MRSA infections were published from Houston (*18*), Chicago (*7*,*19*) and elsewhere in the Midwest (*20*), Minnesota (*21*), Tennessee (*22*), Hawaii (*23*), and California (*9*,*11*). Soon after it began spreading in the community, USA300 became a common cause of infections in the health care setting as well, blurring the epidemiologic distinction between community-associated and health care–associated MRSA (*24*).

No national surveillance program exists that tracks the incidence of community-associated MRSA infections or the molecular epidemiology of MRSA infections more generally. However, anecdotally community-associated MRSA infections were less common on the US East Coast during the early part of the first decade of the 21st century. Single-center studies on the molecular epidemiology of MRSA isolates causing infections in the country became increasingly common after 1999 as new genotyping schemata were developed and as the price decreased for DNA sequencing. These included typing systems for pulsed-field

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gel electrophoresis (PFGE) by the Centers for Disease Control and Prevention (*25*), MLST (*26*), and *spa* typing (*27*).

Using the data available from the literature as a proxy for surveillance of USA300 as a proportion of circulating MRSA isolates, we set out to estimate the geographic spread of USA300 from initial reports of infections during 2000–2013 and compare it to the distribution of USA100 MRSA during that time. We compared USA300 to USA100 because they are the predominant MRSA strain types in the United States, and each is typically associated with different acquisition environments. USA300 is most often community associated, whereas USA100 is usually health care associated (*28*). The availability of extensive genotyping studies during the fourth wave of resistance enabled us to track the emergence of a new, successful strain type in space and time during a 14-year period.

Methods

Literature Review

We systematically reviewed the literature to identify all peer-reviewed publications, including genotyping information, on MRSA isolates. A PubMed search was conducted for citations related to MRSA published during 2000–2014 by using the following search criteria: ("2000/01/01"[date–publication]: "2014/04/01"[date–publication]) AND ((((((MRSA) OR ORSA) OR methicillin-resistant staphylococcus aureus) OR oxacillin-resistant staphylococcus aureus) OR methicillin-resistant staphylococcus aureus)). This search identified 18,615 possible articles for inclusion. A physician (M.Z.D.) reviewed the titles and abstracts of these 18,615 citations; citations were chosen for a review of the full text if we found evidence that genotyping was performed for the study. Genotyping modalities included in this assessment were MLST, PFGE if a reproducible and well-recognized system of nomenclature was used, *spa* typing, or direct repeat unit typing. This assessment resulted in the full-text evaluation of 3,389 articles published worldwide for genotyping information. These studies were then sorted by the country or countries from which reported MRSA isolates were collected. We excluded from further analysis studies with genotyping information about isolates exclusively from countries other than the United States, and all studies that included genotyping information about at least 1 isolate from the United States were further analyzed. We screened the references cited in the selected articles for additional publications, which were evaluated in full text to assess for inclusion in the study. To avoid duplication, we excluded from consideration studies that included only previously published isolates.

Data Abstraction

The search criteria identified isolates from 354 articles for inclusion. Data on 33,543 isolates were then abstracted into an Excel database (Microsoft, Redmond, WA, USA), hereafter called the MRSA TypeCat (an abbreviation of the MRSA Typing Catalogue). For each isolate, the geographic place of collection (city, state, or region of the country), year(s) of collection, source of the culture (specific animal species, human, or fomite), and any unique isolate identifier were recorded. Each isolate obtained from humans was recorded if it was obtained from a site of infection or from a culture assessing for asymptomatic colonization, if this information was available. The MRSA isolate was also recorded for human isolates obtained from a site of infection if we considered the infection to be a community-associated or health care–associated MRSA infection by clinical or epidemiologic criteria. Bibliographic information for each article was recorded, including the first author, the journal, year of publication, journal volume, page numbers, and PubMed unique identifier.

For each isolate, the following genotyping information was recorded if it was provided in the article: SCC*mec* type (with citation for the method used to determine SCC*mec* type); MLST type; *spa* type; coagulase type; direct repeat unit type; *agr* type; PFGE type; capsule type; the presence or absence of PVL; and the presence or absence of a marker for the arginine catabolic mobile element, which is frequently present in USA300 MRSA. Information was available for different combinations of typing schemes (Table).

Definitions

We defined USA300 as any isolate that was considered USA300 by PFGE or any isolate that was 1) PVL positive and 2) either *spa* type t008 or MLST type ST8. These last 2 criteria have an approximate specificity for USA300 of 95% and 98%, respectively (*29*). We defined USA100 as any isolate identified as USA100 by PFGE or any isolate with 1) SCC*mec* type II and 2) either *spa* type t002 or MSLT type ST5.

Table. Types of genotyping* data in the MRSA TypeCat from isolates, indicating number of isolates with data for each genotyping system or result, United States 2000–2013

Data	No. (%), N = 33,543
MLST	7,104 (21.2)
spa typing	7,466 (22.3)
PFGE (USA or USA-like)	22,846 (68.1)
dru Typing	78 (0.23)
SCC*mec* type	13,667 (40.7)
PVL PCR (total tested)	10,782 (32.0)
PVL PCR (positive)	7,370 (22.0)
ACME tested	2,393 (7.68)
Capsule type	70 (0.21)
Coagulase type	48 (0.14)
agr type	476 (1.42)

*Because MRSA isolates could have been subjected to >1 typing method, these categories are not mutually exclusive. ACME, arginine catabolic mobile element; dru, direct repeat unit; MLST, multilocus sequence typing; MRSA, methicillin-resistant *Staphylococcus aureus*; PFGE, pulsed-field gel electrophoresis; PVL, Panton-Valentine leukocidin; SCC, staphylococcal cassette chromosome.

For isolates obtained during a reported period spanning multiple years, the mean year of a study was calculated and was used as the year of collection. If an abstracted published study spanned an even number of years, the mean year was rounded up so that all isolates were assigned an integer year of collection. Eighty-two of the 354 analyzed published studies did not provide dates of collection for the reported MRSA isolates or had assigned years of collection before 2000; we discarded these from further analysis (2,289 isolates). Using these year assignments, we found the 5 most common Ridom *spa* types (*27*) and the 5 most common STs of isolates obtained during the 2000–2004, 2005–2009, and 2010–2013 periods (Figure 1).

Geographic Analysis

Studies were geocoded to the state in which the reported MRSA isolates were collected. We discarded 46 studies that used data from multiple states (i.e., in which no single US state of collection was reported for specific isolates) or that indicated only regional classifications (11,779 isolates). For each state in each study year, we calculated a proportion of isolates that were defined as either USA300 or USA100 to account for variable sample sizes in states over years.

We generated thematic maps of state-level USA300 and USA100 proportions for 2000–2013 to provide a sense of how these types varied spatially and temporally within the dataset. To visualize simultaneously USA300 and USA100 proportions, as well as the presence of non-USA300 or USA100 MRSA isolates, we created pie charts of these 3 categories for each state during each year.

To determine how the detection of USA300 varied over space and time, we calculated a weighted mean geographic center for 2-year nonoverlapping time increments for USA300 proportions (e.g., 2000–2001, 2002–2003). The weighted geographic mean center for each period is influenced by the states reporting MRSA during that time (influencing the starting location of the geographic center) and by the proportion of isolates defined as USA300 (how states are weighted in the calculation of the center).

Statistical Analysis

States were assigned to 1 of 4 US Census geographic regions (Northeast, South, Midwest, or West) to test for regional changes in USA300 proportion over time (*30*). Line graphs with percentage of USA300 and USA100 isolates in each year were generated, and a linear regression line was fit for each strain type. A Cochran-Armitage test for trend was implemented in SAS (SAS Institute, Inc., Cary, NC, USA) to assess whether the proportion of isolates in each of the 4 regions defined as USA300 or USA100, respectively, increased during the study period (2000–2013).

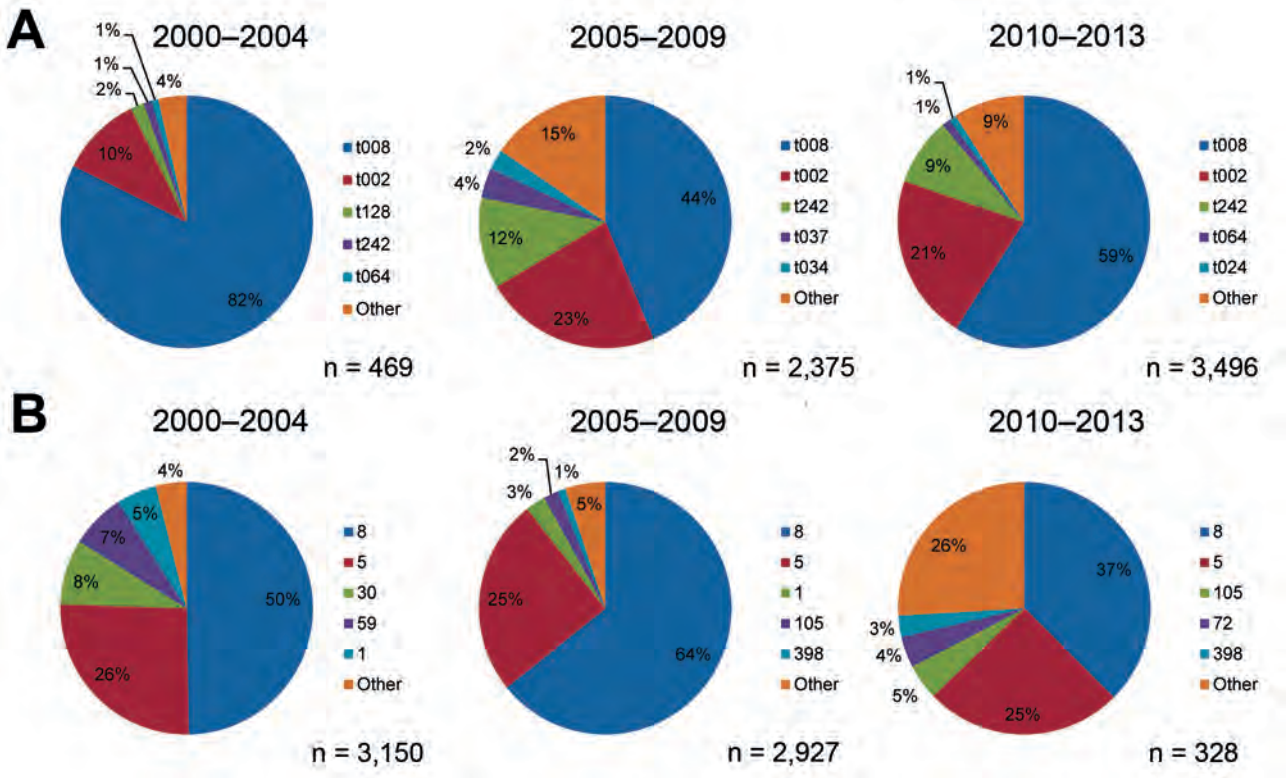

Figure 1. The most frequently reported *spa* types (A) and multilocus sequence types (B) for methicillin-resistant *Staphylococcus aureus* isolates obtained in 2000–2004, 2005–2009, and 2010–2013, United States.

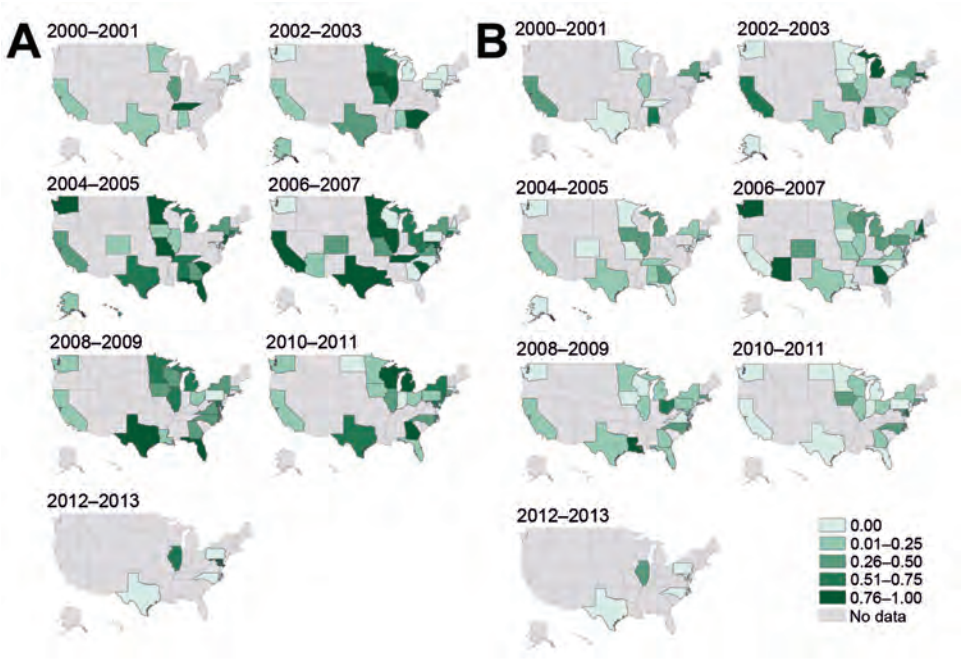

Figure 2. Proportions of methicillin-resistant *Staphylococcus aureus* isolates, United States 2000–2013. A) USA300 strain type. B) USA100 strain type. Darker shading indicates higher proportions of types reported in studies conducted during those years.

Results

Review of the identified 354 articles that included typed MRSA isolates with genotyping data identified 236 studies that included unique years of bacterial isolation and specified the US states where they were isolated. Within these 236 studies, 19,748 MRSA isolates were reported, of which 8,092 were classified as USA300 and 2,595 as USA100. Among isolates with any reported anatomic site of isolation, skin and soft tissue infections accounted for 62.6% of USA300 and 19.1% of USA100 isolates in studies that reported specific years and geographic locations and 58.8% of USA300 and 7.0% of USA100 in all studies, inclusive of those not reporting state locations or study years. Of all the USA300 and USA100 isolates in spatiotemporal analyses that reported a site of isolation, 38.5% of USA300 and 80.9% of USA100 were known invasive infections, whereas the full 33,543-isolate dataset designated 41.7% of USA300 and 93.0% of USA100 isolates as invasive infections.

Of isolates with reported *spa* types under the Ridom typing system, t008 and t002 were the dominant *spa* types during 2000–2004, 2005–2009, and 2010–2013 (Figure 1, panel A). Although t008 isolates made up the largest percentage during all 3 time groups, the share of isolates with this type decreased from 82% during the first period to 59% during the third, even as the total number of isolates with reported *spa* types increased. The share of isolates categorized as t002 rose from 10% during the first period to ≈20% during the 2 subsequent periods. ST8 and ST5 were the 2 most common MLST types reported during all 3 periods (Figure 1, panel B), as the number of isolates with reported MLST results declined during the study.

The proportion of MRSA isolates defined as USA300 increased during the study period and increased in some states earlier than others (Figure 2). Simultaneously, the proportion of isolates defined as USA100 decreased in many states.

Although USA100 isolates were reported during all years of the study period, pie charts indicate a gradual increase in the proportion of all isolates that were USA300 over time and space (Figure 3; online Technical Appendix, http://wwwnc.cdc.gov/EID/article/21/11/15-0452-Techapp1.xlsx). In addition, the proportion of studies reporting USA100 simultaneously declined, and a higher percentage of studies reported other types of MRSA.

The weighted mean geographic center of USA300 shifted from the start of the study period (2000–2001 studies) to the end of the study period (2012–2013 studies) (Figure 4). The general movement was from west to east; the early weight of USA300 isolates was heaviest in the Midwest, gradually moving toward the mid-Atlantic states during the study period. The mean center was pulled westward during 2002–2005 by studies performed in Alaska and Hawaii during those years.

When studies were assigned to 1 of 4 Census regions and the USA300 and USA100 proportions were charted over time (Figure 5), regional differences in the proportions of these 2 types differed by region. The number of isolates reported in each region also differed during the study. For the West, the general pattern over time was a series of peaks and troughs of USA300 reporting and a gradual decline in USA100. In the South and Northeast the general pattern over time was increasing USA300 proportions and

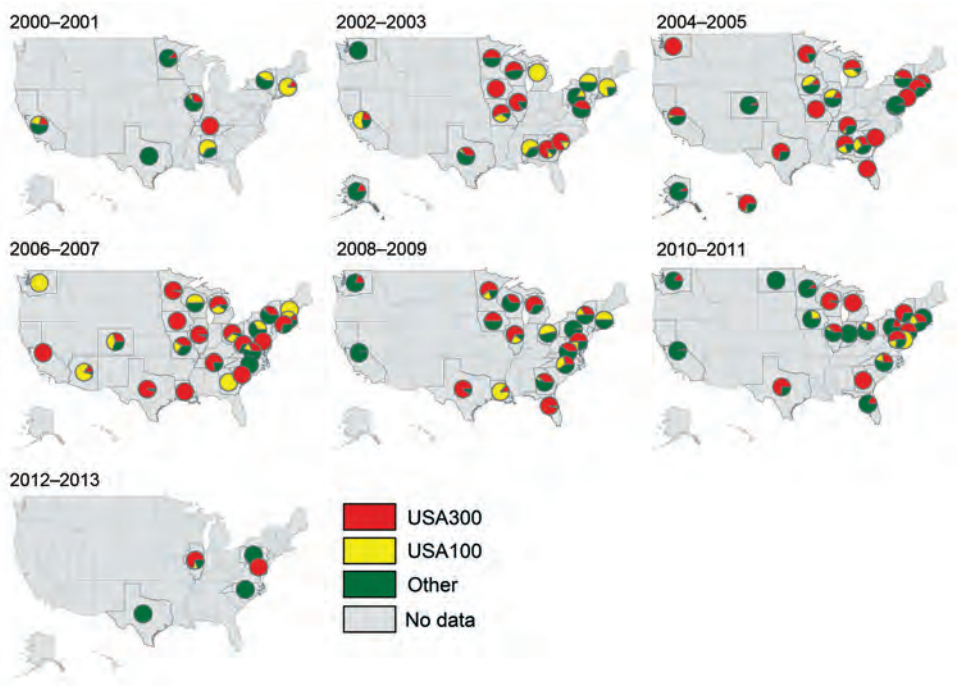

Figure 3. Proportions of methicillin-resistant *Staphylococcus aureus* isolates in each state that were defined as USA300, USA100, or other strain types, United States 2000–2013.

decreasing USA100 proportions. The decrease in USA100 appeared particularly sharp in the Northeast. By contrast, in the Midwest, both USA300 and USA100 gradually increased, although the share of USA300 percentages was consistently higher. Cochran-Armitage tests for trend indicated statistically significant ($p<0.0001$) trends in USA300 and USA100 across all 4 Census areas.

Discussion

We demonstrated that USA300 did not emerge simultaneously throughout the United States. It emerged earlier in the western part of the country and only later on the eastern seaboard. The published literature, used as a proxy for MRSA surveillance, suggests that USA300 appeared during 2000 in several states across the country, including California, Texas, and midwestern states. In subsequent years, USA300 constituted a large share of total reported MRSA isolates.

USA100, the predominant health care–associated MRSA strain type in the United States, in contrast, already constituted a larger proportion of reported MRSA isolates in the earlier years of the study in eastern US states. Over time, USA300 dominated among reported MRSA strain types in the Midwest and East. This finding is most clearly demonstrated by the focus of the geographic center of USA300 in Missouri/Illinois that gradually shifted toward the East during the latter years of the study.

Regionally, USA300 was present in higher proportions during the early years of the study in western states than other Census areas. Although Cochran-Armitage tests indicated statistically significant trends in all regions for both USA300 and USA100, trends toward an increase in the proportion of MRSA isolates that were USA300 were strongest in the Midwest and South, and large declines in USA100 proportions were observed in the Northeast, South, and West. These findings correspond to generally perceived but never formally tested hypotheses on the origins and spread of USA300 MRSA in the United States. The decline in the relative proportion of USA100 isolates reported during the study period most likely resulted from the increased attention to infection control in hospitals and a corresponding decrease in nosocomial health care–associated MRSA (*31*).

Our study is subject to several limitations. Most important, the data were not derived from a single sample of MRSA isolates collected prospectively or with equal representation of all states or regions. The data were derived instead from the extant literature in which authors chose to perform genotyping. Unlike an ideal prospective surveillance study that would include data selected to represent a sample of the entire population, we had available only published studies, which might have biased our results if in a given period. For example, if studies were more or less likely in 1 region of the country to focus on community-associated or health care–associated infections, if few studies were performed in a given region, or if smaller studies predominated in a given region compared with the rest of the country, our results could be biased. We attempted to correct for this lack of complete data by relying on all available data in each studied 2-year period to determine the

Figure 4. Weighted mean geographic center for proportions of methicillin-resistant *Staphylococcus aureus* (MRSA) USA300 strain type, United States, 2000–2013. This map shows the likely trend in the spread of USA300 as a proportion of all MRSA isolates that underwent genotyping, but the trajectory could be biased by large studies or lack of studies in certain states in specific years. The final mean center for 2012–2013 is represented differently to indicate that it is based on a small number of isolates.

geographic center of USA300 among MRSA isolates. The 2012–2013 center in particular was calculated from a small number of studies in the eastern portion of the country.

Second, although the articles included were identified through a rigorous literature review, additional published studies reporting a large number of isolates did not use any genotyping or did not use modalities of genotyping that met our inclusion criteria. Had these studies genotyped their reported isolates or genotyped them using methods that we chose to include, they might have altered the results of the study. Third, we eliminated from consideration in our analysis of geographic spread studies in which no US state of collection was named for studied MRSA isolates. This exclusion might have introduced error into our results, but we have no reason to believe that this error would systematically bias our findings because a specific region is unlikely to be overrepresented or underrepresented given this exclusion criterion. Fourth, although we made every attempt to exclude studies that reported previously published isolates, some isolates might been included more than once in our analyses. We identified specific strain names in the MRSA TypeCat whenever they were included in a published report to avoid repeated entries, but many authors did not identify specific isolate designations in their publications. Finally, many articles with genotyping information were not included because they provided inadequate typing data to identify USA300 or USA100 isolates by the criteria that we used to define these 2 strain types. Some articles, for example, identified isolates that bear the genetic determinants of PVL and SCC*mec* type IV, strongly suggesting a USA300 isolate, but we did not include these isolates in our analysis. We

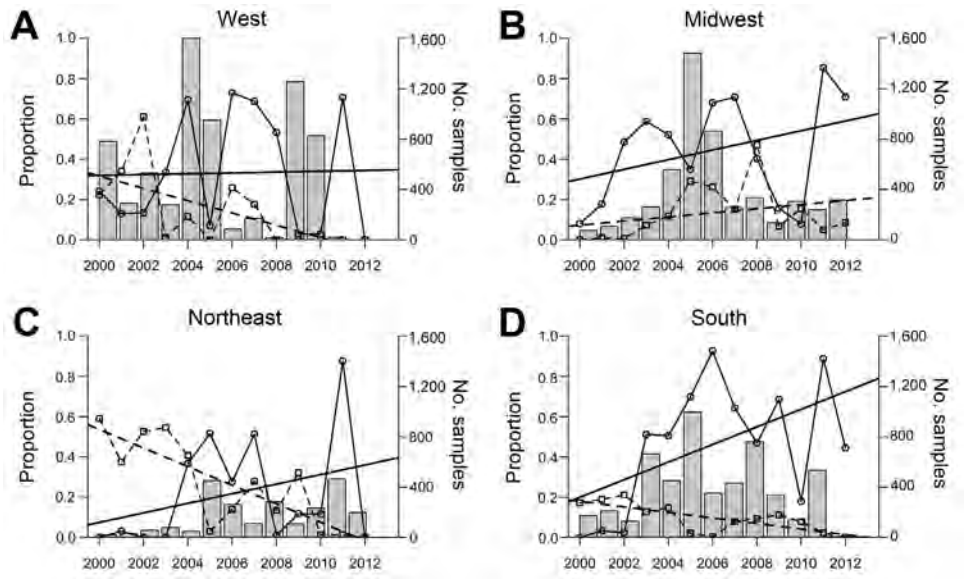

Figure 5. Proportion of methicillin-resistant *Staphylococcus aureus* USA300 and USA100 strain types and total sample size in 4 Census regions, United States 2000–2013. A) West. B) Midwest. C) Northeast. D) South. Linear regression lines are fit for each type. Solid line, USA300; dashed line, USA100.

believe that the criteria that we chose for USA300 isolates, although arbitrary, were appropriately conservative to avoid misclassification of other community-associated MRSA strain types as USA300.

Our study examined the geographic distribution of USA300, a strain type of MRSA that emerged in the late 1990s to cause the fourth wave of resistance in *S. aureus* and came to predominate as a cause of MRSA infections in the United States during the course of approximately a decade. The reasons for the geographic pattern of emergence of USA300 from west to east are not yet known. However, this study represents an attempt to document the movement of a successful epidemic strain type of MRSA geographically over a prolonged period from its earliest emergence to predominance among MRSA strain types in a country. Our study might provide a model for understanding the emergence of a future, novel, fit strain type of antimicrobial drug–resistant *S. aureus* that could make up the fifth wave of resistance in *S. aureus* and is particularly relevant given increased focus and funding from the US government on developing a national strategy to combat antimicrobial drug–resistant bacteria (*32*). Such a strategy should include a national surveillance program that can detect the regional emergence of virulent new strains to inform local empiric therapy.

Acknowledgments

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Molecular Epidemiology of Hospital Outbreak of Middle East Respiratory Syndrome, Riyadh, Saudi Arabia, 2014

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We investigated an outbreak of Middle East respiratory syndrome (MERS) at King Fahad Medical City (KFMC), Riyadh, Saudi Arabia, during March 29–May 21, 2014. This outbreak involved 45 patients: 8 infected outside KFMC, 13 long-term patients at KFMC, 23 health care workers, and 1 who had an indeterminate source of infection. Sequences of full-length MERS coronavirus (MERS-CoV) from 10 patients and a partial sequence of MERS-CoV from another patient, when compared with other MERS-CoV sequences, demonstrated that this outbreak was part of a larger outbreak that affected multiple health care facilities in Riyadh and possibly arose from a single zoonotic transmission event that occurred in December 2013 (95% highest posterior density interval November 8, 2013–February 10, 2014). This finding suggested continued health care–associated transmission for 5 months. Molecular epidemiology documented multiple external introductions in a seemingly contiguous outbreak and helped support or refute transmission pathways suspected through epidemiologic investigation.

Middle East respiratory syndrome (MERS) coronavirus (MERS-CoV) was first recognized as a cause of severe human respiratory disease in 2012 (*1*). As of June 19, 2015, a total of 1,338 confirmed cases of MERS and at least 475 MERS-associated deaths had been reported (*2*). Human zoonotic infections have largely been acquired in the Middle East. Imported cases in Europe, North America, Africa, and Asia have been linked to travel to the Middle East, occasionally with local secondary transmission (*2*).

Although human infections are zoonotic in origin, clusters of human-to-human transmission have been reported, particularly within households or health care settings (*3*–*6*). In an outbreak in Jeddah, Saudi Arabia, in 2014 involving multiple health care facilities, 255 laboratory-confirmed MERS cases were documented during a 2-month period, but intensified infection prevention measures in hospitals terminated that outbreak (*6*,*7*). Available genetic data for these patients showed that they were clustered, which suggested widespread transmission of related viruses (*6*). Of 191 symptomatic patients, 40 were health care workers (HCWs). For the remaining patients for whom data were available, most had some form of contact with a health care facility or patients with suspected MERS. Investigation of outbreaks in health care settings also identified asymptomatic and milder cases, especially in healthy young adults and HCWs with no underlying illnesses (*7*). Dromedary camels have been proposed as a source of human infection; however, the possibility of other reservoirs and intermediate hosts has not been excluded (*2*,*8*).

Molecular epidemiologic analysis of transmission was attempted for a 2013 MERS outbreak at multiple health care facilities in the eastern region of Saudi Arabia (*5*). Combined analysis of genomic and epidemiologic data provided insights into transmission chains that would otherwise not have been apparent. The study on the 2014 Jeddah outbreak included analysis of viral sequences from 2 hospitals in Riyadh and identified a cluster of infections at the Prince Sultan Military Medical City (PSMMC) during March–April 2014 (*6*). In this study, we analyzed viral genetic data for patients and HCWs with MERS at King Fahad Medical City (KFMC), Riyadh, Saudi Arabia, during February 1–May 31, 2014, and available epidemiologic data to better understand transmission within the hospital and place the outbreak in KFMC in the context of contemporaneous MERS outbreaks in other hospitals in Riyadh.

Materials and Methods

Clinical Setting

KFMC is a 1,200-bed tertiary care hospital in Riyadh that comprises 4 hospitals and 4 medical centers on 1 campus. The main hospital is affiliated with specialized women's, children's, and rehabilitation hospitals. The 4 centers are the National Neuroscience, Heart, Oncology, and Diabetes

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[1]These authors contributed equally to this article.

centers. The main hospital, affiliated hospitals, and centers provide nationwide referral services. This study was approved by the Institutional Review Board of KFMC.

The emergency department (ED) is located on the ground floor of KFMC. It accepts patients from throughout Saudi Arabia; in 2014, there were 139,173 recorded visits. Time in the ED is usually brief, but some patients might have extended ED stays depending on availability of isolation rooms in the wards.

Medical wards (MWs) MW-C and MW-D, which have 50 beds combined (online Technical Appendix 1 Figure 1, http://wwwnc.cdc.gov/EID/article/21/11/15-0944-Techapp1.pdf), are located in the main hospital and admit patients from the ED, outpatient clinics, and referrals from elsewhere in Saudi Arabia. Most rooms in these 2 adjacent wards have 4 beds. However, MW-C has 6 isolation rooms, 2 with negative pressure ventilation, and MW-D has 4 isolation rooms, none with negative pressure ventilation. Patients are occasionally moved between the 2 wards, but nurses work only in their assigned wards.

Patients and Specimens

Patients, including HCWs, confirmed to have MERS diagnosed at KFMC during February 1–May 31, 2014, composed the study population. Nasopharyngeal swab specimens and tracheal aspirates or bronchoalveolar lavages were collected for viral diagnosis. A case of MERS, according to the Saudi Arabian Ministry of Health definition, was fever and acute respiratory illness in a patient who had a positive test result for MERS-CoV infection. Criteria for investigation of patients and HCW for MERS-CoV is provided in online Technical Appendix 1.

Laboratory Diagnosis

A reverse transcription PCR diagnostic kit (MERS-Coronavirus EMC Orf1a and SA1 EMC upstream E-gene, Light Mix Modular Assays; TIB MOLBIOL, Adelphia, NJ, USA, and Roche, Mannheim, Germany) was used for the screening and confirmation of MERS-CoV infection. Each sample was also tested simultaneously for 15 respiratory viruses (influenza A and B; parainfluenza viruses 1, 2, 3, and 4; respiratory syncytial virus; adenovirus; enterovirus; human metapneumovirus; human coronaviruses 229E, OC43, NL63 and HKU-1; and human bocavirus) by using the Seeplex RV15 ACE Detection Kit (Seegene Inc., Seoul, South Korea). Samples from the early phase of the outbreak were tested for MERS-CoV at the Ministry of Health laboratories; midway into the outbreak, KFMC developed in-house MERS-CoV testing capability.

Epidemiologic Data

Patient demographics and epidemiologic data on study participants were collected by retrospective chart review, from electronic health records, and from leave or sick leave records of staff. Patients with confirmed cases of MERS were spatiotemporally mapped within the hospital. Additional contact histories were obtained through direct interviews with the infected HCWs or patients. On the basis of date of hospital attendance or admission, date of onset of illness, and reported incubation period for MERS (median 5 days, range 2–14 days) (*9*), the patients were classified into those acquiring infection outside KFMC (externally acquired), long-term patients acquiring infection while at KFMC (long-term patients) and HCWs working at the hospital. HCWs were presumed to have acquired nosocomial infections at KFMC, although infection outside the hospital could not be excluded.

Potential transmission links were identified on the basis of patients or HCW present or working in the same ward or ED concurrently with a MERS patient. Given the retrospective nature of this study, it was not possible to assess whether HCW exposures occurred without use of adequate personal protective equipment (PPE).

Genetic Sequencing and Phylogenetic Analysis

cDNA was synthesized by using gene-specific primers for different regions of the MERS-CoV genome and subsequently subjected to multiple sets of PCR that covered the entire virus genome (primers available on request). Overlapping PCR products generated were sequenced by using MERS-CoV–specific primers. Sequences (without primer sequences) were aligned and assembled by using Geneious version 8.0.5 (http://www.geneious.com). Genomes were sequenced with $\geq$3–5 times coverage.

A time-resolved phylogenetic tree was estimated from a concatenated gene alignment of MERS-CoV genome by using BEAST version 1.8 (http://beast.bio.ed.ac.uk/). Analysis was conducted by using a general time-reversible model and gamma-distributed sites with separate rates for the 3 codon positions under a relaxed lognormal clock model.

Results

Descriptive Epidemiology

The number of specimen tested for MERS-CoV in March, April, and May 2014, were 3, 222 and 1,731, respectively, increasingly markedly during the course of the outbreak. During the study period, 45 patients at KFMC had virologically confirmed MERS. Eight of these patients had externally acquired infections, and 13 long-term hospitalized patients had nosocomial infections; 23 HCWs had MERS-CoV infections, presumably acquired at KFMC. Patient EA-9 (disease onset May 5, first ED visit May 1) might have been infected either at KFMC or at an external source.

Enhanced surveillance identified 4 asymptomatically infected HCWs. Disease onset dates of different patient groups are shown in Figure 1. Thirteen patients died of their infections: 3 of 8 patients with externally acquired infections, 9 of 14 long-term hospitalized patients, and 1 of 23 HCWs. MERS-CoV–infected HCWs had a median age of 35.5 years (range 24–58 years); non-HCWs had a median age of 60 years (range 12–77 years) ($p<0.005$). Demographic characteristics of all patients and work locations of infected HCWs are shown in online Technical Appendix 1 Table 1.

Viral Genetic Analysis

To investigate virus introduction and intrahospital transmission pathways, available archived respiratory specimens from 15 patients who had high viral loads were obtained and genetically analyzed. Whole-genome sequences were obtained from 10 patients and a partial genome was obtained from 1 patient (Table; online Technical Appendix 1 Table 2) (GenBank accession nos. KT121572–KT121581 and KT202801).

A time-resolved phylogenetic tree (Figure 2) shows whole-genome sequences from these 10 patients within the context of other available MERS-CoV whole-genome sequences. Nodes A, B, and C have strong statistical support in this time-resolved phylogeny and in a separate maximum-likelihood phylogenetic tree of these same sequences (aBayes branch support) (*10*). Phylogenetic analysis suggests that patients at KFMC were part of a larger outbreak of MERS that was ongoing in Riyadh at that time, involving, but perhaps not limited to, other hospitals, such as PSMMC and King Khalid University Hospital (KKUH). The dated phylogeny suggests that a putative zoonotic event (node A in Figure 2) occurred on approximately December 31, 2013 (95% highest posterior density (HPD) interval November 8, 2013–February 10, 2014), although the possibility of separate zoonotic events for closely related viruses cannot be excluded.

Virus isolate KFMC-9 clusters phylogenetically with viruses from KKUH and separately with other viruses from KFMC. This isolate has a signature mutation (C26144T KFMC-9) that is also present in KKUH-90b, KKUH-291, and KKUH-368 isolates, indicating that patient from which this virus was isolated was infected with a virus related to those in the ongoing outbreak at KKUH (Figure 3; online Technical Appendix 2, http://wwwnc.cdc.gov/EID/article/21/11/15-0944-Techapp2.xlsx). The ancestral node B has strong statistical support (posterior density ≥ 0.95) and an estimated date of January 28, 2014 (95% HPD interval December 16, 2013–February 27, 2014), which is long before the date of onset of the first known case of MERS at KFMC (March 29, 2014) (Table). Node C in the dated phylogeny (Figure 2) also has strong statistical support, and an estimated date for this node was February 15 (HPD interval January 10–March 16) which is also before the date of disease onset of the first known patient in the outbreak at KFMC. Thus, it is likely that there were multiple introductions of MERS-CoV to KFMC to account for the observed virus genetic diversity in the patients studied at KFMC.

Viruses in node A in the phylogenetic tree have a nucleotide substitution rate of 6.54×10^{-4} nt substitutions/site/year (genome length analyzed 29,897 kb), which is comparable to a previously reported value of 6.3×10^{-4} (*5*). Estimated ancestral sequence at nodes C and E (identical)

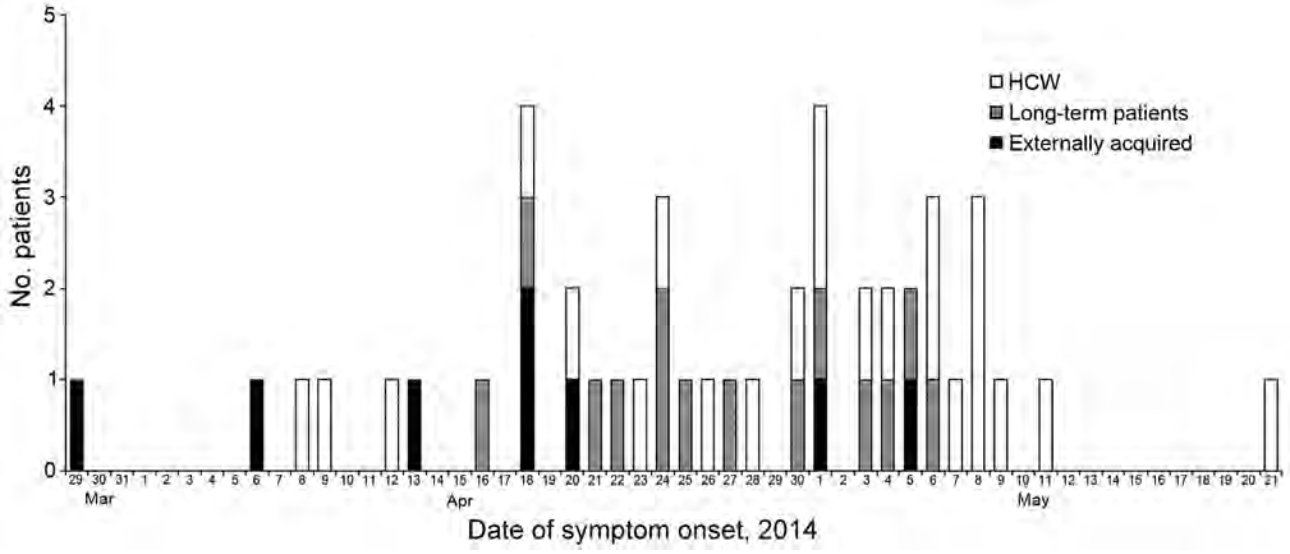

Figure 1. Date of symptom onset for patients with confirmed Middle East respiratory syndrome coronavirus (MERS-CoV) infection hospitalized at King Fahad Medical City, Riyadh, Saudi Arabia, 2014. For 4 asymptomatic health care workers (HCWs) detected by screening, date of virus detection, rather than symptom onset, is indicated.

Table. Characteristics of 21 patients tested for infection with MERS-CoV, King Fahad Medical City, Saudi Arabia, 2014*

Patient	Age, y/sex	Date of illness onset	Date of first ED visit	Date of hospitalization	Patient group	Outcome
Externally acquired infections						
EA-1	32/M	Mar 29	Apr 5	Apr 6	Patient	Recovered
EA-2	65/F	Apr 6	Apr 11	Apr 12	Patient	Deceased
EA-3	46/F	Apr 13	Apr 20	Apr 21	Patient	Recovered
EA-4	70/M	Apr 18	Apr 22	Apr 28	Patient	Deceased
EA-5	64/M	Apr 18	Apr 27	Apr 28	Patient	Recovered
EA-6	22/F	Apr 20	Apr 27	Apr 28	Patient	Recovered
EA-7	28/F	May 1	May 2	Transferred	Patient	Transferred
EA-8	21/F	May 5	May 8	May 9	Patient	Deceased
EA-9†	50/F	May 5	May 1	May 3	Patient	Deceased
Nosocomial infections						
KFMC-0	34/F	Apr 9	Apr 16	Apr 17	ED nurse	Recovered
KFMC-1	45/F	Apr 20	Apr 29	May 2	ED nurse	Deceased
KFMC-2	60/F	Apr 25	Apr 4	Apr 5	Patient	Deceased
KFMC-3	62/F	Apr 27	Feb 1	Jan 12	Patient	Deceased
KFMC-4	63/F	May 1	Apr 21	Apr 22	Patient	Deceased
KFMC-5	56/F	May 3	May 10	May 12	Nurse, MW-D	Recovered
KFMC-6	74/F	May 6	Mar 19	Mar 21	Patient	Transferred
KFMC-7	36/F	Apr 26	Apr 30	May 3	Nurse, MW-C	Recovered
KFMC-8	53/F	Apr 30	Mar 27	Mar 28	Patient	Recovered
KFMC-9	29/M	May 1	May 7	May 9	ED nurse	Recovered
KFMC-10	46/F	Apr 23	Apr 30	May 5	Nurse, MW-C	Recovered
KFMC-11	41/F	Apr 24	Apr 27	Apr 30	Nurse, MW-C	Recovered

*MERS-CoV, Middle East respiratory syndrome coronavirus; ED, emergency department; KFMC, King Fahad Medical City; MW, medical ward.
†This patient visited the ED on May 1 for another illness and was hospitalized on May 3. MERS-related symptoms developed on May 5 while she was hospitalized. The incubation period was compatible with either externally acquired or nosocomial infection.

in the dated phylogenetic tree and nucleotide substitutions observed in virus sequences obtained in this study, together with virus sequences from patients in KKUH and PSMMC hospitals that appear to be related to this outbreak, are shown in Figure 3 and online Technical Appendix 2.

We tested the hypothesis that KFMC-7, KFMC-8, and KFMC-10 viruses diverged from the ancestral virus after April 5, 2014, the date that patient EA-1 came to the ER. Observed nucleotide differences were greater than would be expected if KFMC-7, KFMC-8, and KFMC-10 diverged at KFMC after April 5, suggesting that ≥1 of these 3 viruses were transmitted separately to KFMC (online Technical Appendix 1 Table 3). Conversely, KFMC-1–6 viruses had expected mutation rates, in accordance with observed phylogeny. Node E (including viruses KFMC-1–6) was less robust, but had an estimated date of April 4 (HPD interval March 9–April 25), which as an entry point for transmission at KFMC is more plausible with observed epidemiologic data. Viruses KFMC 1–6 had ≤1 nt difference between them for 29,897 nt sequenced, and the zoonotic time span between the oldest and newest virus specimens was 20 days (online Technical Appendix 2). The partial genome sequence for KFMC-11 is also identical with that of KFMC-1–6 or KFMC-7. However, this partial sequence, although 5,225 nt, cannot optimally resolve transmission pathways.

Epidemiologic Data

Ward locations and patient data are shown in online Technical Appendix 1 Figure 2, and layout of key wards is shown in online Technical Appendix 1 Figure 1. Before admission to KFMC, patient EA-1, the first patient to be identified during the outbreak at KFMC, had regularly visited his father, who was hospitalized at PSMMC, where a MERS outbreak was ongoing.

On the basis of known incubation periods, onset of illness, and presence at the same location (online Technical Appendix 1 Figure 2), the ER was a plausible venue for MERS-CoV transmission from patient EA-1 to KFMC-0 and from patient KFMC-0 to patient KFMC-1. Patients KFMC-0 and KFMC-1 were co-workers in the ER, and patient KFMC-1 provided care for patient KFMC-0 when she was ill in the ER. Patient KFMC-1 also provided care for patient KFMC-0 without PPE in the staff quarters when she was on sick leave (April 15). There were no archived specimens from patients EA-1 and KFMC-0. Patient KFMC-1 was the first patient from this outbreak from whom we have virus genomic data.

Patient KFMC-0 was subsequently treated in MW-C where long-term patients KFMC-2 (illness onset April 25), KFMC-4 (illness onset May 1), and KFMC-6 (illness onset May 6) became ill during April 4–May 3 and were hospitalized, and viruses closely related to the virus from the KFMC-1 cluster (online Technical Appendix 1 Figure 2) were isolated. Patient KFMC-3 was a chronically ill long-term patient in MW-C. A respiratory infection developed, and infection with influenza A(H1N1)pdm09 virus was detected in respiratory specimens on April 27. She was discharged on April 4 but was readmitted on May 6 because of deteriorating respiratory function and was

subsequently given a diagnosis of MERS-CoV infection. Retesting of a predischarge respiratory specimen collected on April 30 showed MERS-CoV infection. Thus, patient KFMC-3 probably had MERS a few days before the testing date. However, the exact onset of illness could not be determined. Patient KFMC-3 used the intensive care unit bed previously used by patient EA-1 on April 15.

Nurse KFMC-5, who worked in MW-D, had disease onset on May 3. Virus isolated from her specimen was closely related to the cluster of viruses isolated in MW-C. Although this nurse had no duties in MW-C, MW-C and MW-D are adjacent general medical wards on the same hospital floor (online Technical Appendix 1 Figure 1).

Genetic analysis suggested that viruses from patient KFMC-9, KFMC-7, KFMC-8, and KFMC-10 were introduced separately into KFMC. Patient KFMC-9 worked in the ER and patients EA-6 and EA-3, who acquired MERS outside the hospital, were admitted to the ER 4 and 11 days, respectively, before onset of disease in patient KFMC-9, which indicated that patients EA-6 and EA-3 were possible sources of infection for patient KFMC-9. Patient EA-2 was hospitalized in a 4-bed room in MW-C where nurses KFMC-7 and KFMC-10 worked. In addition, patient KFMC-8 was a long-term patient in the same ward, which provided opportunities for introduction of a genetically distinct virus (online Technical Appendix 1 Figure 2).

Discussion

We describe a hospital-associated outbreak of ≥45 MERS-CoV infections that occurred at KFMC, Riyadh, Saudi Arabia, during March–May 2014. There appears to be a periodicity in peaks of transmission ≈7 days apart, which is

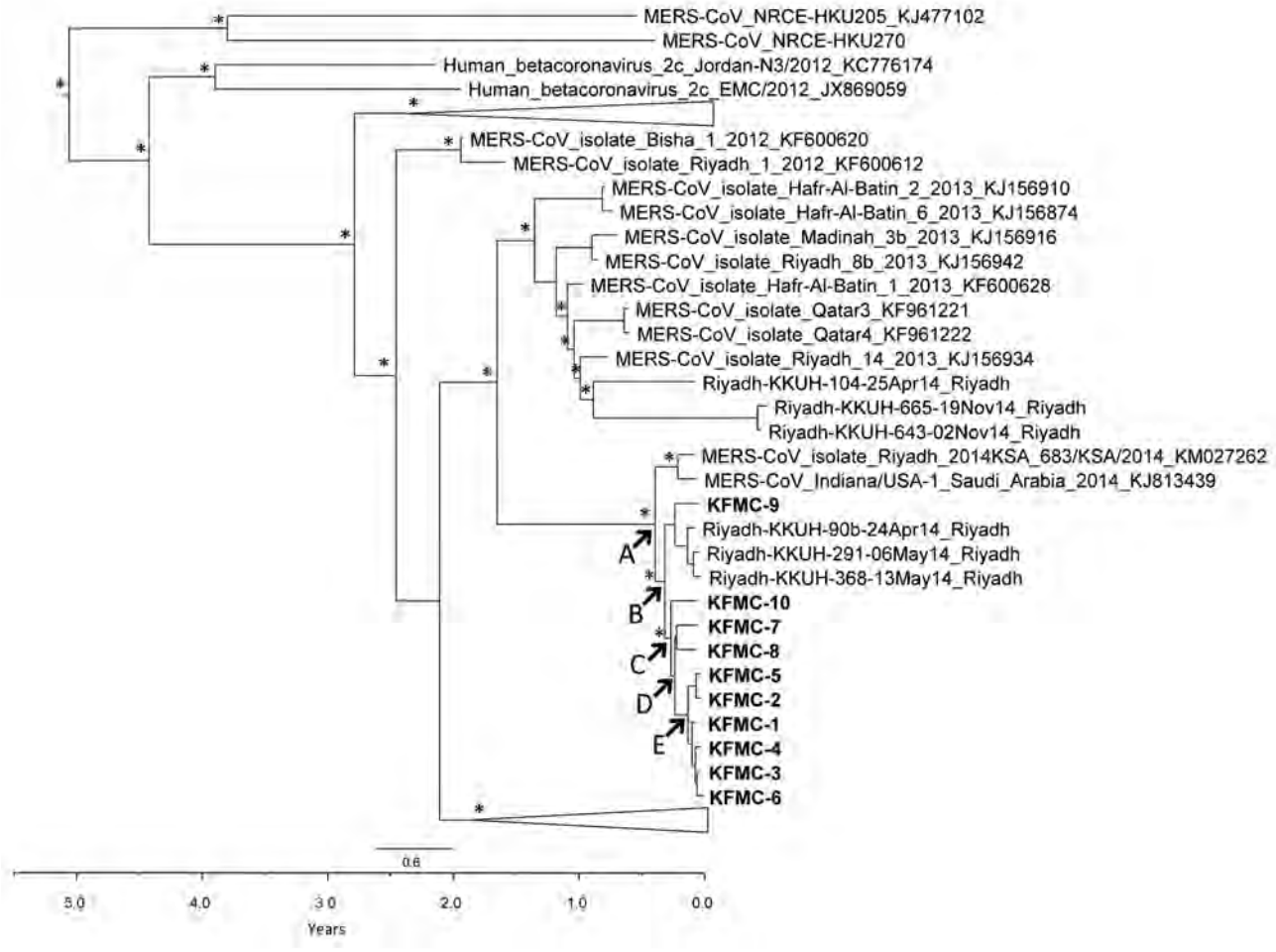

Figure 2. Time-resolved phylogenetic tree of Middle East respiratory syndrome coronavirus (MERS-CoV) genomes, Saudi Arabia, 2014, constructed by using BEAST version 1.8 (http://beast.bio.ed.ac.uk/). Upper scale bar indicates nucleotide substitutions per site. Lower scale bar indicates years in reference to sample KFMC-6 (collected May 18, 2014). Genomes sequenced in this study are indicated in bold. *Indicates major nodes with posterior probabilities >0.95. Estimated median dates for nodes A, B, C, D, and E (95% highest posterior density intervals) are A) Dec 31, 2013 (Nov 8, 2013–Feb 10, 2014), B) Jan 28, 2014 (Dec 16, 2013–Feb 27, 2014), C) Feb 15, 2014 (Jan 10, 2014–Mar 16, 2014), D) Feb 26,2014 (Jan 23, 2014–Mar 25, 2014), E) Apr 4, 2014 (Mar 9, 2014–Apr 25, 2014). KKUH, King Khalid University Hospital; KFMC, King Fahd Medical City.

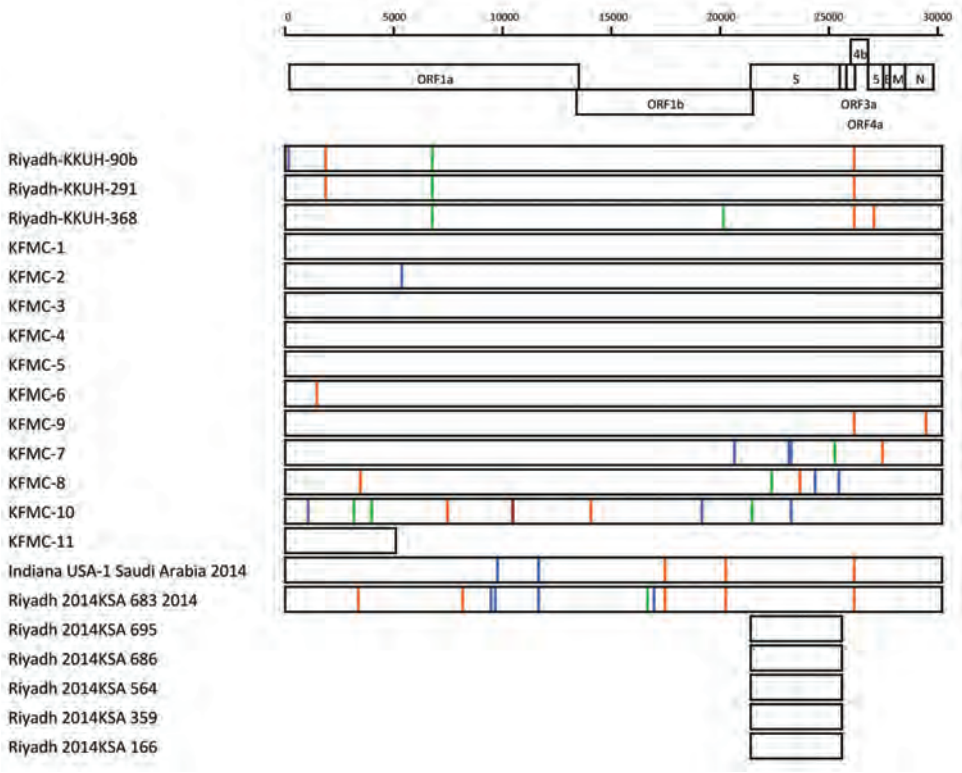

Figure 3. Nucleotide differences from consensus ancestral sequences of Middle East respiratory syndrome coronavirus (MERS-CoV), Saudi Arabia, 2014, estimated at nodes C and E in a time-resolved phylogenetic tree (Figure 2). The region of the genome sequenced is indicated by the length of each box. Exact genome polymorphic nucleotide positions, sampling date, and nucleotide substitutions is shown in online Technical Appendix 2 (http://wwwnc.cdc.gov/EID/article/21/11/15-0944-Techapp2.xlsx). Nucleotide changes are indicated by red (A), orange (T), blue (C), and green (G) vertical bars. ORF, open reading frame; KKUH, King Khalid University Hospital; KFMC, King Fahad Medical City; KSA, Kingdom of Saudi Arabia.

compatible with the known incubation period and case-to-case serial interval reported to be 7.6 days (*4*).

Before this molecular epidemiologic study, the assumption was that the outbreak at KFMC was self-contained and originated from patient EA-1, independent of other outbreaks reported in Riyadh. Viral genomic data obtained during this study generated alternative hypotheses and show that the outbreak of KFMC was linked to ongoing transmission within health care facilities in Riyadh at that time, including, but probably not limited to, PSMMC and KKUH. Data suggest a single zoonotic event that occurred around December 31, 2013 (95% HPD interval November 8, 2013–February 10, 2014), followed by transmission in health care facilities for ≈5 months. However, an alternative possibility of multiple, independent spillover events from closely related viruses in a zoonotic reservoir cannot be excluded. This chain of transmission was spread as far as Indiana in the United States by an HCW from Riyadh (*11*) and 2 travelers returning to the Netherlands (*12*). Viral sequence data for viruses from the 2 travelers was fragmentary and excluded from phylogenetic analysis. However, this cluster of human MERS-CoV in Riyadh was distinct from the large contemporaneous cluster of human-to-human transmission that occurred in Jeddah and represents a separate zoonotic transmission event (*6*). Only 1 of the analyzed sequences from the Riyadh cluster has an amino acid change in the receptor binding domain of the spike protein (*13*), the C23,697T nonsynonymous mutation in KFMC-8, which leads to an R®C amino acid change.

In this outbreak, 36 cases of MERS-CoV infection were putatively acquired through nosocomial transmission. However, given ongoing human-to-human transmission in Riyadh, it cannot be ruled out that some HCWs acquired infection from outside KFMC. Molecular epidemiology indicates 1 definite cluster of transmission associated with KFMC-1–like viruses, which are genetically closely related (KFMC-1–6). There are plausible epidemiologic links for transmission from patient EA-1, the first known patient admitted to KFMC in 2014, in the ER to patient KFMC-0, then to patient KFMC-1, and to patients KFMC-2–KFMC-6. Because no virus sequence data was available patients EA-1 or KFMC-0, the role of these 2 persons in the transmission chain remains presumptive. The nearly identical virus genetic sequences for KFMC-1, -2, -3, -4, -5, and -6 and plausible epidemiologic exposures provide more definite pathways of transmission (online Technical Appendix 1 Figure 2). Although virus KFMC-2 has 1 unique nucleotide substitution (T5321C), that sequence derives from a specimen collected late in the patient's illness and might have originated in her after she transmitted infection to patients KFMC-4 and KFMC-6.

Genetic identity of virus KFMC-3 with viruses in the KFMC-1 cluster led to reassessment of the assumption that infection of patient KFMC-3 was externally acquired

infection. Retesting of 2 archived (April 2014) specimens, 1 of which was positive for influenza A(H1N1)pdm09 virus, showed that patient KFMC-3 was nosocomially infected with influenza A(H1N1)pdm09 virus and MERS-CoV before her discharge on May 4, and this MERS-CoV was closely related to the KFMC-1 virus group. The source of infection for patient KFMC-3 was unclear. This patient used the intensive care unit bed used by patient EA-1 on April 15, and patient KFMC-8 occupied the isolation room vacated by patient EA-2, which raised the possibility of fomite transmission or transmission associated with HCW cases not detected by the surveillance system.

Although epidemiologic linkages would have led us to deduce that patient KFMC-9 may have acquired infection from the KFMC-1 virus cluster, viral genetic analysis conclusively demonstrates that this was a separate introduction into KFMC through a person with an externally acquired infection with a virus closely related to viruses at KKUH. Molecular epidemiology also demonstrated that virus KFMC-7, KFMC-8, and KFMC-10 were not linked to viruses in the KFMC-1 cluster, although there were plausible epidemiologic links with patients infected with viruses from the KFMC-1 cluster. These 3 infections might have resulted from 1, 2, or 3 independent virus introductions from outside KFMC.

Our data suggest that the ER and MW-C at KFMC were major foci of transmission. Although findings are not conclusive, HCWs with mild upper respiratory illness who continued to work might have contributed to transmission. Many of these issues were addressed during and after this outbreak, including, but not limited to, enhancing awareness of MERS through electronic communication, establishing in-house capacity for rapid MERS-CoV testing, active screening of KFMC staff who had influenza-like symptoms through a dedicated influenza clinic, establishing a triage area for patients in the ED, designation of wards for isolation and screening of suspected MERS cases, and strengthening infection control practices among staff by mandatory training.

Our study had limitations. Archived respiratory specimens from patients with MERS acquired outside KFMC (EA-1–EA-9) were unavailable for genomic analysis, which caused us to make assumptions in our putative chains of transmission. Some of the retrospectively retrieved epidemiologic data were obtained through interviews with HCWs and patients 1 year after the outbreak. For example, data on PPE use and extent of exposure to individual MERS-infected patients was difficult to establish with confidence. Thus, risk factors or modes of transmission (i.e., roles of large or small droplets, contact) could not be established. Dates and ward locations of patients and staff were available from the electronic medical record systems at KFMC, and we relied on proximity analysis (e.g., patients being co-housed in the same ward or nursed by the same nursing team members as other known patients with MERS) to provide epidemiologic context to the molecular epidemiologic data.

In summary, we provide molecular epidemiologic data derived from complete virus genome genetic analysis that is suggestive of a large MERS outbreak involving multiple health care facilities in Riyadh, suggesting ongoing human-to-human transmission over many months. Using molecular analysis supplemented by available epidemiologic data, we identified MERS-CoV transmission within a large health care facility and demonstrated the feasibility and value of complete viral genome sequence analysis in outbreak investigations. We showed that what was seemingly a contiguous outbreak within KFMC was caused by multiple introductions of virus from outside the hospital. The small number of mutations observed across the 29,897-nt genome analyzed during this outbreak emphasizes the need for complete genome analysis if molecular epidemiology is to be meaningful in such settings. The ongoing outbreak of MERS in South Korea (*2*), the largest cluster of transmission from a returning traveler to date, highlights the ongoing threat from MERS and the need for understanding pathways of transmission. Detailed molecular epidemiology can contribute to these efforts and thus help minimize transmission.

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Climatic Influences on *Cryptococcus gattii* Populations, Vancouver Island, Canada, 2002–2004

Christopher K. Uejio, Sunny Mak, Arie Manangan, George Luber, Karen H. Bartlett

Vancouver Island, Canada, reports the world's highest incidence of *Cryptococcus gattii* infection among humans and animals. To identify key biophysical factors modulating environmental concentrations, we evaluated monthly concentrations of *C. gatti* in air, soil, and trees over a 3-year period. The 2 study datasets were repeatedly measured plots and newly sampled plots. We used hierarchical generalized linear and mixed effect models to determine associations. Climate systematically influenced *C. gattii* concentrations in all environmental media tested; in soil and on trees, concentrations decreased when temperatures were warmer. Wind may be a key process that transferred *C. gattii* from soil into air and onto trees. *C. gattii* results for tree and air samples were more likely to be positive during periods of higher solar radiation. These results improve the understanding of the places and periods with the greatest *C. gattii* colonization. Refined risk projections may help susceptible persons avoid activities that disturb the topsoil during relatively cool summer days.

Opportunistic fungal infections, such as those caused by *Cryptococcus neoformans*, are common causes of death and illness among persons with compromised immune systems. *C. gattii* is a related fungus that can cause serious illness. Specific genotypes (AFLP4/VGI, AFLP6/VGII) are isolated more commonly from immunocompetent persons, and other genotypes (AFLP5/VGIII, AFLP7/VGIV and AFLP10/VGIV) are isolated more commonly from immunocompromised persons. In 1999, a *C. gattii* genotype that had previously been reported in Brazil and Colombia was first documented on Vancouver Island in the province of British Columbia, Canada (*1*,*2*). The environmental genotypes in British Columbia are primarily VGIIa (AFLP6A, serotype B), VGIIb (AFLP6B, serotype B), and more rarely VGI (AFLP4, serotype B). In 2004, the fungus was identified in the Pacific Northwest region of the United States, and subsequently, *C. gattii* infections have been detected in 8 additional US states (*3*,*4*). Globally, the highest rates of *C. gattii* cryptococcosis incidence among humans and animals and the highest rates of positive environmental samples are reported from Vancouver Island (*5*,*6*). The natural habitat of this fungus seems to be a broad range of native trees and the surrounding soil (*2*,*7*,*8*). The epidemiology, nomenclature, historical climate, and population dynamics of *C. gattii* are summarized in the online Technical Appendix (http://wwwnc.cdc.gov/EID/article/21/11/14-1161-Techapp1.pdf) and Table 1.

In general, previous studies examined seasonal versus short-term (e.g., monthly) *C. gattii* associations and primarily focused on *C. gattii* dynamics on trees versus in the air or soil. A limitation of studying seasonal *C. gattii* changes is that it is difficult to disentangle which biophysical conditions (temperature, sunlight, moisture, momentum) most strongly influence *C. gattii* concentrations. For example, which is the primary driver of airborne *C. gattii* levels in southern Australia: temperature, dryness, or both? More frequent *C. gattii* measurements and longitudinal statistics can help distinguish between competing processes. Most long-term studies documented *C. gattii* dynamics on trees; however, seasonality of *C. gattii* may differ in the soil and air (*5*). In particular, airborne *C. gattii* may have the most relevance for human health and deserves further attention. Furthermore, scrutinizing *C. gattii* dynamics in multiple media may provide additional support for conceptualizations of the *C. gattii* life cycle.

Our goal with this study was to determine the relative strength of associations between biophysical conditions and monthly *C. gattii* dynamics from the air, trees, and soil on Vancouver Island, Canada. The first research question examines specific plots from which repeated measurements were made during 2003–2004, and the second question examines only newly sampled *C. gattii* plots during 2002–2004. Based on environmental samples, these investigations were designed to provide insight into the periods with the greatest *C. gattii* area concentrations. This

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Table 1. Summary of findings from longitudinal *Cryptococcus gattii* studies*

Location (reference)	Genotype, serotype	Medium	Highest isolation frequency	Lowest isolation frequency
British Columbia, Canada (*5*)	VGIIa (AFLP6A, serotype B), VG IIb (AFLP6B, serotype B)	Air	Summer: PPT 31 mm/mo, T 11°C–24°C	Winter: PPT 166 mm/mo, T–1°C to 6°C
Bogotá, Colombia (*9*)	B	Tree	Rainy season: RH ≈85%, PPT 120 mm/mo, T 14.4°C–14.8°C	Dry season: Low RH ≈67%, PPT <5 mm, T 14.0°C
Bogotá, Cúcuta, Medellín, Cali, Colombia (*10*)	B C	Tree Tree	High RH, low T, low EVAP Low RH, high T, high EVAP	Low RH, high T, high EVAP High RH, low T, low EVAP
Punjab, Haryana, Delhi, Chandigarh, India (*11*)	VGIb (AFLP4)	Tree	Autumn: RH ≈54%, PPT 60 mm/mo, T 25°C; summer: RH ≈30%, PPT 20 mm/mo, T 32°C; rainy: RH ≈60%, PPT 150 mm/mo, T 31°C	Winter: RH ≈55%, PPT 10 mm/mo, T ≈17°C; Spring: RH ≈39%, PPT 11 mm/mo, T 23°C
Jabalpur, India (*12*)	B	Tree	Summer: T 32°C, PPT 0.9–141 mm/mo	Rainy: T 6.6°C–30.6°C, PPT 141–589 mm/mo
São Paulo, Brazil (*13*)	B	Tree	November: PPT 244 mm/mo, T 22°C	Other months: PPT 10– 400 mm/mo, T 18°C–26.5°C
Barroso Valley, Australia (*14*)	B	Air	Eucalyptus flowering (Dec–Feb): PPT 0–4.32 mm/mo, T 20.4°C–21.5°C	Other months: PPT 5.08–164 mm/mo, T 8°C–20°C

*Most studies identified the seasons with the greatest or lowest *C. gattii* isolation frequency. Studies commonly examined relative humidity (RH), temperature (T), precipitation (PPT), or evaporation (EVAP).

study expands on previous research in the area by studying changes over time, using representative weather stations, considering more biophysical conditions, and using statistics that control for autocorrelation.

Methods

Concentrations of *C. gattii* in the environmental soil, air, and trees were collected by previously described standardized methods (*5*; online Technical Appendix). We evaluated 2 datasets of *C. gattii* VGIIb (AFLP6B, serotype B) previously collected by different sampling strategies: repeatedly measured and newly sampled. The first strategy sporadically resampled a geographic plot after a positive *C. gattii* sample was obtained for this site during 2003–2004. This dataset is similar to the permanently colonized sites analyzed in an ecologic habitat study (*15*). The definition of a plot refers to a specific tree, soil sample 2 meters from the tree base, and the surrounding air. Plots were initially selected with ≥4 more longitudinal samples. The second strategy analyzed only the first samples from a newly tested plot as analyzed by Kidd et al. (*5*). The sample plots were taken from 9 study areas (Figure). The study areas reported cases in humans, animals, or both or were in biogeoclimatic zones similar to areas with reported cases. Only plots from study areas visited on ≥3 occasions and from which ≥1 *C. gattii*–positive sample was obtained were included in the analysis. In each area, new plots were tested in 16%–41% of the study months. Newly sampled plots may reflect *C. gattii* dynamics across the broader study area.

The study examined a broad range of biophysical conditions that plausibly influence population dynamics of fungi in the phylum Basidiomycota. Environment Canada provided daily temperature and precipitation data from 15 weather stations in 7 study areas (http://climate.weatheroffice.gc.ca/Welcome_e.html) (Figure). The second-generation North American Land Data Assimilation System (NLDAS) provided specific humidity, shortwave solar radiation (0.3–3 μm), and wind speed across the domain. Wind speed and solar radiation were infrequently considered in previous studies. Shortwave radiation was converted into Z-scores (number of SDs away from the mean) to align the range of the independent variables and promote statistical convergence. NLDAS uses weather models to interpolate conditions between stations by using physical laws and processes. The spatial resolution of the gridded NLDAS dataset was ≈14 km^2. Validation shows good agreement between the NLDAS variables used in this study and independent observations (*16*).

There is minimal research to support the selection of periods over which biophysical conditions most strongly influence *C. gattii* dynamics. This analysis broadly considered biophysical conditions over the previous and current day, previous week, and previous month (past 30 days). For each sampling date, *C. gattii* observations for each plot were aligned with the corresponding weather conditions of the surrounding study area.

Statistical Analyses

Long-term *C. gattii* studies may reanalyze data collected for different purposes, such as surveillance and detection. *C. gattii* was rarely sampled continuously from the same plots. More commonly, repeated measurements were sporadically taken from the same plots. For example, tree A might have been sampled in January–March and August–October, tree B in April–July and November–December, and tree C in April–October. Although no tree was continuously sampled throughout the year, standardizing and pooling the sporadic samples can collectively yield

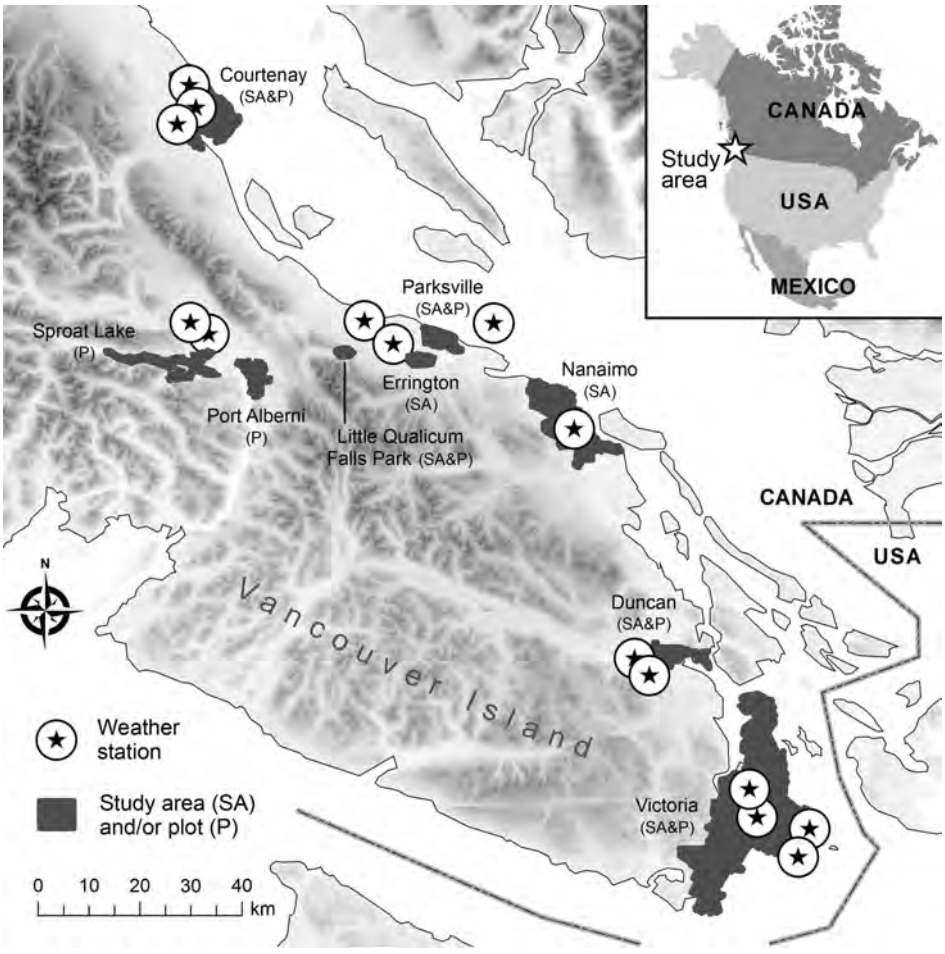

Figure. Areas on Vancouver Island, British Columbia, Canada, in which environmental samples were collected to determine *Cryptococcus gattii* concentrations during 2002–2004. Environment Canada provided weather information from 15 stations across the island.

seasonal *C. gattii* information. The analysis maximized the information available from the sporadic samples by use of hierarchical generalized linear and mixed effect models (GLMMs) that control for repeated measurements and clustered sampling (*17*).

GLMMs were used to investigate association of weather conditions with monthly *C. gattii* CFU counts (soil, air) or *C. gattii* presence/absence (trees). Poisson GLMMs with a random effect for each study observation accounted for overdispersion for the soil and air samples. Logistic GLMMs were used to analyze the tree samples. The analysis was conducted in R version 2.15.3 with use of the LME4 package (http://www.R-project.org/). In the first analysis of longitudinal samples, hierarchical random effects controlled for repeated plot measurements and plots nested within study areas.

The random effect in the second analysis accounted for plots nested within study areas. Both analyses controlled for tree genus (cedar, fir, oak, maple, pine, and other). If *C. gattii* were observed <20 times in trees of a given genus, genera were further aggregated into families or lumped into the "other" category. To control for residual spatial autocorrelation, we considered latitude and longitude as candidate independent variables in the analysis. We also controlled for seasonality with fixed-effect indicator variables for winter (November–February), spring (March–May), summer (June–July), and fall (August–October). The GLMM results were reported when the postvariable selection model residuals were not significantly autocorrelated. Residual autocorrelation was tested by autocorrelation and partial autocorrelation functions that were adjusted for missing data.

Intuitively, the *C. gattii* levels for a given month may be strongly related to the previous month's values. Monthly *C. gattii* samples may exhibit a more complex temporal correlation structure. If the GLMM residuals were significantly autocorrelated, we conducted the analysis on a reduced dataset. For the first analysis, the reduced dataset included plots sampled in sequential months from the plots with ≥4 longitudinal samples. For the second analysis, the reduced dataset included all first samples in a study area, provided that the study area was sampled in the previous month. Thus, the GLMM controlled for seasonality, plot, or study area–specific random effects, and first-order

autoregressive terms for each plot (first analysis) or study area (second analysis). The autoregressive term was the natural logarithm of average *C. gattii* concentration plus 1 (soil, air) or proportion of positive *C. gattii* samples (tree) in the previous month. In the reduced dataset, study areas in which *C. gattii* were observed <20 times were lumped together in the "other area" category.

Because of the relationships among weather conditions, a forward stepwise variable selection procedure involving the Akaike information criterion was used to select the multiple variable models. After a weather variable entered the model, the selection procedure did not consider other temporal aggregations of the same variable. For example, if daily absolute humidity exhibited the most significant *C. gattii* association, weekly or monthly absolute humidity was not tested in the next stepwise iteration. There are minor to moderate differences in the magnitudes of weather conditions across the study area. The statistical results therefore reflect time periods and geographic areas in which weather systematically influences *C. gattii* levels. Weather conditions in the study area were not further standardized to retain the interpretability and biological plausibility of weather conditions for *C. gattii* population dynamics.

Results

Plot Level

Table 2 summarizes the mean *C. gattii* concentrations and sample size for the soil and air samples and the proportion of positive tree swab samples. On a plot level (first analysis), weather systematically influenced soil and airborne *C. gattii* levels (Table 3). The soil results from the reduced dataset with plots sampled in sequential months that controlled autocorrelation are reported. The statistical model controlled for a west-to-east gradient of increasing *C. gattii* concentrations across Vancouver Island and for seasonality. Geographic areas and periods with cooler temperatures, lower wind speeds, or both corresponded to the highest *C. gattii* concentrations. Soil concentrations of *C. gattii* were often elevated in the study areas with the coolest temperatures (Parksville and Little Qualicum Falls Park). Average wind speeds were weakest in the study areas surrounding Courtenay and Errington. During October–April, area-averaged (≈14 km^2) monthly wind speeds were <2 m/s.

Airborne *C. gattii* levels for a given month were not associated with those of the previous month. Therefore all plots sampled ≥4 times were included in the analysis. Similar to the trend for the soil samples, there was an increasing eastward trend of *C. gattii* across the island. Solar radiation intensity was positively associated with airborne *C. gattii* concentrations. The most daily solar radiation is received in the southerly areas (Victoria, Parksville, Duncan) and during May–August. Wind speeds exhibited a more complex, nonlinear relationship to airborne propagules. Moderate daily wind speeds (1.5–3 m/s) may be more likely than less windy days (<1.5 m/s) to entrain *C. gattii* propagules into the air. However, *C. gattii* concentrations were lower on very windy days than on relatively tranquil days. Temperature was not associated with airborne concentrations.

A tree with a positive *C. gattii* sample in a given month was more likely to be positive in the following month. Thus, results are reported from the reduced dataset of trees sampled in sequential months (Table 3). Detection of *C. gattii* in tree samples was not significantly associated with weather conditions. Within the study area, northerly regions were less likely to host *C. gattii*–positive trees.

Study Area Level

Random sampling of new environmental samples during 2002–2004 showed that at the study area level, weather was systematically associated with *C. gattii* in soil and trees (Table 4). Most of the air samples were collected in sequential months, and the small number of air samples from newly sampled plots precluded formal statistical analysis. Consistent with the plot level, concentrations of *C. gattii* in soil were significantly associated with concentrations the previous month. The results of the subset of samples from sequential months are reported. In agreement with the plot-level analyses, higher average temperatures were associated with lower *C. gattii* concentrations in a study area after controlling for seasonality. However, wind speed did not significantly influence concentrations in soil.

Of note, temperature, wind speed, and solar radiation strongly influenced *C. gattii* dynamics on trees at the study area but not the plot level. Across each study area, a higher

Table 2. Mean *Cryptococcus gattii* concentrations for soil and air samples or proportion of positive tree swab samples, Vancouver Island, British Columbia Canada, 2002–2004*

		Mean *C. gattii* concentration†							
Medium	Level	Parksville	Duncan	Courtenay	Errington	LQFP	Nanaimo	Victoria	Other
Soil, CFU (no. samples)	Plot	2,006 (49)	80,139 (18)	–		–		–	1,635 (28)
Soil, CFU (no. samples)	Area	572 (12)	56 (43)	556 (17)	4 (14)	0 (7)	0 (6)	0 (18)	–
Air, CFU (no. samples)	Plot	100 (113)	202 (38)	2 (34)					
Tree, % (no. samples)	Plot	26 (57)	95 (21)	–		–		60 (15)	50 (22)
Tree, %, (no. samples)	Area	55 (55)	10 (42)	15 (34)		13 (9)	0 (4)	5 (110)	

*LQFP, Little Qualicum Falls Park.
†Blank cells indicate areas not included in the analysis. Dashes (–) indicate study areas with a small sample size that were lumped into the column entitled "other." This information is reported for the plot and area analysis levels.

Table 3. Generalized linear and mixed effect model result of the association between weather and *Cryptococcus gattii* in resampled plots in Vancouver Island, British Columbia Canada, 2002–2004

Medium and independent variable	Estimate	SE	95% CI	p value
Soil, CFU*				
Intercept	567.16	167.21	232.7 to 901.5	0.001
Mar–May vs. Nov–Feb	1.06	0.78	–0.50 to 2.626	0.174
Jun–Jul vs. Nov–Feb	15.75	2.38	10.98 to 20.50	<0.001
Aug–Oct vs. Nov–Feb	12.12	1.83	8.45 to 15.78	<0.001
Longitude (°W)	4.47	1.34	1.79 to 7.15	<0.001
Average daily temperature, °C	–1.25	0.19	–1.63 to –0.87	<0.001
Average daily wind speed 1.5–3 m/s	–3.45	0.81	–5.06 to –1.83	<0.001
Average daily wind speed >3 m/s	–5.68	0.99	–7.66 to –3.69	<0.001
Previous month's natural logarithm (*C. gattii* + 1)	0.51	0.11	0.30 to 0.73	<0.001
Garry oak vs. fir/cedar	1.19	1.82	–2.45 to 4.84	0.514
Maple vs. fir/cedar	1.61	1.43	–1.25 to 4.47	0.262
Other tree vs. fir/cedar	–2.82	1.50	–5.82 to 0.18	0.060
Air, CFU†				
Intercept	484.28	94.69	294.8 to 673.6	<0.001
Mar–May vs. Nov–Feb	0.78	1.27	–1.74 to 3.31	0.537
Jun–Jul vs. Nov–Feb	0.89	1.56	–2.23 to 4.01	0.570
Aug–Oct vs. Nov–Feb	2.46	1.05	0.36 to 4.56	0.019
Longitude, °W	3.91	0.76	2.39 to 5.44	<0.001
Daily shortwave solar radiation, watts/m^2, centered	2.32	0.60	1.11 to 3.52	<0.001
Average daily wind speed 1.5–3 m/s	1.53	0.64	0.24 to 2.82	0.017
Average daily wind speed >3 m/s	–3.97	1.37	–6.71 to –1.21	0.004
Garry oak vs. fir/cedar	0.35	0.84	–1.33 to 2.03	0.680
Maple vs. fir/cedar	–0.27	0.87	–1.99 to 1.47	0.760
Other vs. fir/cedar	0.99	0.73	–0.46 to 2.44	0.174
Swab sample, proportion positive‡				
Intercept	145.29	49.42	46.44 to 244.10	0.003
Mar–May vs. Nov–Feb	2.32	0.80	0.71 to 3.93	0.004
Jun–Jul vs. Nov–Feb	2.22	0.88	0.46 to 3.98	0.012
Aug–Oct vs. Nov–Feb	2.62	0.88	0.87 to 4.37	0.003
Latitude, °N	–2.99	1.01	–5.02 to –0.96	0.003
Proportion of *C. gattii*–positive samples previous month	2.38	0.58	1.22 to 3.53	<0.001
Fir/cedar vs. alder	0.18	0.86	–1.53 to 1.91	0.831
Garry oak vs. alder	–0.23	0.97	–2.17 to 1.72	0.817
Other tree vs. alder	–0.80	1.03	–2.85 to 1.26	0.437

*95 samples, 45 plots, 3 study areas,,Akaike Information Criterion = 648.4.
†175 samples, 24 plots, 3 study areas, Akaike Information Criterion = 615.4.
‡115 samples, 44 plots, 4 study areas, Akaike Information Criterion = 117.9.

proportion of positive tree swab samples from the previous month increased the chances of elucidating *C. gattii* in the current month. The weather relationships were largely consistent with the results from the other media (soil and air). As with the soil samples, geographic areas and periods with warmer temperatures were associated with reduced frequency of *C. gattii* isolation. Similar to the air samples, solar radiation and wind speed were positively associated with frequency of *C. gattii* isolation. *C. gattii* isolation was more likely in southern study areas and during May–August, which had the most solar radiation.

Discussion

In British Columbia, Canada, *C. gattii* exhibits specialized habitat preferences. It thrives in the area of the Vancouver Island rain shadow (i.e., southeast coast of Vancouver Island and the southwest coast of mainland British Columbia), where winter temperatures are predominantly above freezing and summer temperatures are not too hot (*15*). In the analysis of resampled plots, weather conditions over the previous and current day most strongly influenced *C. gattii* concentrations. For the first *C. gattii* sample analysis, weekly and monthly weather exhibited the best-fitting associations with detection of *C. gattii* in tree swab samples. Granados and Castañeda suggested that conditions up to 15 days before sampling most strongly influence *C. gattii* concentrations (*18*).

Geographic areas and periods with elevated temperatures decreased isolation of *C. gattii* from tree samples and concentration in soil. The results are consistent with *C. gattii* serotype B in Colombia, where *C. gattii* was sampled from the detritus of trees of species with persistent and elevated *C. gattii* concentrations (*Eucalyptus camaldulensis* and *Terminalia cattapa*) (*18*). In that study, the greatest proportions of positive samples were also found during periods of lower temperatures. Similarly, an elevational transect study conducted at elevations of 300–3,000 m found that *C. gattii* concentrations were greater at high

Table 4. Association between weather and the first *Cryptococcus gattii* sample in study areas, Vancouver Island, British Columbia Canada, 2002–2004*

Medium and independent variable	Estimate	SE	95% CI	p value
Soil†				
Intercept	25.08	15.57	–6.05 to 56.21	0.107
Jun–Juy vs. Mar–May	60.47	25.04	10.39 to 110.50	0.016
Aug–Oct vs. Mar–May	20.24	12.13	–4.02 to 44.49	0.095
Average daily temperature, °C	–4.66	2.15	–8.96 to –0.36	0.030
Cedar vs. alder	1.24	6.56	–11.88 to 14.35	0.850
Fir vs. alder	–2.34	7.44	–17.22 to 12.52	0.753
Oak vs. alder	–1.28	9.49	–20.27 to 17.70	0.893
Maple vs. alder	–0.63	7.07	–14.78 to 13.51	0.929
Other vs. alder	–0.95	7.33	–15.60 to 13.70	0.897
Previous month's natural logarithm(*C. gattii* + 1)	0.65	1.52	–2.38 to 3.69	0.666
Swab sample‡				
Intercept	10.31	2.45	5.42 to 15.19	<0.001
Weekly wind speed, m/s	0.76	0.26	0.24 to 1.28	0.003
Average weekly temperature, °C	–1.23	0.20	–1.63 to –0.82	<0.001
Monthly solar radiation, watts/m^2, centered	6.25	1.34	3.58 to 8.93	<0.001
Mar–May vs. Nov–Feb	0.92	1.18	–1.43 to 3.27	0.435
Jun–Jul vs. Nov–Feb	2.77	1.70	–0.63 to 6.17	0.103
Aug–Oct vs. Nov–Feb	1.24	1.28	–1.31 to 3.80	0.332
Cedar (western red, yellow) vs. alder	–1.00	1.12	–3.24 to 1.25	0.374
Fir (Douglas, other) vs. alder	–0.55	0.72	–2.00 to 0.89	0.444
Garry Oak vs. alder	0.94	0.90	–0.85 to 2.73	0.296
Maple vs. alder	0	0.85	–1.69 to 1.71	0.997
Other vs. alder	–0.61	0.98	–2.55 to 1.35	0.534
Pine vs. alder	–1.70	1.46	–4.61 to 1.21	0.243
Proportion of *C. gattii*–positive samples previous mo	2.21	0.89	0.43 to 3.98	0.013

*Determined by generalized linear and mixed effect model.
†116 samples, 7 study areas, Akaike Information Criterion = 194.7.
‡254 samples, 6 study areas, Akaike Information Criterion = 180.7.

elevations with cold temperatures (12°C–18°C annual average temperatures) than in temperate and tropical regions (*19*). In the Vancouver Island study area, average annual temperatures in *C. gattii*–endemic areas were slightly cooler (9.8°C–11.4°C). Outbreaks of *C. gattii* infection in humans or animals in Western Australia, Mediterranean Europe, and North America have been characterized by dry summers or dry winters with warm but not hot monthly temperatures (<22°C) (*20*). Laboratory studies of the optimum growth rates for *C. gattii* and competitors have not been conducted. This knowledge might provide a stronger mechanistic interpretation of temperature associations. According to research of other Basidiomycota, temperature may influence the ecologic niche by regulating the rate of enzyme-catalyzed reactions (*21*).

The aversion of the *C. gattii* strain in British Columbia to higher temperatures may partially account for the difficulty detecting *C. gattii* in environmental samples in warmer neighboring regions. In general, the proportion of *C. gattii*–positive samples declines with increasing southerly distance from Vancouver Island and the Gulf Islands. Prevalence of *C. gattii* in new soil samples (9.6%) and trees (7.7%) on Vancouver Island is remarkably high (*5*). In Washington, USA, British Columbia's neighbor to the south, *C. gattii* was recovered in 3.0% of air, soil, and tree samples (*5*). This trend continues farther to the south in Oregon, USA, where *C. gattii* was detected in 0–0.6% of tree swab samples (*3*,*22*). The caveat to this trend is that Oregon is host to a different combination of *C. gattii* strains (AFLP6A/VGIIa, AFLP6C/VGIIc) than are British Columbia and Washington (AFLP6A/VGIIa, AFLP6B/VGIIb).

To adapt to biophysical stressors such as temperature, nutrient stress, and radiation, *Cryptococcus* spp. produce melanin. Melanin may increase the integrity of *C. neoformans* cells and make them less susceptible than nonmelanized cells to temperature extremes (*23*). Nutrient stress (glucose and peptone) enhances the production of melanin in *C. gattii* VGI and VGII (*24*). In laboratory *C. neoformans* studies, melanin increases survival to UV-C but not UV-B radiation (*25*,*26*). In our study, periods with more solar radiation (sum of visible, UV, and near-infrared) seem to promote *C. gattii* in the air and trees. Research on *C. gattii* serotype C in Colombia documented a similar association with solar radiation (*18*). To further clarify the role of melanin for mediating environmental stressors, further laboratory studies of *C. gattii* genotypes are needed.

The association between windy days and airborne *C. gattii* concentrations may have >1 interpretation. Very windy conditions may be strong enough to transport *C. gattii* away from the local air monitor. It is also possible

that these periods coincide with depressed soil *C. gattii* concentrations when there are fewer propagules that can be mobilized. Also, the accuracy of the isokinetic air sampler decreases during periods with stronger wind speeds (*27*).

Collectively, the study results support common conceptualizations of the life cycle of *C. gattii*. Trees and the surrounding soil are permanently colonized and seem to act as *C. gattii* reservoirs. Wind may provide a key process for transferring *C. gattii* from the soil into the air and onto trees in the wider study area. Concentrations of *C. gattii* near the soil surface (0 to <15 cm depth) are greater than concentrations deeper (15–30 cm) in the soil (*3*). Moderate wind speeds may mobilize surface soil and increase local airborne *C. gattii* concentrations. Higher wind speeds may transport *C. gattii* from the soil to trees across the broader area. It is also possible that wind is simply a proxy that coincides with life stages in which propagules are more likely to disperse. *C. gattii* colonization seems to be transitory on many of the recently colonized substrates. *C. gattii* flexibly inhabits and colonizes the soil and specific trees during different seasons, which may decrease intraspecific competition.

The primary route of human *C. gattii* exposure is probably the inhalation of infectious propagules. In the study area, the fungus causes ≈25 clinically diagnosed human illnesses and 4 deaths per year (http://www.bccdc.ca/discond/a-z/_c/CryptococcalDisease/Cryptococcus+gattii.html). According to our results, the highest airborne *C. gattii* concentrations occur during August–October on sunny days with moderately windy conditions. The greatest risk for exposure to *C. gattii* from the soil is during relatively cool June and July summer days. Although these associations are consistent, until more research provides information about the infectious dose for humans, the study results characterize the risk for exposure associated with environmental factors, rather than disease risk. Weather and airborne concentrations of *C. gattii* should be associated with human cryptococcosis incidence; however, onset of documented cryptococcosis cases in British Columbia does not vary by season or month (*28*,*29*). The temporal discrepancy may be masked by the long and variable incubation period of this pathogen. Host factors may be stronger predictors of developing disease risk (*30*). Nonetheless, refined risk projections may benefit susceptible humans and animals living in areas of marginal *C. gattii* transmission.

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Coccidioidomycosis among Workers Constructing Solar Power Farms, California, USA, 2011–2014

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Coccidioidomycosis is associated with soil-disruptive work in *Coccidioides*-endemic areas of the southwestern United States. Among 3,572 workers constructing 2 solar power–generating facilities in San Luis Obispo County, California, USA, we identified 44 patients with symptom onset during October 2011–April 2014 (attack rate 1.2 cases/100 workers). Of these 44 patients, 20 resided in California outside San Luis Obispo County and 10 resided in another state; 9 were hospitalized (median 3 days), 34 missed work (median 22 days), and 2 had disseminated disease. Of the 25 patients who frequently performed soil-disruptive work, 6 reported frequent use of respiratory protection. As solar farm construction in *Coccidioides*-endemic areas increases, additional workers will probably be exposed and infected unless awareness is emphasized and effective exposure reduction measures implemented, including limiting dust generation and providing respiratory protection. Medical providers, including those in non–*Coccidioides*-endemic areas, should suspect coccidioidomycosis in workers with compatible illness and report cases to their local health department.

Solar energy generation is an expanding industry, particularly in the southwestern United States (*1*). According to the experience in eastern San Luis Obispo County, California, construction of large-scale solar power–generating facilities (solar farms) in low-population rural areas may often require recruiting skilled laborers from outside the county or state. In California, multiple solar farms are being planned and constructed. During February 2013, after coccidioidomycosis was confirmed in several workers, the California Department of Public Health (CDPH) and the County of San Luis Obispo Public Health Department (SLOPHD) investigated illnesses among workers constructing 2 solar farms (A and B) in the county. We report the results of that investigation.

Coccidioidomycosis (Valley fever) is an infectious disease acquired by inhalation of soil-dwelling *Coccidioides* fungus spores, which are endemic to the southwestern United States, including San Luis Obispo County. After an incubation period of ≈1–3 weeks, ≈40% of infected persons experience an influenza-like illness, which can include cough, difficulty breathing, fever, and fatigue. Fewer than 5% experience disseminated disease, including meningitis, osteomyelitis, or cutaneous lesions (*2*). Severe or disseminated coccidioidomycosis requires treatment and can be fatal.

In recent years, reported cases of coccidioidomycosis and hospitalizations in California increased dramatically, peaking in 2011, particularly in the *Coccidioides*-endemic counties of the southern San Joaquin Valley (*3–7*). Outbreaks, however, have been reported infrequently and are usually associated with soil disruption (*8–15*). During December 2012–February 2013, an outbreak was suspected when SLOPHD notified CDPH of coccidioidomycosis diagnosed for 3 workers who had been constructing solar farm B and when 9 additional workers who had become ill were identified as having a potential connection to solar farms A or B. These additional workers were identified from the CDPH statewide coccidioidomycosis surveillance system and by occupational injury and illness reports on the basis of limited occupational data comments in the records. We investigated the extent of the outbreak, obtained clinical and epidemiologic data, identified work and environmental factors potentially contributing to exposures, and recommended preventive measures. The California Health and Human Services Agency's Committee for the Protection of Human Subjects determined that this investigation was public health practice (i.e., not research).

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Methods

Case Definition

A case was defined as laboratory-confirmed coccidioidomycosis in an employee of either solar farm A or B, with illness onset ≥1 week after beginning work but ≤1 month after the last workday at either solar farm. Criteria for laboratory-confirmed coccidioidomycosis were based on the 2011 Council of State and Territorial Epidemiologists and Centers for Disease Control and Prevention surveillance case definition for coccidioidomycosis (*16*). Criteria included 1) clinical presentation with influenza-like signs and symptoms, pneumonia, meningitis, or involvement of bones, joints, or skin; and 2) laboratory confirmation with cultural, histopathologic, or molecular evidence of *Coccidioides*, or positive serologic test results for coccidioidal IgM (by immunodiffusion, enzyme immunoassay [EIA], latex agglutination, or tube precipitin) or for coccidioidal IgG (by immunodiffusion, EIA or complement fixation).

Case Finding

During December 2012–July 2014, we identified cases by using multiple sources. In California, health care providers and laboratories are required to report coccidioidomycosis diagnoses to the local health jurisdiction where the patient resides (i.e., reported as their permanent residence). Local health jurisdictions subsequently report cases to CDPH. Additionally, health care providers in California who evaluate an employee with a possible work-associated medical condition are required to complete a Doctor's First Report of Occupational Injury or Illness (DFR) (*17*). We reviewed reports of coccidioidomycosis and DFRs to identify cases of coccidioidomycosis possibly associated with either solar farm. Additionally, we reviewed employee rosters and employer logs of injury and illness, mandated by the California Department of Industrial Relations, Division of Occupational Safety and Health (Cal/OSHA), from both solar farms' contractors and subcontractors to match names with those reported to CDPH by local health jurisdictions and to a statewide database of workers' compensation claims. We also sent a nationwide email alert to state and local health departments requesting information about coccidioidomycosis possibly acquired among workers constructing solar farms in San Luis Obispo County. Last, we sent a letter to all identified employer-designated health care providers and all health care providers who had evaluated or provided treatment for solar farm employees with a diagnosis of coccidioidomycosis to request that they report any solar farm employees with suspected coccidioidomycosis.

Epidemiologic Investigation

During March 5–6, 2013, Cal/OSHA led a multiagency site visit at the solar farms to interview employers and employees, identify employer-referred health care providers, review employer injury and illness logs, observe work practices, identify potential exposure routes, and observe protective measures used. On the basis of that information, we developed a standardized telephone patient questionnaire for collection of data regarding demographics, work practices, worksite characteristics, patient clinical presentation and outcome, and preexisting medical conditions. For permanent residence, we defined the California *Coccidioides*-endemic counties as Fresno, Kern, Kings, Madera, San Luis Obispo, and Tulare and the less *Coccidioides*–endemic counties as all others, as previously described (*7*). We defined other states with possible coccidioidomycosis endemicity as Arizona, Nevada, New Mexico, Texas, and Utah. We also asked about dust exposure during the 4 weeks before disease onset, including work activities, dust exposure at or away from the worksite, and individual and worksite dust control and safety measures. From published literature (*18*), we identified manual digging, working in a ditch or trench, and operating heavy machinery as probable soil-disruptive exposures at solar farms. If patients were able to provide only an estimate for date or duration of these activities, we used the range midpoint for analyses; if patients were unable to provide an estimate, or if the date of symptom onset reported by the patient was later than the date of diagnosis, we ascertained this information from medical or employer records when possible. Patients' estimates of frequency of performing soil-disruptive duties and high dust levels at the worksite were categorized as frequently (every day and once a week) or infrequently (rarely and never); respirator use frequency and hygienic practices were categorized as frequently (always and often) or infrequently (sometimes, rarely, and never).

We obtained employee rosters from as many employers as possible and determined the number of workers at each site. We received information about first and last day worked on rosters from 2 predominant employers at solar farm A (employers A1 and A2); using these rosters, we estimated employee time at risk for *Coccidioides* infection. Employee time at risk was defined as the number of days from the first to the last day worked onsite. Incidence among employees was compared with the incidence rate for San Luis Obispo County in 2012, which was calculated per 100,000 population (*19*). The number of incident cases among employer A1 and A2 employees was also compared with the average worker count by month by using the Spearman rank test. Statistical analyses were conducted by using SAS version 9.3 (SAS Institute, Inc., Cary, NC, USA), and p values <0.05 were considered significant.

Results

Among 3,572 known employees at the 2 solar farms, we identified 44 patients with illness onset during October

4, 2011–April 15, 2014 (attack rate 1.2 cases/100 workers) (Figure 1, panel A). Cases were identified from multiple sources (Figure 2). Most were identified after the site visit, by review of employer records and comparison of rosters to CDPH coccidioidomycosis reports. Other cases were reported to CDPH by health care providers, by an employer, or by a union, or were identified by interviewed patients. Of note, no cases had been reported by employers to Cal/OSHA, SLOPHD, or CDPH before the site visit.

Of the 44 patients, 43 were interviewed; 41 completed the interview, and some refused or were unable to answer specific questions. Patients were interviewed a median of 335 (range 77–621) days after symptom onset. A total of 41 (93%) of the 44 patients were men; patients' median age was 48 years. Of the 41 patients who completed the interview, 11 (26%) were Hispanic and 28 (68%) were white (Table 1). Varying job titles were reported by 43 patients; the most common titles reported were electrician (33%) and heavy equipment operator (26%).

Of the 44 patients, 17 (39%) had a permanent address in the *Coccidioides*-endemic counties of California (14 [32%] in San Luis Obispo County), 17 (39%) in the less *Coccidioides*–endemic counties, 6 (14%) in another state with possible *Coccidioides* endemicity, and 4 (9%) in a non–*Coccidioides*-endemic state (Table 1; Figure 1, panel B). All interviewed patients with a permanent address outside of California reported a temporary address in San Luis Obispo County. Of the 20 interviewed California residents with permanent residence outside of San Luis Obispo County, 11 (55%) reported a temporary address: 8 in San Luis Obispo County, 2 in Kern County, and 1 unknown. Of the 3,572 identified workers, 39% resided in a California county other than San Luis Obispo and 21% resided outside California.

Patients' clinical characteristics at time of illness are displayed in Table 2. Of the 44 patients, 17 (39%) visited an emergency department at some point during their illness and 9 (20%) were hospitalized for 2–17 (median 3) days. For 2 (5%) patients, the diagnosis was disseminated

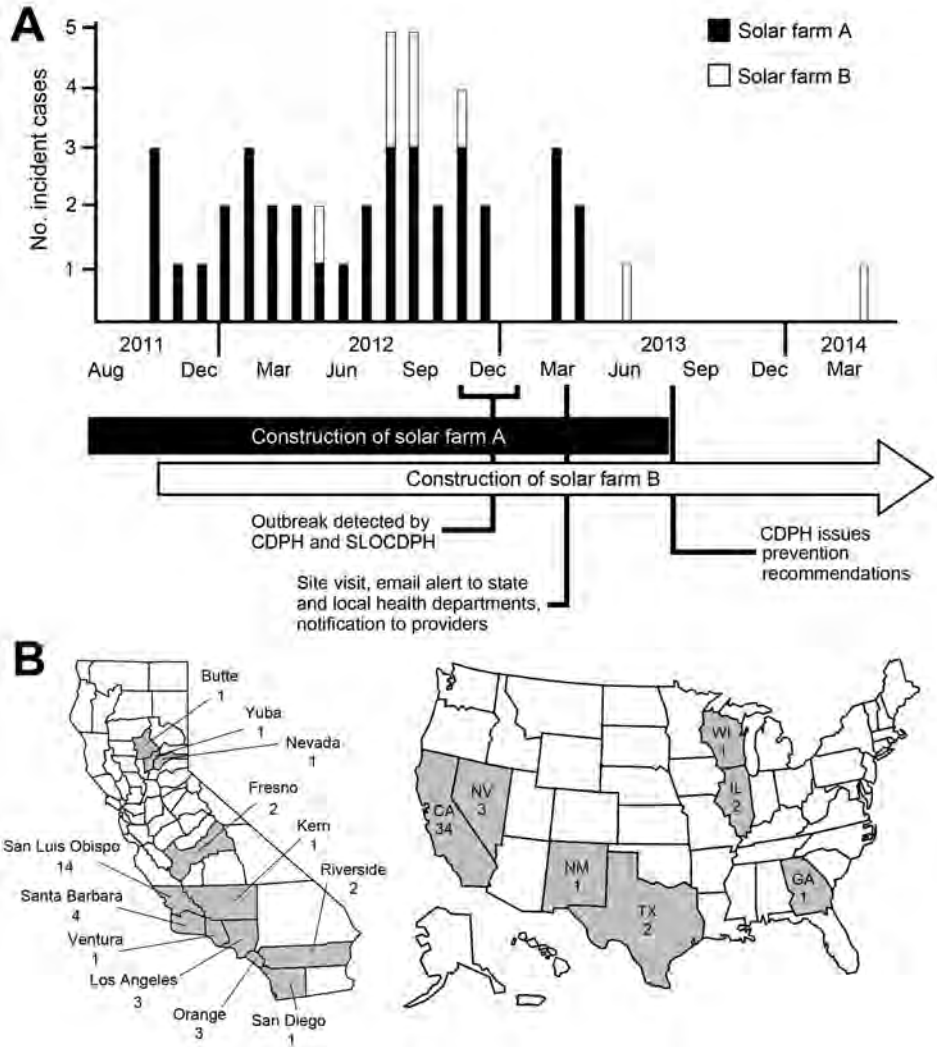

Figure 1. Distribution of outbreak cases of coccidioidomycosis among solar farm workers, by month of symptom onset and patients' residence, San Luis Obispo County, California, USA, October 2011–April 2014. A) Number of cases listed by month of onset and by solar farm. Investigation timeline is displayed below the *x*-axis. B) Number of cases during this outbreak listed by patient-reported California county or state of permanent residence (gray shading). CDPH, California Department of Public Health; SLOPHD, County of San Luis Obispo Public Health Department.

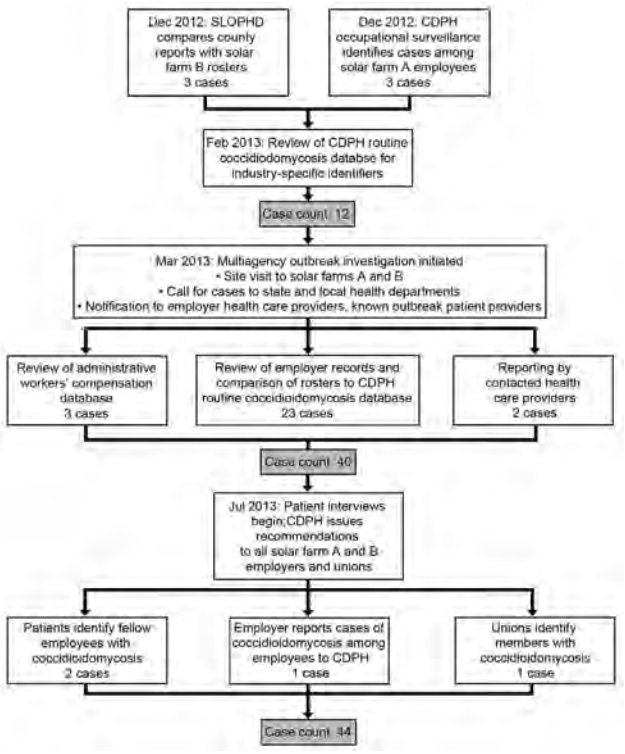

Figure 2. Flowchart of outbreak investigation of coccidioidomycosis among solar farm workers, San Luis Obispo County, California, USA, October 2011–April 2014. CDPH, California Department of Public Health; SLOPHD, County of San Luis Obispo Public Health Department.

disease: 1 to skin and 1 to bone. The median time from first day of work to symptom onset was 105 (range 10–638) days; 10 (23%) patients reported symptom onset ≤2 months after beginning work. Of the 41 patients who provided symptom data, ≥80% reported fatigue, night sweats, weakness, difficulty breathing, fever, cough, joint/muscle pain, and weight loss.

A limited number of interviewed patients reported chronic medical conditions (excluding hypertension) before symptom onset. None reported corticosteroid use, and only 1 reported an immunocompromising condition (cancer, diagnosed after coccidioidomycosis onset).

Of the 41 patients who completed the interview, 34 (83%) reported missing work because of illness (median 22 days); 2 had missed work for ≥18 months at the time of interview. We estimate that a minimum of 9.1 person-years of work were lost because of illness. A total of 11 (27%) patient reported that as of the interview date they were still experiencing health effects that interfered with work or other physical activity.

Only 9 (22%) of 41 patients reported having received a diagnosis of coccidioidomycosis after their first symptom-related visit to a health care provider. The median time from symptom onset to laboratory diagnosis was 23 (range 5–267) days. Of the 44 patients, 37 (84%) received a diagnosis by using ≥1 of the following serologic tests for *Coccidioides* exposure: complement fixation, immunodiffusion, or tube precipitin. Of the remaining 7 patients, 4 received a diagnosis by serologic IgG or IgM EIA results only and 3 by only immunohistochemical test or *Coccidioides* culture results.

Site and Work Characteristics

Both solar farms are located near the southwestern side of San Joaquin Valley, ≈10 miles apart in sparsely populated, arid grassland where it is frequently windy and dusty. Construction of solar farm A occurred during September 2011–October 2013 and of solar farm B during November 2011–December 2014 (Figure 1, panel A). Both solar farms encompass thousands of acres. Installation of solar photovoltaic panel arrays involved leveling the ground, digging trenches for installation of underground electric cables, performing electrical work in trenches, driving piles to install panel rigs, and ancillary digging manually or by machine. Employees can be exposed to dust by wind or by being in close proximity to soil-disruptive activities (Figure 3). The San Luis Obispo County permit approval for solar farm projects requires minimizing exposure to dust and providing coccidioidomycosis safety training to all employees (*20*,*21*). It further requires monitoring for visual opacity caused by suspended dust and shutting down work if the opacity exceeds 20%.

The 43 interviewed employees reported working outdoors a median of 10 (range 0–11) hours/day. A total of 25 (58%) reported frequently performing soil-disruptive exposure activities, including digging, working in a ditch or trench, or operating heavy machinery (Table 3); of these 25, only 6 (24%) reported frequently using respiratory protection. Of 42 patients, 39 (93%) reported substantial dust levels at the worksite most days. Of 41 patients, most (31 [76%]) reported frequently driving home in work clothes, potentially increasing exposure to *Coccidioides*. None reported exposure to dust outside of work, and only 3 (7%) reported working at a construction site other than the solar farms ≤4 weeks before symptom onset.

Of the 44 patients, 36 (82%) were employees at solar farm A (Figure 1, panel A); of these, 29 worked for employer A1, 3 worked for employer A2, and 4 worked for other employers. The number of incident cases by month for employers A1 and A2 combined was associated with the average worker count onsite (Spearman $r = 0.62$; $p<0.01$), indicating that individual workers' temporal risk for infection was relatively constant. The incidence rate among employees of employers A1 and A2 combined was 5,168 cases/100,000 person-years, 135 times (95% CI 84–215 times) the 2012 incidence rate of 38.4 cases/100,000

Table 1. Demographic characteristics of 44 coccidioidomycosis patients who worked at 2 solar power–generating facilities, San Luis Obispo County, California, USA, with symptom onset October 2011–April 2014*

Characteristic	Value
Male sex, no. (%)	41 (93)
Median age, y (range)	48 (21–63)
Ethnicity, no. (%), n = 41	
Hispanic	11 (26)
Not Hispanic	28 (68)
Don't know or data missing	2 (5)
Race, no. (%), n = 41	
White	28 (68)
Not white†	6 (15)
Don't know or declined to state	7 (17)
Job title, no. (%), n = 43	
Electrician, lineman, or wireman	14 (33)
Heavy equipment operator	11 (26)
Laborer	6 (14)
Carpenter, ironworker, millwright, or mechanic	5 (12)
Manager or superintendent	4 (9)
Other	3 (7)
Permanent residence, no. (%)	
California, San Luis Obispo County	14 (32)
California, other *Coccidioides*-endemic county‡	3 (7)
California, less *Coccidioides*–endemic county	17 (39)
Other state with possible *Coccidioides* endemicity§	6 (14)
Any other state	4 (9)

*Of the 44 patients, 43 were interviewed and 41 completed the interview.
†Including African American, Filipino, Samoan, Native American, and multiracial.
‡Other *Coccidioides*-endemic counties are Fresno, Kern, Kings, Madera, and Tulare.
§Other possibly *Coccidioides*–endemic states are Arizona, Nevada, New Mexico, Texas, and Utah.

person-years for San Luis Obispo County (*19*) and 109 times (95% CI 82–145 times) the 2012 incidence rate of 47.4 cases/100,000 person-years for adult men in San Luis Obispo County (A. McDowell, unpub. data). We were unable to compare incidence rates among employers and across worksites because we were unable to obtain rosters that included first and last day of work from employers with coccidioidomycosis-diagnosed employees, other than employers A1 and A2.

Of 41 patients, 36 (88%) reported having received safety training regarding Valley fever; however their descriptions of the training provided ranged widely, from comprehensive safety training that addressed how to minimize dust exposure to more limited notification (required by San Luis Obispo County, condition of the permit) about the risk for Valley fever, symptom recognition, and diagnosis. Although not specifically asked during the interview, 8 patients volunteered that when seeking care for symptoms, they had asked their health care provider to test for coccidioidomycosis.

Public Health Response

On July 22, 2013, CDPH provided interim recommendations for preventing and reporting coccidioidomycosis among workers to all identified employers at both solar farms and associated unions based, in part, on the CDPH published guidelines (*22*). The recommendations extended beyond the original (local) permitting requirements, and included the following:

- Improve worksite dust-control measures, including stabilizing disturbed soil areas, increasing soil watering frequency, using soil binders, and covering excavated soil.
- Equip all earth-moving equipment and trucks with high-efficiency particulate air ([HEPA]-filtered) enclosed cabs to protect the operator.
- Implement and enforce criteria for suspending work on the basis of wind and dust conditions.
- Provide all outdoor workers access to National Institute for Occupational Safety and Health–approved respiratory protection (respirators with particulate filters designated N95, N100, P100, or HEPA) within the context of a comprehensive, OSHA-compliant respirator program including provision of medical clearance, fit testing, and training (*23*). (Respirator use was recommended when conducting or in close proximity to soil-disturbing work, and for exposure to excessive wind-blown dust, but not necessarily for the entire work shift in recognition of possible variation in exposure levels and the potential risk of heat stress.)
- Provide clean coveralls daily to employees; encourage workers to remove coveralls and work shoes before entering vehicles to leave the worksite.

Table 2. Clinical characteristics of 44 coccidioidomycosis patients who worked at 2 solar power–generating facilities, San Luis Obispo County, California, USA, with symptom onset October 2011–April 2014*

Characteristic	Value
Visited emergency department, no. (%)	17 (39)
Hospitalized, no. (%)	9 (20)
Length of hospitalization, median no. days (range)	3 (2–17)
Disseminated disease, no. (%)	2 (5)
No. days to symptoms from first day at worksite, median (range), n = 43	105 (10–638)
Symptoms, no. (%), n = 41	
Fatigue	41(100)
Night sweats	39 (95)
Weakness	38 (93)
Difficulty breathing	37 (90)
Fever	35 (85)
Cough	33 (80)
Joint or muscle pain	33 (80)
Weight loss	33 (80)
Chest pain	29 (71)
Headache	29 (71)
Rash or other skin lesions	24 (59)
Missed work, no. (%), n = 41	34 (83)
Days missed work, median no. (range), n = 34	22 (1–547)
Diagnosed following first visit to provider, no. (%), n = 41	9 (22)
No. days to laboratory diagnosis from symptom onset, median (range), n = 43	23 (5–267)
Frequency of positive laboratory result by diagnostic method, no. (%)	
Complement fixation, immunodiffusion, or tube precipitin	37 (84)
Complement fixation only	10 (23)
Immunodiffusion only	10 (23)
Tube precipitin only	2 (5)
Immunohistochemistry or culture only	3 (7)
IgG/IgM enzyme immunoassay only	4 (9)

*Of the 44 patients, 43 were interviewed and 41 completed the interview.

- Improve compliance by employers and their designated health care providers with reporting cases to local health jurisdictions, workers' compensation carriers, and Cal/OSHA.

Solar farm B, which continued construction after solar farm A was completed, reported implementing the CDPH recommendations and having reduced the amount of grading. During September 2013, multiple employers at both solar farms were cited by Cal/OSHA for failure to implement appropriate control measures for coccidioidomycosis as part of their injury and illness prevention programs, failure to provide adequate employee respiratory protection, and failure to report serious employee illnesses and hospitalizations resulting from coccidioidomycosis (*24*). Despite the general contractors' awareness of *Coccidioides* in the soil and implementation of some dust-reduction steps, the measures used were insufficient to prevent an excess of laboratory-confirmed cases over expected rates, including illnesses among patients who reported infrequently engaging in soil-disruptive work. Therefore, more effective methods to control dust and better adherence to exposure-control standards were deemed necessary.

Discussion

This outbreak of coccidioidomycosis among workers in the growing solar power industry is concerning because additional solar farms are being planned for *Coccidioides*-endemic areas. Large-scale construction, including solar farm construction, might involve substantial soil disturbance for months, and many employees, particularly from non–*Coccidioides*-endemic areas, probably lack immunity to *Coccidioides*.

Given the frequently dusty conditions, as well as the fact that coccidioidomycosis can be a mild and self-limiting illness for which persons do not seek medical attention, we believe that additional employees at these 2 solar farms were probably exposed to *Coccidioides* and had undiagnosed illness of mild or moderate severity. Thus, the number of confirmed cases is probably an underestimate of the extent of the outbreak. Even so, the health consequences, medical resources required, and lost productivity for the identified patients have been substantial.

Although some patients may have been exposed to *Coccidioides* spores away from the worksite, no patients reported exposure to dust outside of work. Furthermore, the high incidence rate among these workers relative to background rates demonstrates that occupational exposure played a predominant role in disease incidence. Because nearly half of the patients reported infrequent or no soil-disruptive work, it is likely that factors other than direct work activities contributed to coccidioidomycosis risk on this worksite. Dust-producing activities and high winds in this area expose workers to ambient dust from inside and

outside the work area (Figure 3, panel B); therefore, employers should assess whether respirators (in addition to wetting the local soil and other dust control measures) are necessary for reducing dust exposure for outdoor workers. Respiratory protection may be particularly advisable in the near field, such as work in trenches where other dust-reduction techniques may be less effective. Admittedly, no published study has compared incidence among 2 similarly exposed groups to address the effectiveness of air-purifying respirators for preventing coccidioidomycosis, and wearing an N95 filtering facepiece or half-face elastomeric respirator for a prolonged period is uncomfortable. However, because air-purifying respirators greatly reduce exposure to airborne particles (*25*,*26*), outdoor workers in *Coccidioides*-endemic areas should be protected from exposure to a recognized hazard and provided this personal protective equipment to use during soil-disturbing activities or when outdoors during dust storm or high winds. Also, employers should be required by local permitting agencies to prepare (and adhere to) comprehensive coccidioidomycosis-prevention strategies, including plans for minimizing dust generation, limiting workers' exposure, and establishing criteria for stopping work when wind or dust is excessive or uncontrollable. Prompt reporting of employee illnesses to local health jurisdictions and to OSHA programs will help identify worksites where control measures need improvement.

A previous study determined that patients in Arizona who were aware of coccidioidomycosis received a diagnosis earlier than those who were unaware (median 20 vs. 25 days) (*27*). The relatively rapid diagnoses of illnesses during the outbreak described in this article demonstrates the value of training workers to recognize and report their illness to supervisors and for employers to promptly refer ill employees to health care providers. Health care providers in non–C*occidioides*-endemic areas might not consider coccidioidomycosis in the differential diagnosis of prolonged febrile respiratory illnesses. Reports such as this one can help increase the index of suspicion for this condition and prompt providers to ask patients about potential environmental dust exposures acquired elsewhere.

Describing the occupational burden of coccidioidomycosis is challenging because existing data sources are not comprehensive. Although incidence rates estimated by using statewide workers' compensation claims have been reported (*18*), these estimates include only those employees who sought workers' compensation; in the outbreak described in this article, few cases were initially identified by review of workers' compensation claims. Similarly, in a 2012 outbreak among outdoor workers, only 2 of 10 cases were initially identified by review of DFRs (*15*). The variety of data sources necessary to describe the scope of this outbreak highlights the need for improved recognition and reporting of occupational illness by medical providers to public health jurisdictions as required under public health regulations and, in instances of serious illness involving hospitalization, for employer compliance with mandated reporting to occupational safety and health regulatory agencies.

This investigation had limitations. The calculated relative risk for workers, although impressive, compares denominators of total person-time worked at the solar farm for workers and assumes a stable population for San Luis Obispo County. We were unable to obtain rosters from all employers and unable to estimate person-years of work time for all employees at the 2 solar farms. As a consequence, we recommend that in the future,

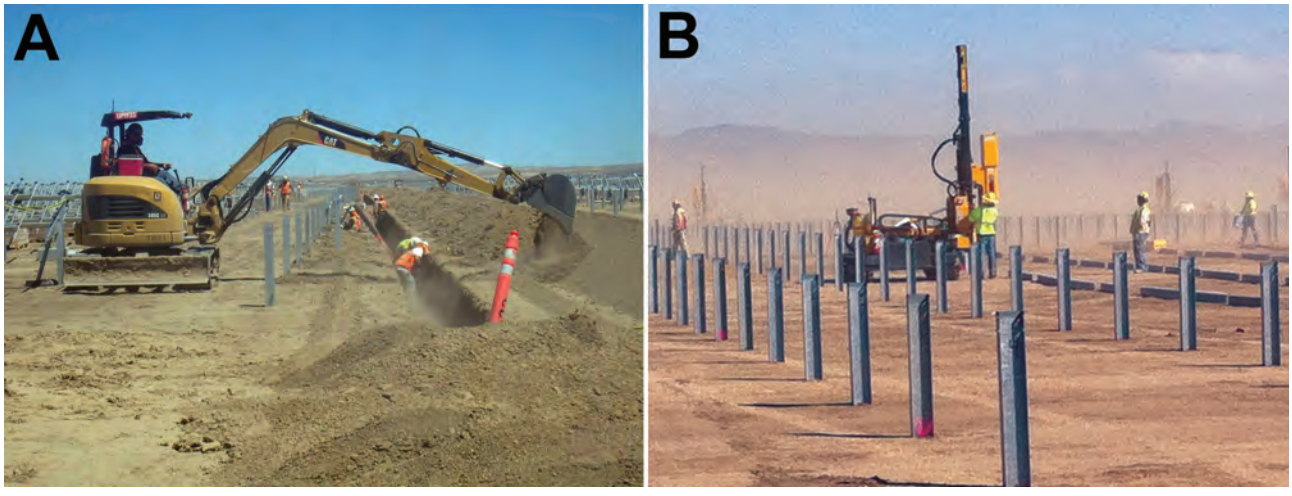

Figure 3. Conditions during solar farm construction in San Luis Obispo County, California, USA. A) Localized dust generation associated with a soil-disruptive activity. Photograph was taken during the week of July 28–August 3, 2013 (courtesy of Aspen Environmental Group). B) Ambient dust exposure because of high-wind conditions. Photo was taken on March 5, 2013 (courtesy of Dennis Shusterman).

Table 3. Potential worksite *Coccidioides* exposures and use of control measures during 4 weeks before symptom onset by workers at 2 solar power–generating facilities, San Luis Obispo County, California, USA, October 2011–April 2014*

Exposure	No. (%)		
	Frequently	Infrequently	Don't know
Work duties involved, n = 43†			
Manual digging	12 (28)	31 (72)	0
Working in a ditch or trench	19 (44)	24 (56)	0
Operating heavy machinery‡	8 (19)	35 (81)	0
Any of the above	25 (58)	18 (42)	0
High dust levels, n = 42†	39 (93)	0	3 (7)
Used a respirator, n = 41§¶	10 (24)	31 (76)	0
At the end of work, did you†			
Drive home in work clothes, n = 41	31 (76)	9 (22)	1 (2)
Wash up before leaving work, n = 39	19 (49)	20 (51)	0
Shower immediately after getting home, n = 41	34 (83)	5 (12)	2 (5)

*Of the 44 patients, 43 were interviewed and 41 completed the interview. Some patients did not answer certain questions.
†Frequently includes the responses of "every day" and "once a week"; infrequently includes the responses of "rarely" and "never."
‡One of 8 worked with heavy equipment with an enclosed cab and high-efficiency particulate air (HEPA)–filtered air-conditioning every day.
§Frequently includes the responses of "always" and "often"; infrequently includes the responses of "sometimes," "rarely," and "never."
¶Does not include 1 employee who reported operating heavy equipment with an enclosed cab and HEPA-filtered air-conditioning every day.

conditions for permit approval for large construction projects in *Coccidioides*-endemic areas should require that complete employee rosters including date of birth be provided to the local health jurisdiction. Case-finding was also complicated because a substantial number of employees resided outside San Luis Obispo County and California. In addition, interviews were conducted a median of 335 days after symptom onset, giving rise to the potential for recall bias.

In conclusion, unless awareness is emphasized and effective prevention measures are implemented, additional construction in *Coccidioides*-endemic areas, including solar power facility construction, will probably expose workers to *Coccidioides*, thus leading to additional infections. Awareness and prevention of coccidioidomycosis among all personnel at these and other similar construction sites should be included among the priorities for employee safety. Medical providers should consider work-related coccidioidomycosis when evaluating construction workers with prolonged febrile respiratory illness, particularly after work in central or southern California or in Arizona, and medical providers should follow all statutory requirements for documenting and reporting occupational illness.

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Dr. Wilken was an Epidemic Intelligence Service Officer with the Centers for Disease Control and Prevention, assigned to CDPH, at the time of this investigation. His primary research interests are environmental, occupational, and infectious disease epidemiology, and emergency preparedness and response.

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Shigella Infections in Household Contacts of Pediatric Shigellosis Patients in Rural Bangladesh

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To examine rates of *Shigella* infections in household contacts of pediatric shigellosis patients, we followed contacts and controls prospectively for 1 week after the index patient obtained care. Household contacts of patients were 44 times more likely to develop a *Shigella* infection than were control contacts (odds ratio 44.7, 95% CI 5.5–361.6); 29 (94%) household contacts of shigellosis patients were infected with the same species and serotype as the index patient's. Pulsed-field gel electrophoresis showed that 14 (88%) of 16 with infected contacts had strains that were indistinguishable from or closely related to the index patient's strain. Latrine area fly counts were higher in patient households compared with control households, and 2 patient household water samples were positive for *Shigella*. We show high susceptibility of household contacts of shigellosis patients to *Shigella* infections and found environmental risk factors to be targeted in future interventions.

In South Asia and Africa, an estimated 88.5 million diarrhea episodes are attributed to *Shigella* infections annually (*1*). Shigellosis occurs most often in children <5 years of age (*2*,*3*). Two recent multicountry studies found that Bangladesh has the highest rates of shigellosis (*4*,*5*). In the recent Global Enteric Multicenter Study (GEMS) conducted at a study site in Mirzapur, Bangladesh, *Shigella* was the third leading cause of moderate-to-severe diarrhea in children 12–23 months of age and the second leading cause of moderate-to-severe diarrhea in children 24–59 months of age (*5*). In addition, hospital-based surveillance of *Shigella* in Bangladesh found that patients from rural health facilities have higher rates of *Shigella* isolates than patients from urban facilities (e.g., 3% in urban Dhaka vs. 12% in rural Mirzapur during 2011) (*6*). Previous studies have identified risk factors for developing shigellosis, such as age (*7*–*9*), high fly counts (*10*–*12*), contaminated food (*13*,*14*), and recent overnight travel (*8*). Furthermore, a recent study in rural Bangladesh found that 10% of tube wells sampled had detectable *Shigella* (*15*).

Household studies have indicated that family members of shigellosis patients are at much higher risk for developing a *Shigella* infection than the general population (13–19 cases/100 shigellosis patient households vs. 7 cases/100 control households) (*7*,*8*). However, little research has been done to identify clinical and environmental transmission routes for *Shigella* infection in this susceptible population.

Shigella includes 4 species and numerous serotypes: *S. flexneri* (17 serotypes), *S. dysenteriae* (16 serotypes), *S. boydii* (20 serotypes), and *S. sonnei* (1 serotype) (*16*). A study in Wisconsin found that isolates from family members of index shigellosis patients were always the same serotype as the index patient's (*7*). In contrast, studies in rural (*8*) and urban (*9*) Bangladesh found that 75% and 72%, respectively, of infected household contacts of shigellosis patients excreted serotypes different from the index patient's serotype. These studies suggest that *Shigella* infections in Bangladesh are attributable to both secondary transmission and external infecting sources. To examine the rate of *Shigella* infection within households of shigellosis patients and to investigate risk factors for infection, we prospectively observed a cohort of household contacts of pediatric shigellosis patients and community controls in rural Mirzapur, Bangladesh.

Methods

This study was conducted in Mirzapur, a subdistrict of Bangladesh's Tangail district, at a field site of the icddr,b. Mirzapur is the Bangladesh site of the GEMS Demographic Surveillance System. We received ethical approval for this study from the icddr,b ethical review committee and an exemption from the Institutional Review Board at

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Table 1. Characteristics of household contacts of pediatric shigellosis patients and of community controls, rural Bangladesh

Characteristic	Median ± SD (range) or no. (%)		p value
	Patient contacts, n = 81	Control contacts, n = 77	
No. enrolled contacts per household*	3.0 ± 0.73 (2.0–5.0)	3.0 ± 0.95 (2.0–6.0)	0.25
No. persons living in the household for past 6 mo*	5.0 ± 1.4 (3.0–9.0)	6.0 ± 2.7 (3.0 –15.0)	0.16
Age of contacts, y†	27.0 ± 16.9 (1.8–72.0)	30.0 ± 18.6 (3.5–89.0)	0.47
Hours contacts spent outside their home in the past 48 h during surveillance period†	2.0 ± 1.8 (0–6.3)	1.0 ± 1.8 (0–7.3)	0.09
Female sex‡	48 (58)	46 (61)	0.75
Drank water outside their home during surveillance period‡	57 (69)	48 (62)	0.41
Consumed food outside their home during surveillance period‡	51 (61)	36 (47)	0.08
Consumed uncooked vegetables or fruits during surveillance period‡	22 (27)	12 (16)	0.12

*For household characteristics, a Wilcoxon signed-rank test was used for paired continuous variables.
†For individual characteristics, a Wilcoxon rank-sum test was used for continuous variables.
‡For individual characteristics, a Fisher exact test was used for categorical variables.

the Johns Hopkins Bloomberg School of Public Health (Baltimore, MD, USA). Written informed consent was obtained from all study participants or their guardians.

A cohort of household contacts of index shigellosis patients and matched community controls was followed prospectively for 7 days after each index patient visited the Kumudini Women's Medical College and Hospital in Mirzapur for health care. Our sample size was determined by the number of pediatric shigellosis patients that could be recruited during October 2013–July 2014. Patients with suspected pediatric shigellosis were identified as children <5 years of age with dysentery, which was defined as having ≥1 stool containing blood during the previous 24 hours, as reported by a guardian or observed by research personnel. Community controls were matched to index shigellosis patients on the basis of age (within 3 months) and village location and were randomly selected by using the GEMS Demographic Surveillance System. Stool samples were collected from patients with suspected shigellosis and from community controls at time of enrollment in the study and were immediately sent in a cooler to the icddr,b Enteric and Food Microbiology Laboratory in Dhaka, Bangladesh, for bacterial culture analysis to detect *Shigella.*

Households of suspected shigellosis patients whose samples were found to be negative for *Shigella* by culture were excluded from the study. All index patients enrolled in the study received ciprofloxacin as part of their standard course of care at Kumudini Hospital.

After enrollment of pediatric shigellosis patients, we recruited household contacts of shigellosis patients and of community controls. Household contacts were defined as persons sharing the same cooking pot as the index shigellosis patient or control during the previous 3 days. To be eligible for the study, household contacts had to report that they would be present in the household of the patient or control for the next 7 days and be present for most household visits. Household contacts were followed prospectively for clinical and environmental surveillance by conducting household visits at days 1, 3, 5, and 7 after the initial visit of the index shigellosis patient at Kumudini Hospital.

For clinical surveillance, household contacts were asked to report whether, during the previous 48 hours, they had diarrhea (≥3 loose stools during a 24-hour period), dysentery (blood in stool observed by caregiver or research personnel), or vomiting. A stool sample was collected from enrolled household contacts at every visit. In addition, at each household visit, a questionnaire was administered to enrolled contacts to collect information on previously identified risk factors for *Shigella* infection. For environmental surveillance, on days 1 and 5, a water sample was collected directly from the household's primary drinking water source, and a second sample was collected from drinking water stored in the home for immediate consumption. The water samples were tested for *Shigella* spp. by bacterial culture and PCR. Trained field assistants also conducted fly counts by using a Scudder grill at all surveillance visits over a period of 30 minutes at the kitchen and latrine area of each household, according to previously published methods (*12*). Weekly fly counts for each household were the total number of flies observed during surveillance visits.

All stool and water samples collected were analyzed at the icddr,b Enteric and Food Microbiology Laboratory in Dhaka. The laboratory received no information identifying whether samples were from patient or control households. For isolation of *Shigella*, stool and water samples were cultured on MacConkey and *Shigella-Salmonella* agar media, and *Shigella* was isolated and serotyped by using standard microbiologic and biochemical methods described previously (*4*). In brief, water samples of 1,000 mL were filtered through 0.22 μm pore-size filters. The filter paper was enriched in 25 mL gram-negative broth at 37°C overnight and then analyzed by culture. Template DNA was prepared from the enriched broth and tested for the *ipa*H gene, according to previously published methods (*16*).

To determine genetic relatedness of *Shigella* strains isolated within households, pulsed-field gel electrophoresis (PFGE) was performed on all *Shigella*–positive water and stool samples, according to the PulseNet protocol (*17*). Agarose–embedded genomic DNA of *Shigella* strains was digested by using *Xba*I, and fragments were separated by

Table 2. Demographic and environmental characteristics of households of pediatric shigellosis patients and of community controls, rural Bangladesh

Characteristic	No. (%) or median ± SD (range) Patient households, n = 27	Control households, n = 27	p value
Demographic*			
Age of child, patient or control†			
0–11 mo	3 (11)	3 (11)	1.00
12–23 mo	11 (41)	11 (41)	
24–35 mo	6 (22)	6 (22)	
36–47 mo	6 (22)	6 (22)	
48–59 mo	1 (4)	1 (4)	
Female sex, patient or control†	13 (48)	13 (48)	
Primary caregiver educational level‡			
No formal education	2 (7)	4 (15)	0.48
Less than primary school	2 (7)	3 (11)	
Completed primary school or greater	23 (86)	20 (74)	
Electricity in home*	20 (74)	19 (70)	0.75
Environmental			
Main source of drinking water*			
Shallow tube well	16 (59)	16 (59)	1.00
Deep tube well	11 (41)	11 (41)	
Households with water source S*higella* positive by PCR for *ipa*H gene*	0	2 (7)	0.48
Households with stored water S*higella* positive by culture*	2 (7)	0	0.48
Households with stored water S*higella* positive by PCR*	2 (7)	1 (4)	1.00
Households with no soap observed at any surveillance visit*§	18 (67)	19 (70)	0.75
Floor type*			
Earth	18 (67)	23 (85)	0.13
Concrete	9 (33)	4 (15)	
Latrine type‡			
Ventilated improved pit latrine	14 (52)	12 (44)	0.49
Pour flush toilet	6 (22)	6 (22)	
Traditional pit latrine	6 (22)	8 (30)	
No facility	1 (4)	1 (4)	
Latrine area weekly fly counts¶	27 ± 20 (0–84)	16 ± 13 (0–48)	0.0014
Kitchen area weekly fly counts¶	59 ± 55 (0–216)	44 ± 48 (0–192)	0.47

*McNemar test was used for paired categorical variables.
†All patient–control pairs were the same.
‡Friedman test was used for paired categorical variables with >2 levels.
§Soap within 10 steps of location reported to be used for household defecation.
¶Wilcoxon signed-rank test was used for paired continuous variables.

using a CHEF-DR II apparatus (Bio-Rad, Hercules, CA, USA) (*18*). Genetic relatedness was determined on the basis of previously published methods (*19*). To compare strains within a single household, 4 categories of genetic relatedness were used: a) "indistinguishable" (all fragments matched); b) "closely related" (1–3 fragments differed); c) "possibly related" (4–6 fragments differed); and d) "unrelated" (≥7 fragments differed).

A *Shigella*-infected person was defined as a person with a stool sample positive for *Shigella* spp. by culture. Various tests were used for household-level variables: a McNemar test for paired binary variables; a Friedman test for clustered categorical variables with >2 levels; and a Wilcoxon signed-rank test for paired continuous variables (Tables 1, 2). For individual-level variables, a Fisher exact test was used for categorical variables

Table 3. Household infection characteristics of pediatric shigellosis patients and of community controls, rural Bangladesh*

Characteristic	Patient households, no. (%), n = 27	Control households, no. (%), n = 27	p value†
Households with ≥1 infected contact	16 (59)	1 (4)	<0.0001
Households with ≥1 contact with infection at visit 1	9 (33)	1 (4)	0.02
Households with ≥1 contact with initial infection detected at visits other than visit 1	11 (41)	0	0.001
Households with >1 infected symptomatic contact‡	4 (15)	0	0.07
Households with ≥1 infected contact with same species and serotype as index patient's	15 (94)	–	–
Households with ≥1 contact with different species and serotype than index patient's	2 (12)	–	–

*–, not applicable because control households had no index patient.
†McNemar test was used for paired categorical variables, and Wilcoxon signed-rank test for continuous paired variables.
‡Defined as a *Shigella* infection with diarrhea, vomiting, or blood in stool during previous 48 hours.

Table 4. Characteristics of household contacts with *Shigella* infections for pediatric shigellosis patients and community controls, rural Bangladesh

Characteristic	Patient contacts		Control contacts		p value*
	No. (%)	Total no.	No. (%)	Total no.	
Contacts infected	31 (37)	83	1 (1)	77	<0.0001
Contacts with symptomatic infections†	6 (7)	83	0	77	0.03
Contacts with infection detected on visit 1 of surveillance	13 (16)	83	1 (1)	77	0.0013
Contacts with initial infection detected on visits other than visit 1 of surveillance	18 (22)	83	0	77	
Infected contacts by sex					
M	18 (51)	35	0	31	0.44
F	13 (27)	48	1 (2)	46	
Infected contacts by relation to patient or control child					
Mother	9 (35)	26	0	26	0.09
Father	8 (53)	15	0	16	
Brother	6 (55	11	0	12	
Sister	2 (17)	12	1 (10)	10	
Other relative	6 (32)	19	0	13	
Infected contacts by *Shigella* species and serotype					
S. flexneri	20 (65)	31	0	1	0.63
S. flexneri 1b	2 (6)	31	0	1	
S. flexneri 1c	3 (10)	31	0	1	
S. flexneri 2a	12 (39)	31	0	1	
S. flexneri 3a	3 (10)	31	0	1	
S. flexneri 4X	0	31	0	1	
S. sonnei	9 (29)	31	1 (100)	1	
S. boydii	2 (6)	31	0	0	
S. boydii 7	1 (3)	31	0	0	
S. boydii	1 (3)	31	0	0	
S. dysenteriae	0	31	0	0	

*By Fisher exact test.
†Defined as a *Shigella* infection with diarrhea, vomiting, or blood in stool during previous 48 hours.

and a Wilcoxon rank-sum test for continuous variables (Tables 1, 2).

Logistic regression was used to estimate the odds of developing a *Shigella* infection. Generalized estimating equations were used to account for clustering within households and in patient–control pairs and to estimate an odds ratio (OR) and approximate 95% CIs. Clusters in this analysis are the 27 patient–control pairs. A bivariate analysis was performed in which index patient or control child status in the household was used as the single predictor, and a binary outcome was used to determine whether household members developed a *Shigella* infection. All analyses were performed by using SAS, version 9.3 (SAS Institute, Inc., Cary, NC, USA).

Results

During October 2013–July 2014, a total of 27 shigellosis patients with 83 household contacts and 27 community controls with 77 household contacts were followed prospectively. Of the initial 61 suspected shigellosis patients who were screened, 29 were excluded because cultures were negative for *Shigella*, and 5 were excluded because the caregiver was too busy or uninterested in the study. Of the 33 community controls screened, 6 were excluded because they did not pass stool on visit 1. Of 88 shigellosis–patient household contacts and 81 control household contacts who were screened for eligibility, 5 (6%) and 4 (5%), respectively, were excluded from the study because they did not pass stool on visit 1.

Median age for household contacts was 27 years for patient households and 30 years for control households (Table 1). Of the 83 household contacts in patient households, 48 (58%) were women, compared with 47 (61%) of the 77 contacts in control households. Patient and control households did not differ significantly by age, sex, number enrolled, or number of total contacts (Table 1). Among household contacts of patients and control children, 52 (33%) were mothers, 31 (19%) fathers, 23 (14%) brothers, 22 (14%) sisters, and 32 (20%) other relatives. Contact relationship to the patient or control child did not differ significantly ($p = 0.88$).

During the surveillance period, patient contacts reported spending more time outside the home than did control contacts (median time outside home during previous 48 hours, 2 hours vs. 1 hour; $p = 0.09$); eating more food outside the home (61% vs. 47%; $p = 0.08$); and consuming more uncooked vegetables and fruits (27% vs. 16%; $p = 0.12$). These differences were not statistically significant (Table 1). The rate for acquiring stool samples was 97% for enrolled household contacts and did not differ significantly for patient and control households ($p = 0.15$).

We found no significant differences between patient and control households in caregiver's education level, type of latrine or floors, or presence of soap at the

Table 5. Patient households with *Shigella*-infected household contacts by *Shigella* species and serotype and visit number at which infection was detected, rural Bangladesh*

Household	Household member	Species and serotype	Visit no.	No. PFGE genotypes within household	Household genetic relatedness of strains†
Household 1	Patient	*S. flexneri* 2a	1	2	Closely related
	Contact 1	*S. flexneri* 2a	3		
	Contact 2	*S. flexneri* 2a	1		
	Contact 2	*S. flexneri* 2a	2		
Household 2	Patient	*S. flexneri* 2a	1	2	Unrelated
	Contact 1	*S. flexneri* 2a	3		
	Contact 1	*S. flexneri* 2a	4		
	Contact 2	*S. boydii*	1		
Household 3	Patient	*S. sonnei*	1	1	Indistinguishable
	Contact 1	*S. sonnei*	1		
	Contact 1	*S. sonnei*	2		
	Contact 2	*S. sonnei*	4		
	Stored water	*S. sonnei*	1		
Household 4	Patient	*S. sonnei*	1	1	Indistinguishable
	Contact 1	*S. sonnei*	1		
	Contact 1	*S. sonnei*	2		
Household 5	Patient	*S. flexneri* 2a	1	2	Closely related
	Contact 1	*S. flexneri* 2a	4		
	Contact 2	*S. flexneri* 2a	2		
	Contact 2	*S. flexneri* 2a	3		
	Contact 2	*S. flexneri* 2a	4		
Household 6	Patient	*S. flexneri* 2a	1	2	Closely related
	Contact 1	*S. flexneri* 2a	1		
	Contact 1	*S. flexneri* 2a	2		
	Contact 2	*S. flexneri* 2a	1		
	Contact 2	*S. flexneri* 2a	2		
	Contact 3	*S. flexneri* 2a	1		
Household 7	Patient	*S. flexneri* 1c	1	3	Closely related
	Contact 1	*S. flexneri* 1c	4		
	Contact 2	*S. flexneri* 1c	3		
	Contact 3	*S. flexneri* 1c	2		
	Contact 3	*S. flexneri* 1c	4		
Household 8	Patient	*S. flexneri* 4X	1	2	Unrelated
	Contact 1	*S. boydii* 7	2		
Household 9	Patient	*S. sonnei*	1	1	Indistinguishable
	Contact 1	*S. sonnei*	1		
	Contact 1	*S. sonnei*	2		
	Contact 2	*S. sonnei*	2		
Household 10	Patient	*S. flexneri* 1b	1	3	Closely related
	Contact 1	*S. flexneri* 1b	1		
	Contact 1	*S. flexneri* 1b	2		
	Contact 1	*S. flexneri* 1b	3		
	Contact 2	*S. flexneri* 1b	1		
Household 11	Patient	*S. flexneri* 2a	1	1	Indistinguishable
	Contact 1	*S. flexneri* 2a	3		
	Contact 1	*S. flexneri* 2a	4		
	Contact 2	*S. flexneri* 2a	3		
Household 12	Patient	*S. flexneri* 3a	1	1	Indistinguishable
	Contact 1	*S. flexneri* 3a	4		
	Contact 2	*S. flexneri* 3a	4		
	Contact 3	*S. flexneri* 3a	2		
Household 13	Patient	*S. sonnei*	1	2	Closely related
	Contact 1	*S. sonnei*	2		
	Contact 1	*S. sonnei*	3		
Household 14	Patient	*S. sonnei*	1	1	Indistinguishable
	Contact 1	*S. sonnei*	1		
	Contact 1	*S. sonnei*	2		
Household 15	Patient	*S. flexneri* 2a	1	2	Closely related
	Contact 1	*S. flexneri* 2a	1		
	Contact 2	*S. flexneri* 2a	1		
Household 16	Patient	*S. sonnei*	1	1	Indistinguishable
	Contact 1	*S. sonnei*	4		
	Contact 2	*S. sonnei*	4		

*Excluding household contact with different *Shigella* species. PFGE, pulsed-field gel electrophoresis.
†Strain relatedness determined by using criteria in (*19*).

household latrine area, a proxy measure for handwashing with soap (Table 2). All households relied on tube wells as their primary drinking water source. The latrine areas of patient households had significantly higher weekly fly counts compared with those of control households ($p = 0.001$), but weekly kitchen fly counts did not differ significantly ($p = 0.47$). In patient households, 33% had concrete floors compared with 15% of control households; this difference was not statistically significant ($p = 0.13$). Household water samples from 2 (7%) patient households were positive by PCR for the *ipaH* gene of *Shigella* and were culture positive for non–type 1 *S. dysenterae* and *S. sonnei* during the surveillance period, compared with 1 PCR-positive (for the *ipaH* gene) household water sample and 2 PCR-positive samples from household water sources in control households.

Of the 27 patient households, 16 (59%) had ≥1 *Shigella*-infected contact during the 7-day surveillance period, compared with 1 (4%) control household, in which an asymptomatic *Shigella* infection was detected on day 1 of clinical surveillance (Table 3). In a bivariate model that used patient household as the predictor, the odds of developing a *Shigella* infection were 44 times higher for patient contacts than for control contacts (OR 44.7, 95% CI 5.5–361.6). The 16 patient households had 31 *Shigella*-infected contacts, compared with 1 *Shigella*-infected contact in 1 control household (Table 4). Four (15%) patient households had 6 contacts with symptomatic *Shigella* infection (i.e., having diarrhea, vomiting, or blood in stool during the previous 48 hours). *Shigella* infections for 13 (42%) of 31 *Shigella*-infected patient contacts (asymptomatic and symptomatic) were first detected on day 1 of clinical surveillance; 6 (19%) were detected on day 3, 5 (16%) on day 5, and 7 (23%) on day 7.

In patient households, a *Shigella* infection developed in 18 (51%) of 35 male contacts and in 13 (27%) of 48 female contacts during the surveillance period ($p = 0.02$). Five of the 6 symptomatic *Shigella* infections were in men, and 4 of the infections were first detected on day 1 or 3 of surveillance. Difference in day of initial detection by sex was not significant ($p = 0.53$), but male contacts spent significantly more time outside their homes during the surveillance period ($p<0.0001$) and reported drinking significantly more water outside their homes ($p<0.0001$) than did female contacts.

During the surveillance period, 4 patient contacts reported using antibacterial drugs; 3 of those had a symptomatic *Shigella* infection, 1 of whom was hospitalized for symptoms. This person tested positive on visit 1. To estimate duration of shedding for patient household contacts, we observed the time during which shedding occurred for 6 household contacts with a *Shigella* infection first detected on visit 2. Of these 6 contacts, 5 (83%) had detectable shedding for ≤2 days: 3 for 1 day, 2 for 2 days, and 1 for 3 days.

Among the 31 patient household contacts in whom *Shigella* infection developed, 29 (94%) were infected with the same species and serotype as the index patient in their household (Table 5). Twenty (65%) of the 31 patient household contacts with detectable *Shigella* in stool by culture had *S. flexneri* (2 *S. flexneri* 1b, 3 *S. flexneri* 1c, 12 *S. flexneri* 2a, and 3 *S.flexneri* 3a); 9 (29%) had *S. sonnei*; and 2 (6%) had *S. bodyii* (1 *S. boydii* 7 and 1 *S. boydii* of an unknown serotype) (*16*–*18*). In the 16 patient households with infected household contacts, 7 (44%) contacts had strains indistinguishable from those of the index patient by PFGE analysis; 7 (44%) had closely related strains; and 2 (12%) had unrelated strains (Table 5).

Discussion

We found that the odds of developing a *Shigella* infection were >44 times higher for contacts of pediatric shigellosis patients than for control contacts (OR 44.7, 95% CI 5.5–361.6). We also observed that 94% of patient household contacts had the same species and serotype as the index patient. Consistent with this finding, PFGE analysis found that 88% of households with infected household contacts had strains that were indistinguishable from or closely related to the index patient's strain. In contrast, 2 previous studies in Bangladesh found that only one quarter of household contacts had the same species and serotype as the index patient in their home (*8*,*9*). Our finding suggests that a single infectious pathogen is being spread in study households; however, whether the infections are caused by a shared environmental source or secondary transmission from an infected household member is unclear.

We observed that most (59%) patient households had ≥1 household contact with a *Shigella* infection over the 7-day surveillance period, and 25% of these households had contacts who had symptomatic infections. In comparison, 1 (4%) control household contact had a *Shigella* infection, which was asymptomatic. The proportion of shigellosis patient households with *Shigella*-infected contacts in our study is higher than has been previously reported. In a study in Peru, 34% of households of index shigellosis patients during the 1-week surveillance period had infected contacts (*20*). Another study conducted in rural Bangladesh in 1974 found that 19% of members of household compounds (i.e., multiple households living together) developed *Shigella* infection over a 10-day period after identification of the index shigellosis patient, compared with 7% of members of control household compounds (*21*). The reason our study found a higher rate of infection in household contacts of shigellosis patients is unknown but may reflect differences in environmental risk factors or population immunity.

Despite no significant difference in toilet type, we found significantly higher fly counts in the latrine areas of patient households than in control households during the

surveillance period (Table 2). This finding likely suggests that patient households had latrines with poorer sanitary conditions than did control households. Similarly, a recent study conducted in the same site found a significant association between seasonal fly densities and peaks in pediatric shigellosis patients (*12*). Furthermore, these significant associations are consistent with the growing body of literature that implicates houseflies as vectors of shigellosis. The 2 flies thought to be responsible for transmission of *Shigella* are *Musca domestica*, because of its mobility, and *M. sorbens*, because it commonly breeds in human feces. Previous studies in Bangladesh, Myanmar, Egypt, and the United States have detected *Shigella* in flies by culture (*11*). An intervention study conducted on 2 military field bases in Israel found that baited fly traps reduced fly counts by 64% and rates of shigellosis by 85% compared with fly counts and shigellosis rates on the control base (*10*). These studies suggest that fly control could be a promising intervention to reduce *Shigella* transmission in the study population. Future studies should more closely evaluate the potential of flies to be a vector of *Shigella* in our study population by culturing flies and conducting fly species identification.

Our study also found 2 (7%) patient households with stored water samples positive for *Shigella*. In 1 household, detectable *S. sonnei* by culture was found in stool from the patient and 2 household contacts and in stored water. This finding suggests potential secondary contamination of stored water by a household member, particularly because none of the corresponding source water samples had detectable *Shigella* by culture. In another household, *S. dysenteriae* was found in stored water but was not detected in any household members. A potential explanation for this finding is that the *Shigella* came from the household tube well. A previous study in rural Bangladesh identified *Shigella* by PCR in 10% of tube wells; that study also found that 40% of these tube wells were contaminated with rotavirus, 10% with *Vibrio* spp., and 8% with pathogenic *Escherichia coli* (*15*). The mostly likely source of *Shigella* in tube well water is fecal contamination from latrines, which are commonly located near tube wells used for drinking in rural Bangladesh. Further research is needed to evaluate whether groundwater is a major environmental transmission route for shigellosis and other enteric infections in this population.

We observed that male patient contacts were twice as likely as female contacts to develop *Shigella* infection during the surveillance period (51% vs. 27%); all but 1 symptomatic infections in contacts were in men. The reason for this higher rate of infection among male household contacts is unknown, but a possible explanation is that men may introduce the infection into the home. Future studies should investigate the role of sex in susceptibility and transmission of *Shigella* infection.

Among patient households, 41% had ≥1 contact who developed an initial *Shigella* infection after day 1 of surveillance. This finding suggests a potential opportunity to intervene in *Shigella* transmission in households with shigellosis patients. An intervention study that promoted handwashing with soap in Dhaka reduced the secondary infection rate for *Shigella* by 69% in the 10-day period after identification of the index patient compared with a control group (*22*). Future studies should evaluate whether this intervention would be effective in rural settings such as Mirzapur.

This study has several limitations. First, our small sample size limited our ability to detect significant differences in environmental risk factors for *Shigella* infection at the household level and to detect differences in behavioral risk factors at the individual level. Second, our analysis focused on pediatric index shigellosis patients, so our findings are not necessarily generalizable to older index patients. Third, we did not collect longitudinal stool samples from index patients and therefore cannot determine how long their shedding may have continued through the 1-week surveillance period. However, because all index patients received antibacterial drugs, we suspect that the shedding was minimal and that the source of *Shigella* infection in these households during the surveillance period was more likely from household members already infected or from a shared environmental source that we did not measure. Fourth, we used bacterial culture to detect *Shigella* in the stool samples. This method limited our analysis to infections with sufficiently high bacteria quantity in stool to be detected by culture. Future studies should use bacterial culture and quantitative PCR on collected stool samples. Finally, future studies should obtain >1 isolate from stool samples collected from each household contact to determine whether a person can shed multiple PFGE genotypes.

A main strength of our study was the environmental surveillance of household water sources, stored household water, and fly counts in study households. Second, we included households of both shigellosis patients and controls; this approach enabled us to examine rates of *Shigella* infection in patient households, compared with control households, and to investigate household-level risk factors for shigellosis. Third, we followed up with study households at 4 specific times during a 1-week period to obtain detailed information on potential behavioral and environmental risk factors for *Shigella* infection. Fourth, we used PFGE to conduct genetic characterization of strains within households.

In rural Bangladesh, household contacts of shigellosis patients are highly susceptible to *Shigella* infection during the week after the index patient visits a health facility for care. Our findings suggest that each shigellosis patient household represents the spread of a single infectious pathogen. Therefore, interventions for household-level risk

factors, such as fly control, water treatment, and hygiene practices, could potentially reduce *Shigella* transmission in this high-risk population.

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Carbapenem-Resistant *Enterobacteriaceae* in Children, United States, 1999–2012

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The prevalence of carbapenem-resistant *Enterobacteriaceae* (CRE) infections is increasing in the United States. However, few studies have addressed their epidemiology in children. To phenotypically identify CRE isolates cultured from patients 1–17 years of age, we used antimicrobial susceptibilities of *Enterobacteriaceae* reported to 300 laboratories participating in The Surveillance Network–USA database during January 1999–July 2012. Of 316,253 isolates analyzed, 266 (0.08%) were identified as CRE. CRE infection rate increases were highest for *Enterobacter* species, blood culture isolates, and isolates from intensive care units, increasing from 0.0% in 1999–2000 to 5.2%, 4.5%, and 3.2%, respectively, in 2011–2012. CRE occurrence in children is increasing but remains low and is less common than that for extended-spectrum β-lactamase–producing *Enterobacteriaceae*. The molecular characterization of CRE isolates from children and clinical epidemiology of infection are essential for development of effective prevention strategies.

Gram-negative bacteria belonging to the family *Enterobacteriaceae* are major causes of health care-acquired and community-acquired infections. In the past 3 decades, antimicrobial drug resistance in this family of bacteria has increased dramatically, specifically because of enzymes that hydrolyze broad-spectrum β-lactam antimicrobial drugs (*1*,*2*). Genes encoding AmpC cephalosporinases (AmpC) may be chromosomal or plasmid-based in origin, whereas genes encoding extended-spectrum β-lactamases (ESBLs) are most often carried on mobile genetic elements, such as plasmids or transposons, and cause resistance to all β-lactams except carbapenems and cephamycins (*1*–*4*). However, ESBLs and AmpC can confer carbapenem resistance when associated with alteration or loss of porins, a family of proteins on the outer membrane of gram-negative bacteria (*2*,*5*).

In recent years, the rapid global spread of carbapenem-resistant *Enterobacteriaceae* (CRE) has been facilitated by mobile genetic elements harboring genes encoding for carbapenemases, such as *Klebsiella pneumoniae* carbapenemase (KPC) and metallo-β-lactamases (*6*,*7*). More recently, oxacillinase 48 (OXA-48)–producing *Enterobacteriaceae* have emerged in the United States, adding to major increases in CRE infections (*8*). Carbapenem-resistant organisms often carry additional plasmid-borne genes against other antimicrobial drug classes, rendering them multidrug resistant (MDR).

Few, if any, antimicrobial drugs are able to treat these infections, and their prevalence is increasing in the United States, including in children (*9*). The National Healthcare Safety Network of the US Centers for Disease Control and Prevention (CDC) reported that the proportion of *Enterobacteriaceae* that were carbapenem-resistant increased from 1.2% in 2001 to 4.2% in 2011, and that in 2012, 4.6% of acute-care hospitals reported ≥1 CRE hospital-acquired infection (*10*). In a 2013 Threat Report on Antimicrobial Resistance, the CDC prioritized CRE as an urgent threat (the highest level), requiring concerted commitment and action, and noted that ≈50% of hospitalized patients with bloodstream infection caused by CRE die from the infection (*10*,*11*).

Despite this increased attention for CRE in the United States (*6*,*12*–*14*), limited data are available on the epidemiology of these infections in children (*9*,*15*,*16*). In this study, our primary objective was to describe the national and regional epidemiology of CRE in children in the United States.

Methods

Antimicrobial drug susceptibility data were obtained from The Surveillance Network (TSN) database-USA (Eurofin-Medinet, Herndon, VA, USA). This database has been used to characterize national antimicrobial drug susceptibility trends (*14*,*17*–*19*). The network includes ≈300 clinical microbiology laboratories that serve one or more patient

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care facilities. Laboratories included in the network were selected to be representative of hospitals in each of the 9 US Census Bureau regional divisions. To be included in the TSN database, the laboratories must submit results from all routine antimicrobial drug susceptibility testing performed on site. Categorical result interpretations are based on the Clinical Laboratory Standards Institute (http://clsi.org/) criteria adopted by the reporting facilities at the time of testing and reflect susceptibilities as reported to clinicians. The data are electronically validated and merged into a central TSN database.

The database includes records with the following information: identified organism; tested drug and susceptibility result: susceptible, intermediate resistance, or resistant; source of the isolate: blood, urine, wound, lower respiratory tract, or other (upper respiratory tract and skin cultures); patient characteristics: age, sex; the health care setting where the patient sample was collected: outpatient (ambulatory), inpatient intensive care unit (ICU), inpatient (non-ICU), and long-term care settings; the geographic location of the laboratory where the specimen was tested; and the date of the drug susceptibility test.

Our analysis considered relevant isolates obtained from all children (age range 1–17 years) in outpatient (ambulatory), inpatient ICU, inpatient non-ICU, and long-term care settings during January 1, 1999-June 30, 2012. The included pathogens were *Escherichia coli*, *K. pneumoniae*, *Proteus mirabilis*, *Enterobacter cloacae*, *E. aerogenes*, *Citrobacter freundii*, *C. koseri*, and *Serratia marcescens*. *K. oxytoca* and *Providencia* species were not included in the TSN database. A separate analysis was performed on isolates from infants (age <1 year) because data were only available from 2010 onwards.

We defined the CRE phenotype by using CDC criteria to include relevant isolates that were resistant to all third-generation cephalosporins (ceftriaxone, cefotaxime, or ceftazidime), and nonsusceptible to ≥1 carbapenem (ertapenem, meropenem, imipenem, or doripenem) (*20*). Isolates that were not tested against all 3 third-generation cephalosporins were still classified as CRE if they were resistant against all tested third-generation cephalosporins. For bacteria with intrinsic imipenem nonsusceptibility (*P. mirabilis*, *Providencia* spp.), the CRE criteria required nonsusceptibility to meropenem, doripenem, or ertapenem (*20*).

Data were filtered to retain isolates that were tested against ≥1 third-generation cephalosporin and ≥1 carbapenem of those considered for the CRE phenotype. When duplicate records (with same identification number, drug susceptibility test, and source location) existed, the first record was kept and the other records were discarded. The frequency of the CRE phenotype is reported as the proportion of positive isolates of all tested isolates included in the analysis. Individual susceptibility results were stratified by location (ICU, inpatient non-ICU, and outpatient); patient age (1–5, 6–12, and 13–17 years); patient sex, isolate source (blood, urine, wound, lower respiratory tract, and other); 2-year intervals; and geographic region on the basis of the location of the laboratory (West, Northeast, South Atlantic, South Central, East North Central, and West North Central). These 6 regions correspond to the 4 US Census regions (West, Northeast, South, Midwest). The South and Midwest regions were split (into South Central and South Atlantic, and East and West North Central, respectively) to achieve a more even regional distribution of isolates.

The χ^2 (Cochran-Armitage) test for linear trend was used to test the significance of 2-year trends. A quadratic term was added to test for a nonlinear shape of the trend. If the parameter estimate for the square of the time variable was significant and positive (negative) ($p<0.05$), that implied that the trend was nonlinear and the frequency of resistance was changing at an increasing (decreasing) rate. Susceptibility patterns of CRE isolates to other antimicrobial drugs were also assessed. Data were analyzed by using the R statistical software environment (*21*).

Results

Of the 438,600 isolates from children corresponding to pathogens of interest during 1999-2012, a total of 316,253 (72.1%) met the inclusion criteria that they had been tested against ≥1 third-generation cephalosporin and ≥1 carbapenem of those considered for the CRE phenotype. Of these 316,253 isolates analyzed, 266 (0.08%) met the CRE criteria (Table 1). The median age of children for all analyzed isolates was 8 years; 120,500 (38.1%) of the isolates were from children 1–5 years of age, 100,198 (31.7%) were from children 6–12 years of age, and 95,555 (30.2%) were from children 13–17 years of age. When we considered only CRE isolates, the age distribution was skewed toward younger patients (median age 4 years), and 145 (54.5%) of isolates were from children 1–5 years of age.

For all analyzed isolates from children, 255,181 (80.7%) were from female patients, and for the subset of CRE isolates, 154 (57.9%) were from female patients (Table 1). When we categorized all isolates by organism, isolate source, and health care setting, we found that most (239,274 [75.7%]) were *E. coli*, from urinary sources (265,690 [84%]), and from the outpatient setting (245,257 [77.6%]) (Table 1). However, among CRE isolates, the largest number of isolates were *Enterobacter* species (98 [36.8%]), from urinary sources (85 [31.9%]), and from the inpatient non-ICU setting (116 [43.6%]) (Table 1). Of the 6 geographic regions in the dataset, the largest number of isolates was from the West (78,795 [24.9%]), and for CRE isolates, the highest number of isolates was from the Northeast (63 [23.7%]) (Table 1).

Table 1. Characteristics of *Enterobacteriaceae* isolates and children from which they were isolated, The Surveillance Network–USA database, 1999–2012*

Characteristic	No. (%) isolates analyzed, N = 316,253	No. (%) CRE isolates analyzed, n = 266	% CRE, 266/316,253 (0.084)	Met inclusion criteria,† 316,253/438,600 (72.11)
Organism				
Escherichia coli	23,9274 (75.66)	58 (21.80)	0.02	70.60
Klebsiella pneumoniae	23,442 (7.41)	83 (31.20)	0.35	76.91
Proteus mirabilis	19,506 (6.17)	2 (0.75)	0.01	71.35
Enterobacter species‡	17,215 (5.44)	98 (36.84)	0.57	80.84
Serratia marcescens	10,086 (3.19)	17 (6.39)	0.17	85.77
Citrobacter species§	6,730 (2.13)	8 (3.01)	0.12	76.42
Health care setting				
Outpatient	245,257 (77.55)	89 (33.46)	0.04	71.28
Inpatient	53,832 (17.02)	116 (43.61)	0.22	71.71
Inpatient–ICU	10,048 (3.18)	55 (20.68)	0.55	88.12
Unknown	6,041 (1.91)	5 (1.88)	0.08	88.09
Nursing home	1,075 (0.34)	1 (0.38)	0.09	90.34
Isolate source				
Urine	265,690 (84.01)	85 (31.95)	0.03	70.30
Wound	23,269 (7.36)	66 (24.81)	0.28	80.16
Lower respiratory tract	14,400 (4.55)	74 (27.82)	0.51	86.64
Blood	8,605 (2.72)	37 (13.91)	0.43	87.56
Other¶	4,289 (1.36)	4 (1.50)	0.09	82.91
Age group, y				
1–5	120,500 (38.10)	145 (54.51)	0.12	72.43
6–12	100,198 (31.68)	63 (23.68)	0.06	71.68
13–17	95,555 (30.21)	58 (21.80)	0.06	72.14
Sex				
F	255,181 (80.69)	154 (57.89)	0.06	70.49
M	56,105 (17.74)	105 (39.47)	0.19	78.76
Unknown	4,967 (1.57)	7 (2.63)	0.14	5.37
Region				
West	78,795 (24.92)	47 (17.67)	0.06	73.62
South Atlantic	69,066 (21.84)	53 (19.92)	0.08	78.97
East North Central	57,846 (18.29)	18 (6.77)	0.03	56.13
South Central	44,414 (14.04)	28 (10.53)	0.06	82.22
North East	41,892 (13.25)	63 (23.68%)	0.15	71.35
West North Central	24,240 (7.66)	57 (21.43)	0.24	67.49

*Data for patients <1 year of age were not available for all years and excluded from analysis. CRE, carbapenem-resistant *Enterobacteriaceae*. CRE is defined as resistance to all tested third-generation cephalosporins (ceftriaxone, cefotaxime, or ceftazidime), and nonsusceptiblity to ≥1 carbapenem (ertapenem, imipenem, meropenem, or doripenem). For bacteria with intrinsic imipenem nonsusceptibility (*P. mirabilis*), the CRE criteria required nonsusceptibility to ≥2 of the carbapenems listed. ICU, intensive care unit.
†Isolates were tested against ≥1 third-generation cephalosporin and ≥1 carbapenem of those considered for the CRE phenotype.
‡*E. aerogenes* and *E. cloacae*.
§*C. freundii* and *C. koseri*.
¶Includes upper respiratory tract and skin cultures.

When analyzing for linear and quadratic trends during 1999–2012, we found a significant increase ($p<0.0001$) in the frequency of CRE isolates (Figure 1). From the 1999–2000 period until the 2011–2012 period, the frequency of CRE isolates (across all of the included *Enterobacteriaceae*) increased from 0% to 0.47%. The greatest increases were seen among *Enterobacter* species (from 0.0% in 1999–2000 to 5.2% in 2011–2012) (online Technical Appendix Table 1, http://wwwnc.cdc.gov/EID/article/21/11/15-0548-Techapp1.pdf). Likewise, there was a major increase in CRE across the ICU, inpatient non-ICU, and outpatient health care settings; the greatest increase was seen among ICU isolates (from 0.0% in 1999–2000 to 4.5% in 2011–2012) (Figure 2).

The frequency of CRE among analyzed isolates throughout the study period was also highest among male patients, children 1–5 years of age, and blood cultures (online Technical Appendix Figures 1–3). Blood and lower respiratory tract cultures showed large increases of CRE over time, increasing from 0% in 1999–2000 to 3.2% and 2.3% in 2011–2012, respectively (online Technical Appendix Figure 2).

Regional data are shown in Figure 3. Before 2007, the frequency of CRE was consistently low across all regions (<0.1%). In the last 2-year period (2011–2012), all regions except East North Central reached CRE prevalences >0.1%; South Central had the highest prevalence of 1.1%.

When we compared CRE counts between inpatient and outpatient settings across species, 64% (171/266) of CRE isolates were obtained from hospitalized patients (Table 2). CRE isolates were frequently resistant to additional antimicrobial drugs: 142 (54%) were resistant to

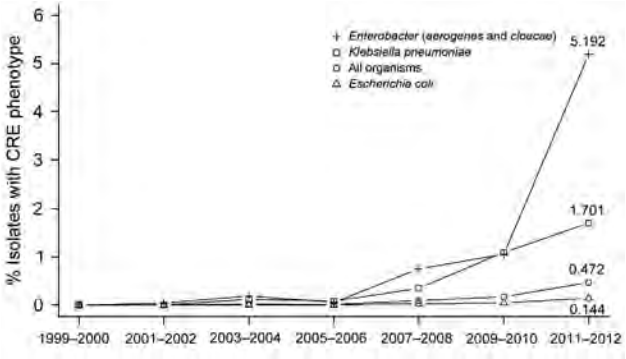

Figure 1. National trends in prevalence of carbapenem-resistant *Enterobacteriaceae* (CRE) isolates from children, The Surveillance Network-USA database, 1999–2012. Markers show the percentage of isolates that belonged to the resistance phenotype for each 2-year period. Data for patients <1 year of age were not available for all years and were excluded from this analysis. The All Organisms trend encompasses *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterobacter cloacae*, *E. aerogenes*, *Citrobacter freundii*, *C. koseri*, and *Serratia marcescens*. Each trend had a significant positive quadratic term: All Organisms ($p = 1.3 \times 10^{-40}$), *E. aerogenes* and *E. cloacae* ($p = 1.4 \times 10^{-29}$), *K. pneumoniae* ($p = 6.6 \times 10^{-11}$), and *E. coli* ($p = 2.4 \times 10^{-11}$). Trends for *C. freundii* and *C. koseri*, *S. marcescens*, and *P. mirabilis* are not shown but they all had significant positive quadratic terms ($p = 0.0006$; $p = 0.002$; and $p = 1.0 \times 10^{-7}$).

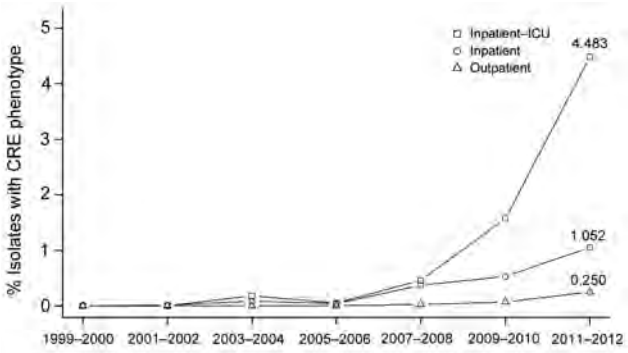

Figure 2. Prevalence of carbapenem-resistant *Enterobacteriaceae* (CRE) isolates from children by health care setting, The Surveillance Network-USA database, 1999–2012. Health care setting was determined by patient location at the time a microbiological sample was collected. Data for patients <1 year of age were not available for all years and were excluded from this analysis. There was a significant positive quadratic trend for intensive care unit (ICU) ($p = 1.1 \times 10^{-18}$), outpatient ($p = 8.6 \times 10^{-26}$), and inpatient non-ICU ($p = 5.0 \times 10^{-11}$). There was no significant trend for the nursing home setting, which made up 0.34% of total isolates (Table 1).

trimethoprim/sulfamethoxazole, 139 (52.3%) were resistant to ≥1 aminoglycoside, 122 (48.2%) were resistant to ciprofloxacin, and 127 (48.3%) were multidrug resistant (nonsusceptible to ≥3 antimicrobial drug classes) (Table 3). CRE isolates retained the lowest phenotypic resistance to amikacin (21.3%). However, CRE isolate data did not contain information on susceptibility to other CRE treatment options, including colistin, tigecycline, polymyxin B, and fosfomycin (*23*). When the distribution of MDR isolates among CRE isolates was considered by species, we found that MDR strains were more common in *K. pneumoniae* (89.2%) and *E. coli* (50.9%) and less common in *Enterobacter* species (20.4%) (Table 4).

CRE trends were not analyzed for isolates from children <1 year of age because of lack of data before 2010. However, data for this age group collected during 2010–2012 demonstrated resistance levels consistent with those seen in other age cohorts (online Technical Appendix Table 2). Of the 8,319 isolates, 70 (0.8%) met CRE criteria. *E. aerogenes* and *E. cloacae* isolates represented the largest group of CRE isolates (41 [58.6%]) compared with other organisms, as did male patients (42 [60%]) compared with female patients and isolates from urinary sources (32 [45.7%]) compared with other sources.

Discussion

In our nationally representative sample, we found that CRE in US children showed a major increase during 1999–2012, and the most substantial increases were in children 1–5

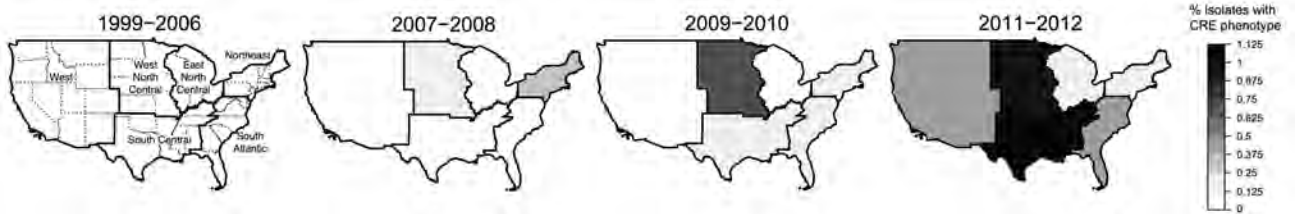

Figure 3. Regional trends in the prevalence of carbapenem-resistant *Enterobacteriaceae* (CRE) isolates from children, The Surveillance Network-USA database, 1999–2012. A) Percentage of isolates with CRE phenotype, 1999–2006 (0%). The 6 regions shown correspond to the 4 US Census regions (West, Northeast, South, Midwest). However, the Midwest and South regions, respectively, were split into East and West North Central and South Central and South Atlantic. Isolates from Alaska and Hawaii are included in the West region. B–D) Percentage of isolates with CRE phenotype, by 2-year period, 2007–2012. There was a significant positive quadratic trend for West ($p = 4.1 \times 10^{-15}$), South Atlantic ($p = 9.4 \times 10^{-12}$), East North Central ($p = 0.0002$), South Central ($p = 5.2 \times 10^{-17}$), and West North Central ($p = 7.2 \times 10^{-8}$). There was a significant linear trend for North East ($p = 5.8 \times 10^{-8}$). Data for patients <1 year of age were not available for all years and were excluded from this analysis.

Table 2. Inpatient and outpatient CRE counts by species *Enterobacteriaceae*, The Surveillance Network–USA database, 1999–2012*

Organism	Outpatient		Inpatient and inpatient–ICU	
	No. (%) isolates analyzed, n = 245,257	No. (%) CRE isolates analyzed, n = 89	No. (%) isolates analyzed, n = 63,880	No. (%) CRE isolates analyzed, n = 171
Escherichia coli	197,807 (80.65)	27 (29.35)	36,139 (56.57)	31 (18.13)
Proteus mirabilis	15,738 (6.42)	2 (2.17)	8,747 (13.69)	56 (32.75)
Klebsiella pneumoniae	14,115 (5.76)	22 (23.91)	8,620 (13.49)	70 (40.94)
Enterobacter species†	8,225 (3.35)	27 (29.35)	4,907 (7.68)	8 (4.68)
Serratia marcescens	4,879 (1.99)	9 (9.78)	3,364 (5.27)	0
Citrobacter species‡	4,493 (1.83)	2 (2.17)	2,103 (3.29)	6 (3.51)

*CRE, carbapenem-resistant *Enterobacteriaceae*. CRE is defined as resistance to all tested third-generation cephalosporins (ceftriaxone, cefotaxime, or ceftazidime), and nonsusceptiblity to ≥1 carbapenem (ertapenem, imipenem, meropenem, or doripenem). For bacteria with intrinsic imipenem nonsusceptibility (*P. mirabilis*), the CRE criteria required nonsusceptibility to ≥2 of the carbapenems listed. ICU, intensive care unit.
†*E. aerogenes* and *E. cloacae*.
‡*C. freundii* and *C. koseri*.

years of age, male patients, blood culture isolates, and the ICU setting. However, overall CRE occurrence in children remained low and relatively uncommon compared with ESBL-producing *Enterobacteriaceae*. The proportion of ESBL-producing *Enterobacteriaceae* from the same TSN database was much higher than that of CRE (0.47% ESBL vs. 0.08% CRE) in children (*19*).

Dissemination of KPC accounts for most of the increasing prevalence of CRE in the United States (*24*). However, in the past 5 years, other carbapenemases that are also rapidly spread by mobile genetic elements harboring genes encoding carbapenemases, such as metallo-β-lactamases (MBLs), including New Delhi MBLs, Verona integron-encoded MBLs, and IMPs (active on imipenem), as well as Class D OXA-producing enzymes (such as OXA-48), have also been reported in clinical *Enterobacteriaceae* isolates from the United States (*8*,*24*). CRE infections have been associated with high rates of illness and death (*25*).

Risk factors for CRE are well described in adults and include critical illness, immune compromise, exposure to health care, residence in long-term health care facilities, longer length of stay before infection, and prior exposure to antimicrobial drugs (*13*,*25*,*28*). However, little is known about the epidemiology of these factors for children (*9*), and published data on the prevalence of CRE in children have been scarce. Moreover, the increase in prevalence in children over the past decade has not been well described. Unlike for adults, where increases were greater than for children, we did not find that the increase in CRE in children appeared to be related to residence in long-term care facilities, because only 0.1% of CRE isolates came from this setting (*13*). However, long-term care facilities have been described as a potential risk factor for colonization in children (*16*), and it is possible that this factor was missed in our study because patient location entered in laboratory information systems might not correspond to the clinical setting in which patients ultimately received care. We observed that CRE are more commonly isolated with hospitalized patients (Table 2).

There are few treatment options for CRE infections. This armamentarium is further reduced for children and pediatric clinical data are lacking (*29*). Side effect profiles limit tigecycline use for persons <18 years of age, and the US Food and Drug Administration discourages routine use because of increased risk for death (*30*). Several questions remain about optimal pediatric dosing of polymyxins, such as colistin. Oral fosfomycin is available for the treatment of CRE cystitis; however, standard dosing guidelines are available only for older children and adolescents (*29*).

Furthermore, CRE are known to harbor additional drug-resistance genes to other antimicrobial drug classes, which may also be carried on mobile genetic elements. *K.*

Table 3. Co-resistance of 266 carbapenem-resistant *Enterobacteriaceae* isolates, The Surveillance Network–USA Database 1999–2012*

Drug class or drug	No. nonsusceptible/ no. tested (%)
Aminoglycosides	139/266 (52.26)
Gentamicin	108/265 (40.75)
Tobramycin	116/235 (49.36)
Amikacin	49/230 (21.30)
β-lactam/β-lactamase inhibitors	236/249 (94.78)
Ampicillin/sulbactam†	188/194 (96.91)
Piperacillin/tazobactam	201/231 (87.01)
Cefepime	125/241 (51.87)
Ciprofloxacin	122/253 (48.22)
Trimethoprim/sulfamethoxazole	142/263 (53.99)
Multidrug resistant‡	127/263 (48.29)

*Rows showing drug classes (aminoglycosides, β-lactam/β-lactamase inhibitors) indicate number of isolates that were tested against ≥1 drug listed in the class and the number of isolates that were nonsusceptible to ≥1 drug listed in the class. Tigecycline susceptibility test results were not recorded in the database. Polymyxin B and colistin susceptibility test results were recorded in the database, but none of the carbapenem-resistant *Enterobacteriaceae* (CRE) isolates were tested against those drugs. Only 1 CRE-positive isolate was tested against fosfomycin. CRE is defined as resistance to all tested third-generation cephalosporins (ceftriaxone, cefotaxime, or ceftazidime), and nonsusceptiblity to ≥1 carbapenem (ertapenem, imipenem, meropenem, or doripenem). For bacteria with intrinsic imipenem nonsusceptibility (*P. mirabilis*), the CRE criteria required nonsusceptibility to ≥2 of the carbapenems listed.
†*Citrobacter* (n = 8) and *Enterobacter* (n = 98) species are intrinsically resistant (*22*) to ampicillin/sulbactam. However, they have been included in the table.
‡These CRE isolates were nonsusceptible to ≥1 drug from each of the following 3 drug classes: aminoglycosides (gentamicin, tobramycin, amikacin), β-lactams (ampicillin/sulbactam, piperacillin/tazobactam), fluoroquinolones (ciprofloxacin), and trimethoprim/sulfamethoxazole.

Table 4. CRE and non-CRE multidrug-resistant isolates by species, The Surveillance Network–USA database, 1999–2012*

Organism	Non-CRE, no. MDR†/no tested‡ (%)	CRE, no. MDR†/no. tested‡ (%)
All species	11,718/314,573 (3.73)	127/263 (48.29)
Escherichia coli	8,402/238,709 (3.52)	29/57 (50.88)
Klebsiella pneumoniae	1,223/23,263 (5.26)	74/83 (89.16)
Proteus mirabilis	390/19,458 (2.00)	0/2 (0.00)
Enterobacter species§	775/16,867 (4.59)	20/98 (20.41)
Serratia marcescens	513/9,629 (5.33)	4/15 (26.67)
Citrobacter species¶	415/6,647 (6.24)	0/8 (0.00)

*CRE, carbapenem-resistant *Enterobacteriaceae*. CRE is defined as resistance to all tested third-generation cephalosporins (ceftriaxone, cefotaxime, or ceftazidime), and nonsusceptiblity to ≥1 carbapenem (ertapenem, imipenem, meropenem, or doripenem). For bacteria with intrinsic imipenem nonsusceptibility (*P. mirabilis*), the CRE criteria required nonsusceptibility to ≥2 of the carbapenems listed. ICU, intensive care unit.
†These isolates were nonsusceptible to ≥1 drug from each of the following 3 drug classes: aminoglycosides (gentamicin, tobramycin, amikacin), β-lactams (aAmpicillin/sulbactam, piperacillin/tazobactam), fluoroquinolones (ciprofloxacin), and trimethoprim/sulfamethoxazole.
‡These isolates were tested against ≥1 drug from each of the following 3 drug classes: aminoglycosides (gentamicin, tobramycin, amikacin}, β-lactams (ampicillin/sulbactam, piperacillin/tazobactam), fluoroquinolones (ciprofloxacin, and trimethoprim/sulfamethoxazole.
§*E. aerogenes* and *E. cloacae.*
¶*C. freundii* and *C. koseri.*

pneumoniae sequence type 258 strains are KPC-producing clones that harbor Tn*4401*-bearing plasmids. These clones are highly effective in plasmid transfer across bacteria and are known to carry other plasmid-based antimicrobial drug resistance genes such as those that encode resistance to trimethoprim/sulfamethoxazole, aminoglycosides, and fluoroquinolones (*6*). For *Enterobacter* species, various typing methods have been used to study clonal lineages, including pulse-field gel electrophoresis (*31*), repetitive sequence PCR (*32*), and, more recently, multilocus sequence typing (*33*) and partial sequencing of the housekeeping gene *hsp*60, which suggests that KPC-producing *Enterobacter* strains are clustered within specific genetic groups (*34*).

It has been well documented that carbapenemase-bearing plasmids frequently carry determinants of resistance to multiple drug classes (*1*,*6*,*9*). Thus, we propose that the high levels of multidrug resistance in carbapenem-resistant *K. pneumoniae* and *E. coli* isolates from children and lower levels in *Enterobacter* species argue in favor of increased prevalence of plasmid-mediated carbapenemase production as the basis for increases in carbapenem resistance in *K. pneumoniae* and *E. coli*, and increased prevalence of other mechanisms (e.g., chromosomal AmpC cephalosporinase induction/derepression or porin modification) as the basis in *Enterobacter* species.

For MDR strains, this resistance could be reflective of dissemination of the sequence type 258 clone. However, the increase in CRE in children might also be caused by additional MDR genetic clusters, because single-center molecular studies of KPC isolates from children have reported that a more polyclonal epidemiology may be responsible for dissemination of KPC in children (*16*,*35*). Among the *Enterobacter* species, MDR strains were less common when compared with *K. pneumoniae* and *E. coli*. Thus, the sensitivity of current surveillance definitions may capture nosocomial ecology and not reflect true carbapenemase production among *Enterobacter* species.

In addition, *S. marcescens* (comprising 6.4% of CRE isolates in this study) is known to harbor SME, a serine carbapenemase, as a mechanism of carbapenem resistance (*1*,*2*). Isolates with this phenotype may retain susceptibility to cefepime. Of the *S. marcescens* in this dataset, 40% were resistant to cefepime.

Although data for infants (children <1 year of age) were only available in the last 2 years of the study (2010–2012), resistance levels in infants were similar to those in other age cohorts, suggesting increases in this age group. The epidemiology of colonization and infection in this age group might differ from that of the overall pediatric population because cases have been described as being caused by vertical transmission or by other risk factors associated with neonatal ICU acquisition; however, data remain limited (*9*,*36*). Available data on colonization with MDR *Enterobacteriaceae* in pediatric patients suggest that intestinal carriage of these organisms can last for months to years in some children and that this might be associated with reinfection or potential spread to other family members (*37*,*38*).

Our study has major limitations. First, we cannot distinguish between true infection and colonization, especially among non-blood isolates. Second, because of the nature of the TSN data, it is not possible to avoid bias caused by multiple health care visits made by the same patient over the course of an infection because each time a patient is admitted, a new identification number is assigned that is used to tag any specimens obtained during that health care visit. Third, in June 2010, Clinical Laboratory Standards Institute clinical breakpoints for carbapenems against *Enterobacteriaceae* were decreased. Because MICs were not available, we ran truncated analyses that included all isolates collected before June 2010. All pediatric CRE prevalence trends were still significant. In addition, many clinical laboratories have been slow to adopt these breakpoint revisions. Fourth, because the overall numbers of CRE isolates in the study were small, resistance trends could be potentially affected by an outbreak at 1 or a small number of institutions. Fifth, molecular mechanisms of carbapenem resistance among the isolates could not be determined.

Sixth, although we used CDC criteria to define the CRE phenotype, some mechanisms of resistance other than carbapenemase production might account for carbapenem resistance in isolates.

In summary, the prevalence of CRE infections is increasing among children in the United States but CRE remain relatively uncommon. Molecular characterization is necessary to determine specific CRE genotypes associated with this spread. Continued vigilance for CRE and initiation of the CDC 4 core actions to prevent antimicrobial drug resistance (*11*) should be emphasized for all patient populations, including children.

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Contact Tracing Activities during the Ebola Virus Disease Epidemic in Kindia and Faranah, Guinea, 2014

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The largest recorded Ebola virus disease epidemic began in March 2014; as of July 2015, it continued in 3 principally affected countries: Guinea, Liberia, and Sierra Leone. Control efforts include contact tracing to expedite identification of the virus in suspect case-patients. We examined contact tracing activities during September 20–December 31, 2014, in 2 prefectures of Guinea using national and local data about case-patients and their contacts. Results show less than one third of case-patients (28.3% and 31.1%) were registered as contacts before case identification; approximately two thirds (61.1% and 67.7%) had no registered contacts. Time to isolation of suspected case-patients was not immediate (median 5 and 3 days for Kindia and Faranah, respectively), and secondary attack rates varied by relationships of persons who had contact with the source case-patient and the type of case-patient to which a contact was exposed. More complete contact tracing efforts are needed to augment control of this epidemic.

During March 23, 2014–July 8, 2015, Guinea reported 3,748 Ebola virus disease (EVD) cases and 2,499 EVD-related deaths (*1*), as part of what is the largest reported EVD epidemic to date (*2*). Thorough case identification and contact tracing are necessary to end this epidemic (*3*). Contact tracing involves locating all persons who have been exposed to someone infected with Ebola virus (case-patients) or their body fluids and monitoring them daily for EVD symptoms during the 3 weeks after the last exposure (*4*). This tracing permits immediate identification and isolation of symptomatic contacts (suspected case-patients). Incomplete contact tracing and delayed time to isolation of suspected case-patients may result in transmission of EVD to others in the community, perpetuating the epidemic.

Excluding Conakry, the capital, Guinea is divided into 33 prefectures, which are subdivided into >300 subprefectures; these divisions are large and smaller administrative governmental units, respectively. Of all Ebola cases nationwide, 3.0% and 1.9% have been identified in Kindia and Faranah (Organisation Mondiale de la Sante, unpub. data), respectively, where respective populations are 4.1% and 2.6% of the national population (Institut National de la Statistique, Guinée, unpub. data). At the time of data collection, neither prefecture had its own Ebola treatment unit (ETU) or laboratory with Ebola virus testing capabilities; suspected case-patients were transported by ambulance to the nearest ETU, which was at minimum a 3-hour drive from either prefecture (Figure 1). We conducted a retrospective review of case and contact tracing data collected from a convenience sample from 2 Guinea prefectures, Kindia and Faranah, during the EVD epidemic response from September 20 through December 31, 2014. We provide descriptive analyses of case and contact tracing for these 2 prefectures to identify gaps in reporting and the yield of contact tracing; we also propose actions for improvement of the contact tracing process.

Methods

Case Identification

EVD cases in Guinea are categorized into 1 of 3 case definitions modified from World Health Organization recommendations: 1) suspected case (in a living person

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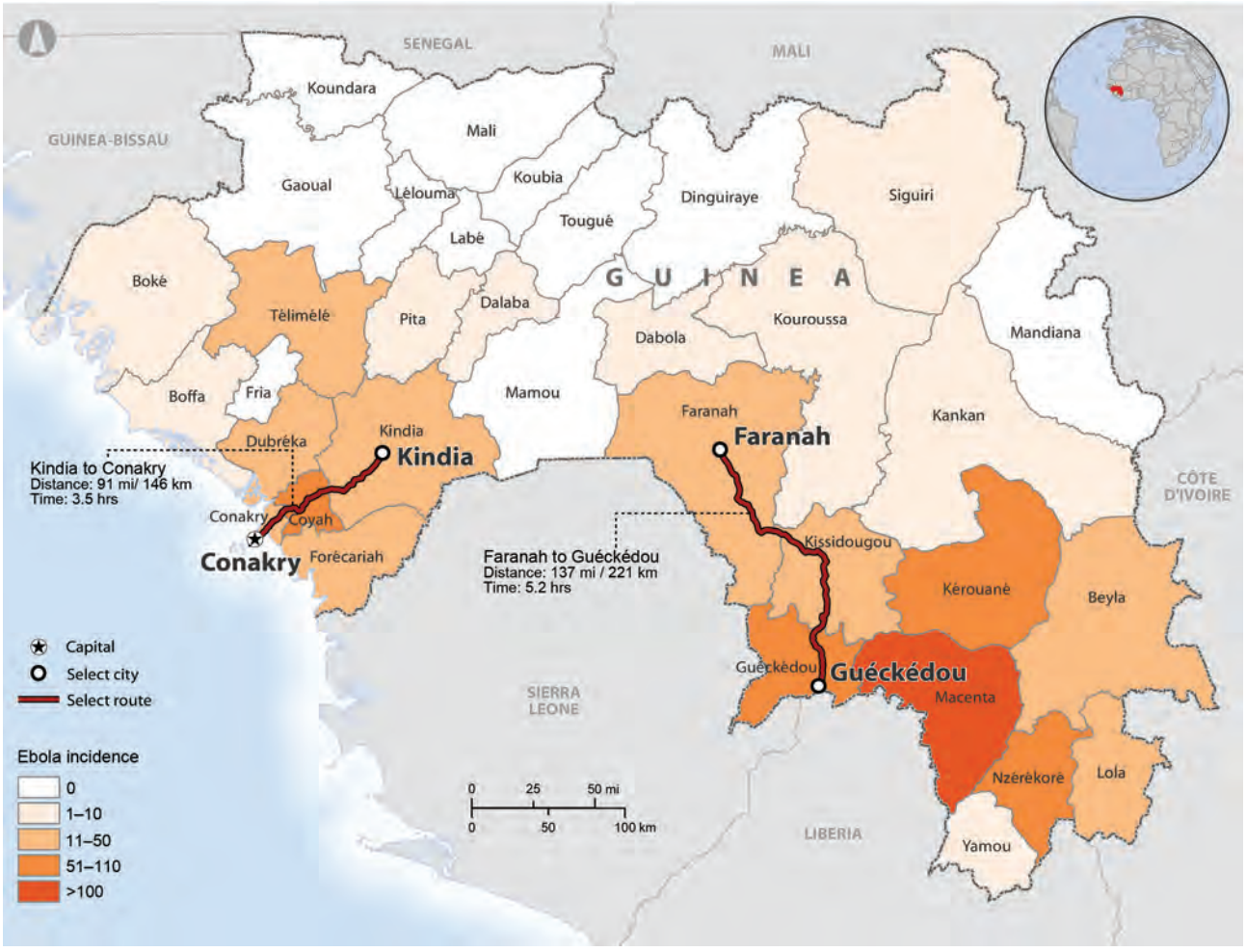

Figure 1. Ebola virus disease incidence (confirmed cases per 100,000 population), by prefecture, Guinea, 2014. Distances and driving times for the transport of suspect case-patients from Kindia or Faranah to the nearest Ebola treatment unit are shown (red lines). Data sources: Guinea Ministry of Health; Guinea Ministry of Planning; Database of Global Administrative Areas (GADM); Europa.

with fever and ≥3 of these symptoms: headache, anorexia, lethargy, aching muscles or joints, difficulty breathing, vomiting, diarrhea, stomach pain, difficulty swallowing, hiccups; or with fever and a history of contact with a person with hemorrhagic fever or a dead or sick animal; or with unexplained bleeding); 2) probable case (in a deceased person who otherwise met the suspect case definition and has an epidemiologic link to a confirmed or probable case); or 3) confirmed case (suspected or probable case that also has laboratory confirmation) (*5*). Cases are reported by using a standardized case reporting form, data from which are submitted to the national viral hemorrhagic fever (VHF) case database.

Cases from the 2 prefectures were identified and cross-referenced between the national VHF case database and the prefecture case database. Demographic information (sex, age) and case classification (confirmed, probable) were abstracted. Because this investigation was part of a public health response and considered to be nonresearch, it was not subject to US Centers for Disease Control and Prevention Institutional Review Board review.

Contact Identification

To identify and register contacts of persons infected with EVD, prefecture public health officials and ETU staff interviewed case-patients, their families, and community members and documented resulting information on standardized contact registration forms (*6*). A contact is defined as someone at risk for infection with EVD because he or she has slept in the same household as a confirmed or probable EVD case-patient, had direct physical contact with the case-patient during that person's illness, had direct physical contact with the body of a case-patient at a funeral or during burial preparation, touched the body fluids of a case-patient during illness, touched the case-patient's clothes or linens, or is an infant breastfed by the case-patient (*6*).

Demographic data of contacts (name, age, sex, relationship to the presumed source case-patient, and prefecture and subprefecture of residence) and daily follow-up data (presence or absence of symptoms) were obtained through use of standardized contact tracing forms (*6*), which populated a prefecture contact database.

We performed demographic descriptive analyses using nonduplicated contact data; the individual person was the unit of analysis. We performed other nondemographic descriptive analyses using contact event data; the contact event was the unit of analysis because a single contact may have had contact with several case-patients, resulting in several contact events per person. The case and contact databases may show differing numbers of source cases because contacts in a prefecture might have contacted source case-patients in another prefecture and data quality issues could exist. The date of isolation was the date a suspected case-patient was transported to an ETU. We created the following definitions: time to isolation (days) was calculated by subtracting the date of first symptom onset from the date of isolation; family/household member was anyone related by blood or marriage or who lived in the same household as the case-patient or was described as being a caregiver, excluding health care workers; safe burial was a burial with placement of the body in an impermeable bag and interment by a team wearing personal protective equipment (*7*). Secondary attack rate was calculated as the proportion of new cases among contact events × 100 (*8*). Because variables had nonparametric distributions, medians were analyzed. Relative risks were used to quantify the risk for becoming a secondary case-patient after exposure to a case-patient by relationship status or a case-patient by epidemiologic case classification. We used χ^2 tests to measure associations between categorical variables; specifically, to compare attack rates among family members and non–family members and among contacts to confirmed versus probable cases. A p value of ≤ 0.05 was considered statistically significant. Epidemiologic weeks were in accordance with those designated by in-country situation reports.

Results

Kindia

Cases

During September 20–December 31, 2014, a total of 90 EVD cases were reported in Kindia; 63 (70%) were confirmed and 27 (30%) probable cases (Table 1). The median case-patient age was 35 (interquartile range [IQR] 20–50) years; 21 (23.3%) case-patients were <18 years of age, and 52 (57.8%) were female. No case-patients were health care workers. Case-patients resided permanently in 23 villages in 7 subprefectures of Kindia. Median time to isolation for suspect case-patients was 5 (IQR 3–7) days; this time varied by epidemiologic week. Seventy-one (78.9%) case-patients died; of those, 35 (49.1%) died in the community, of whom 30 (85.7%) underwent unsafe burial. The number of community deaths per epidemiologic week fluctuated (range 1–7) and peaked during weeks 49 and 51 (Figure 2).

Table 1. Demographic characteristics of Ebola virus disease case-patients in 2 prefectures, Guinea, September 20–December 31, 2014*

Characteristic	Prefecture	
	Kindia, n = 90	Faranah, n = 62
Case classification, no. (%) patients		
Confirmed	63 (70.0)	39 (62.9)
Probable	27 (30.0)	23 (37.1)
Registered as contacts before case identification, no. (%) patients	28 (31.1)	17 (27.4)
Age, y		
Median	35.0	30.0
IQR	20.0–50.0	14.0–47.0
<18 y, no. (%)	21 (23.3)	19 (30.6)
Female sex, no. (%) patients	52 (57.8)	33 (53.2)
Villages, no.	23	11
Subprefectures, no.	7	4
Median time to isolation, d (IQR)	5 (3–7)	3 (1–6)
Final outcome, no. (%) patients		
Deceased	71 (78.9)	52 (83.9)
Place of death, no. (%) patients		
Ebola treatment unit	36 (50.7)	28 (53.8)
Community	35 (49.1)	24 (46.1)
Burial type for community deaths, no. (%) patients†		
Safe	5 (14.3)	20 (90.9)
Unsafe	30 (85.7)	2 (9.1)
Case-patients for whom contacts are registered, no. (%)	35 (38.9)	20 (32.2)

*Data in this table originate from the prefecture case database. The variables "registered as contacts before case identification" and "No. (%)" were created in the prefecture case database by cross-referencing with the contact database. IQR, interquartile range.
†Burial data from Faranah missing for 2 case-patients.

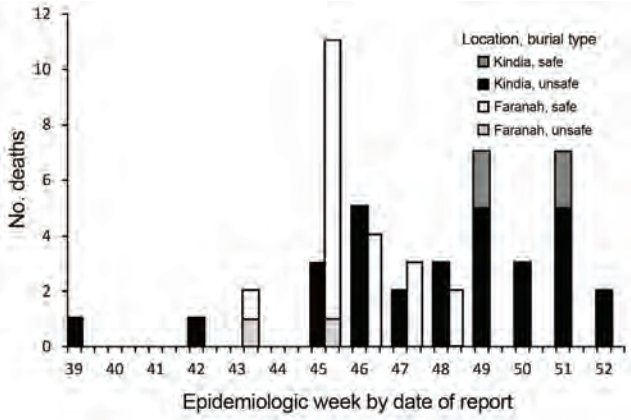

Figure 2. Community deaths by burial type for case-patients with confirmed and probable cases of Ebola virus disease in Kindia and Faranah, by epidemiological week, Guinea, 2014. Safe burial was defined as placement of the body in an impermeable bag and interment by a team wearing personal protective equipment (*9*).

Contact Tracing

Twenty-eight (31.1%) of 90 case-patients in Kindia were identified as contacts and registered in the contact database before being identified as a case-patient (Table 1). Fifty-five (61.1%) of 90 case-patients had no contacts listed in the contact database (Table 1). Thirty-five (38.9%) of the 90 case-patients in the case database had contacts listed in the contact database; 25 (71.4%) were confirmed and 10 (28.6%) had probable cases. For the 35 case-patients for whom contacts were registered, the median number of contacts per case was 16 (IQR 11.2–28.0) (Table 2).

The Kindia contact database contained data on 1,137 contacts of 50 source case-patients (29 confirmed, 11 probable; 10 had unknown classification) (Table 2). Some of the 50 source case-patients did not reside in Kindia but were source case-patients of contacts followed in Kindia. The median age of contacts was 22 (IQR 10–40) years; 450 (39.6%) were <18 years of age, and 611 (53.7%) were female. Family or household members accounted for 470 (41.3%) contacts.

Among the 1,137 contacts and case-patients with 50 source case-patients, 1,233 contact-events occurred; 26 contacts became ill with EVD, for an overall secondary attack rate of 2.1%. Nineteen (73.1%) of these patients had confirmed cases and 7 (26.9%) had probable cases. Eighteen of 829 contacts exposed to a confirmed case-patient and 7 of 226 contacts exposed to a patient who had a probable case became infected, for secondary attack rates of 2.2% and 3.1%, respectively (p = 0.4). Data on the epidemiologic classification of the source case were missing for 1 secondary case-patient.

The median age of secondary case-patients was 28 (IQR 9–60) years; 7 (26.9%) were <18 years of age, and 15 (57.7%) were female. The secondary attack rate was 4.2% (20 cases among 470 contact-events) when the contact was a family or household member of the source case-patient but only 0.4% (2 of 507) when the contact was not a family or household member (relative risk 10.8, 95% CI 2.5–45.9). There was no statistically significant difference in the risk for becoming a secondary case-patient for contacts who were <18 years of age or according to sex. No contacts who were traditional healers or health care workers became secondary case-patients.

Faranah

Cases

During September 25–December 12, 2014, a total of 62 EVD cases were reported in Faranah; 39 (62.9%) were confirmed and 23 (37.1%) probable cases (Table 1). The median case-patient age was 30 (IQR 14–47) years; 19 (30.1%) case-patients were <18 years of age, and 33 (53.2%) were female. One case-patient was a health care worker. Patients resided permanently in 11 villages in 4 subprefectures of Faranah. Median time to isolation for suspect case-patients was 3 (IQR 1–6) days; this time varied by epidemiologic week. Fifty-two (83.8%) case-patients died; of those, 24 (46.1%) died in the community, of whom 2 (9.1%) underwent unsafe burial (burial data were missing for 2). The number of community deaths per epidemiologic week decreased over time after week 45 (Figure 2).

Contact Tracing

Seventeen (27.4%) of 62 case-patients in Faranah were identified as contacts and registered in the contact database before being identified as a case-patient (Table 1). No contacts were listed in the contact database for 39 (62.9%) of 62 cases, and contact data were missing for 3. Of the 20 (32.2%) cases for which contacts were listed in the contact database, 10 (50.0%) were confirmed and 10 (50.0%) were probable cases. For the 20 case-patients for whom contacts were registered, the median number of contacts per case-patient was 9 (IQR 5.5–15.5) (Table 2).

The Faranah contact database contained data for 289 contacts of 27 source case-patients (8 confirmed, 10 probable; 9 had unknown epidemiologic classification) (Table 2). The median age of contacts was 20 (IQR 8–35) years; 124 (42.9%) were <18 years of age, and 146 (50.5%) were female. Family or household members accounted for 152 (52.6%) contacts.

Among 289 contacts and 27 source case-patients, 317 contact events occurred. Twenty-five contacts became ill with EVD; the overall secondary attack rate was 7.9%. Of these patients, 21 (84%) had confirmed cases and 4 (16%) had probable cases. Seven of 92 contacts exposed to a confirmed case-patient and 13 of 132 contacts exposed to a patient who had a probable case became infected, accounting

Table 2. Demographic characteristics of contacts of Ebola virus disease case-patients in 2 prefectures, Guinea, September 20-December 31, 2014*

Characteristic	Prefecture	
	Kindia	Faranah
No. contacts	1,137	289
No. source case-patients	50	27
No. contact events	1,233	317
Median no. contacts per case-patient (IQR)	16 (11.2–28)	9 (5.5–15.5)
Age, y		
Median	22	20
IQR	10–40	8–35
<18 y, no. (%)	450 (39.6)	124 (42.9)
Female sex, no. (%)	611 (53.7)	146 (50.5)
Village, no.	58	24
Subprefecture	10	8
Relationship to source case-patient, no. (%)		
Family/household member	470 (41.3)	152 (52.6)
Neighbor	464 (40.8)	6 (2.1)
Health care worker	22 (1.9)	0
Teacher	1 (0.1)	0
Other	17 (1.5)	39 (13.5)
No data	163 (14.3)	92 (31.8)

*Data in this table are from the contact databases; source case-patients listed here are not necessarily the same as case-patients listed in Table 1. IQR, interquartile range.

for secondary attack rates of 7.6% and 9.8%, respectively (p = 0.8). The median age of secondary case-patients was 30 (IQR 16–45) years; 8 (32%) were <18 years of age, and 11 (44%) were female. The secondary attack rate was 12.3% (19 cases among 154 contact-events) when the contact was a family or household member of a case-patient and 4.8% (3 cases among 63 contact events) when the contact was not a family or household member of a case-patient (relative risk 2.6, 95% CI 0.6–10.8). There was also no statistically significant difference in the risk for having a confirmed case by persons <18 years of age or by sex. No transmission was reported between the health care worker who had a confirmed case and contacts, although at the time of data collection, contacts had not completed their 21-day follow-up review.

Discussion

This evaluation of 2 EVD-affected prefectures of Guinea documents 2 major gaps in contact tracing activities: 1) most case-patients were not previously registered and followed up on as contacts before case identification, and 2) most case-patients, once identified, had zero contacts registered, so any contacts they had were not properly investigated. Time to isolation of suspect case-patients was suboptimal in both prefectures. Many deaths occurred in the community, and a high percentage of unsafe burials occurred in Kindia. Somewhat higher secondary attack rates occurred among contacts who were family or household members of their source case-patient and among contacts of probable case-patients.

One third of case-patients were previously identified and followed up on as contacts before onset and confirmation of EVD. Without identification of all contacts of a case-patient, it is not possible to provide adequate follow-up and ensure prompt isolation if those contacts become symptomatic. Suspected case-patients that are not isolated from the community, if infected, can transmit EVD and thus serve as reservoirs of infection. In addition, we identified suboptimal time for isolation of persons who were suspect case-patients. Not having been followed up on initially as registered contacts may have contributed to this finding. Although this evaluation was not sufficiently designed to measure the contribution of community or individual reluctance to participate in contact tracing or time to isolation of suspect case-patients, situation reports from Kindia identify these factors as barriers to contact tracing activities. Further, a recent report noted that violence related to response control efforts has been particularly problematic in Guinea, compared with Liberia and Sierra Leone, and has been a barrier to community access (*9*). These barriers to implementation of optimal disease control interventions need further evaluation to identify effective community engagement strategies.

Similarly, only one third of cases had contacts registered and followed up on according to contact tracing guidelines. There are multiple reasons for which a contact may not be registered or followed up on, including: case-patients are sometimes incapacitated or die before providing complete data; interviewed case-patients and community members might not disclose complete contact data; and families and communities might not permit public health officials in their homes or communities for contact tracing purposes because of fear or stigma. In addition, identified contacts may not cooperate with public health officials and community health agents may cease efforts to engage uncooperative or threatening contacts.

Probable cases and community deaths, especially among known contacts, represent missed opportunities for case confirmation and isolation in the response effort. Unsafe burials perpetuate EVD transmission because persons who have EVD are highly viremic before death. In Kindia and Faranah, we identified a high percentage (49% and 46%, respectively) of community deaths; a high percentage of probable cases (37% and 30%) as compared to a Guinea national proportion of 11.8% (*1*); and a high proportion of unsafe burials (86%) in Kindia. Evidence from previous outbreaks reveals a relative risk of 2.1 for virus transmission from a deceased EVD case-patient to adult family members when controlling for direct contact and exposure to body fluids (*10*). Unsafe burials have been associated with large local outbreaks; in December 2014, one unsafe burial in Guinea led to 85 confirmed cases (*11*). Both prefectures reported higher attack rates among contacts exposed to a probable versus a confirmed case, although these differences were small and not statistically significant. Probable cases are likely to be underreported, so the actual percentage of these among all cases is likely higher than reported here.

Higher secondary attack rates among family and household members of case-patients demonstrate the need for larger analyses to assess the effect of the relationship between a contact and source case-patient on disease transmission risk. For this large and complex response, which at times has been hampered by human resources limitations, it would be useful to know if stratification of contacts could be performed to more efficiently focus response efforts on those most at risk. Conversely, enrolling persons who are at minimal risk might increase community reticence and overburden local contact tracing teams. Our investigation found that a large proportion of registered contacts (47.6%) in Kindia were neighbors, whereas a small proportion (2.1%) of contacts in Faranah were neighbors. Although the relationship between family status and attack rate should be further studied, contact tracing teams should adhere to strict use of the contact definition to ensure follow-up of those truly at risk, while minimizing unnecessary follow-up of persons who do not fit the contact definition.

Limitations include, but are not restricted to, the factors provided herein. Data collection during an emergency response, especially of this scope and magnitude, is difficult. There were missing data, data of poor quality, and data that may have been in paper format only. For example, no contacts listed in the contact database may mean that no data was collected electronically or that the task was never performed. These issues were compounded by data management challenges, most specifically the lack of unique identifiers with which to link source case-patients and contacts. Thus, manual linkage of case and contacts was necessary; this task was time-consuming, onerous, and had the potential to introduce errors because of multiple data sources and common use of a small number of given and family names within a restricted geographic region. Also, because of inconsistent data flow and incompleteness, the national contact database was not used in this analysis. Thus, additional contacts named in other prefectures could have been missed, which may contribute to lower attack rates in this analysis.

Another limitation is underreporting. Field investigations in early fall 2014 across 5 prefectures in Guinea determined that probable EVD cases comprised 30%–50% of cases (M.G. Dixon, unpub. data), whereas situation reports from October 2014–April 2015 showed that probable cases represented only 10%–12% of all cases nationwide (*1*). This difference in proportion of probable cases in overlapping time periods could mean that a higher percentage of cases are being tested and confirmed but more likely represents underreporting of probable cases. This underreporting could artificially lower the attack rates discussed here. Similarly, the inclusion of source case-patients for whom epidemiologic classification is missing may also underestimate the effects of secondary transmission on the basis of epidemiologic source case classification being probable.

In addition, sample size limited our ability to show statistical significance for some associations. These results represent contact tracing activities from 2 prefectures of Guinea and should be generalized with caution.

The case and contact tracing descriptive analyses for these 2 prefectures in Guinea demonstrate that most case-patients were not previously registered and observed as contacts before case identification and that most case-patients had no contacts that were registered and followed up on. Individual control measures should be performed more completely to end the epidemic in Guinea. Every EVD case-patient not previously observed as a contact represents an unidentified chain of transmission. Every contact not identified or followed up on represents a possible transmission opportunity. Whereas no single control measure in this epidemic in Guinea will achieve the goal of "Getting to Zero" (http://www.unicef.org/emergencies/ebola/75941_81198.html), our findings show that the control measures of case identification and contact tracing individually were not reaching target levels of 100% and thus need improvement to assist in termination of this outbreak in Guinea.

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Association of Higher MERS-CoV Virus Load with Severe Disease and Death, Saudi Arabia, 2014

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Middle East respiratory syndrome coronavirus (MERS-CoV) causes a spectrum of illness. We evaluated whether cycle threshold (C_t) values (which are inversely related to virus load) were associated with clinical severity in patients from Saudi Arabia whose nasopharyngeal specimens tested positive for this virus by real-time reverse transcription PCR. Among 102 patients, median C_t of 31.0 for the upstream of the E gene target for 41 (40%) patients who died was significantly lower than the median of 33.0 for 61 survivors (p = 0.0087). In multivariable regression analyses, risk factors for death were age >60 years, underlying illness, and decreasing C_t. Results were similar for a composite severe outcome (death and/or intensive care unit admission). More data are needed to determine whether modulation of virus load by therapeutic agents affects clinical outcomes.

Middle East respiratory syndrome coronavirus (MERS-CoV) was first reported in September 2012 in a patient from the Kingdom of Saudi Arabian (KSA) who had pneumonia (*1*). From September 20, 2012 through January 2, 2015, a total of 825 MERS-CoV cases (356 fatal) have been reported from KSA, representing most of the cases worldwide (*2*,*3*). Initial reports of clinical course among MERS-CoV patients from KSA indicated high case-fatality rates (>50%) (*4*,*5*), but the subsequent increase in testing of symptomatic and asymptomatic persons as part of contact investigations has shown that approximately one fifth to one fourth of patients are mildly symptomatic or asymptomatic (*6*,*7*).

The factors dictating severity of illness and outcome among MERS-CoV patients are still not well defined. Underlying illness and older age have been associated with more severe disease and death (*4*,*5*,*8*,*9*). In some other viral respiratory illnesses, the amount of virus measured in the respiratory tract has been associated with more severe disease (*10*–*16*). However, whether virus load of MERS-CoV is associated with severity of illness is unknown. We evaluated this association among a large cohort of MERS-CoV patients from the Jeddah region of KSA during 2014.

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Methods

Study Population

Eligible persons were children and adults admitted to Jeddah area hospitals during March–May 2014. Patients were included if they met the following criteria: 1) tested positive for MERS-CoV by real-time reverse transcription PCR (RT-PCR) from nasopharyngeal swab, sputum, or bronchoalveolar lavage samples at the Jeddah Regional Laboratory during March 26–May 16, 2014, and 2) were matched to a line list of Jeddah MERS-CoV patients with the most complete clinical and outcome data (*17*)

Data Collection

Jeddah Regional Laboratory maintained a database of all patients tested that included cycle threshold (C_t) values for the upstream of the envelope E (upE) gene and open reading frame (ORF) 1a targets. A composite of MERS-CoV patients for whom medical history and clinical information were obtained by case report forms and medical records review or by phone calls was compiled in late May 2014 (*17*). Laboratory and clinical datasets were linked by matching the national identification number for Saudis or foreign identification number for foreigners. If this number was a close but not exact match, confirmatory evidence of identity included sex, age, and date of specimen collection. All personal identifiers, including identification numbers, were stripped from the merged, analytic dataset.

Specimen Collection and Laboratory Testing

Patients with suspected MERS-CoV infection were tested as soon as possible after admission to Jeddah area hospitals at the discretion of the treating clinicians, under guidance from the KSA Ministry of Health (MOH) (*18*). In addition,

some close contacts of confirmed MERS-CoV patients and health care workers who cared for MERS-CoV patients were sampled as part of contact investigations. KSA MOH guidelines recommended collection of nasopharyngeal swab as the screening specimen for all patients suspected to have MERS-CoV but enabled additional collection of lower respiratory tract specimens for intubated patients (*5*,*18*). Swab samples were obtained by using Dacron flocked swabs and placed in 2–3 mL of virus transport media. Specimens were stored at 2°C–8°C and transported to the Jeddah Regional Laboratory within 48 hours after collection.

At the Jeddah Regional Laboratory, total nucleic acid extraction from clinical specimens was performed by using the MagNA Pure LC 96 or the MagNA Pure Compact (Roche, Basel, Switzerland). Both instruments were programmed for the DNA_Blood_100_400_V3_2 protocol (Roche) with a 200-µL sample volume and 100-µL elution volume. Real-time RT-PCR amplification was performed by using the ModularDx Coronavirus SA1 (EMC) upstream E-gene kit (TIB Molbiol LLC, Berlin, Germany) for primary detection, and the ModularDx MERS-Coronavirus (EMC) ORF1a kit (TIB Molbiol LLC) was used to confirm positive results (*5*,*19*). Discordant results needed to be confirmed with a second clinical specimen. C_t values were read as positive if the amplification curve crossed the threshold set above the background fluorescence levels. Standard curves were not generated for each real-time RT-PCR run, and absolute virus concentrations in the specimens were not calculated. Because upE and ORF1a C_t values were highly correlated (Spearman ρ) and because the upE region is noncoding and would be less influenced by the presence of mRNA, all primary analyses were run for upE C_t values.

Outcome and Risk Factor Variables

The main outcome of interest for this study was severity of illness and the main predictor being assessed was the MERS-CoV virus load, as reflected by the C_t values. The following severity outcomes were assessed: death by the time of follow-up chart review or phone contact, a composite severe outcome (death and/or admission to the intensive care unit [ICU]), admission to ICU versus admission to the general ward among hospitalized patients, and symptomatic versus asymptomatic infection among surviving patients. Other independent variables in the analysis as risk factors and/or potential confounders of the association between C_t value and outcome were underlying illness, age, sex, and week of specimen collection.

C_t values were used as a relative indicator of virus load, in that lower C_t values were considered to reflect higher virus load than were higher C_t values, an intrinsic characteristic of real-time RT-PCR (*12*,*20*). Analysis of the association between C_t values and severity was restricted to nasopharyngeal specimens because C_t values from lower respiratory tract specimens (e.g., sputum and bronchoalveolar lavage) can be lower (i.e., higher virus load) than upper respiratory tract specimens, thereby introducing a possible bias when evaluating severity (*20*). If multiple specimens were available for a patient, the nasopharyngeal sample with the earliest date of collection was used.

Underlying illness was defined as heart disease, chronic lung disease, renal diseases, and/or diabetes. Age was divided into quartiles based on the age distribution of MERS-CoV–positive patients. To assess whether changes might have occurred over time during the period of testing that confounded the results (e.g., specimen collection or transport methods), we created a categorical variable based on the date of specimen collection for each 2-week period during March 26–May 16. "Days since illness onset" was the difference in the date of the first symptom of MERS (i.e., cough, dyspnea, fever) and the date of specimen collection.

Statistical Analysis

Because C_t distributions were not normally distributed, we compared the C_t values between 2 groups using the Wilcoxon rank sum test and for ≥3 groups using the Kruskal-Wallis test. C_t values also were grouped into high virus load ($C_t \leq 26$), medium virus load (C_t 27–33), and low virus load ($C_t \geq 34$) values for categorical analysis, and groups were compared by using the χ^2 test (Brett Whittaker, pers. comm. on grouping of C_t values). Correlation between other continuous variables and C_t values were compared after the values were ranked and were assessed by Spearman rank correlation coefficient (Spearman ρ).

Univariate and multivariate logistic regression was performed to identify risk factors for death, as well as for a composite severe outcome (death and/or ICU admission) among MERS-CoV–positive patients. Categorical variables with missing data were coded to include missing as a valid response category.

Variables with $p<0.10$ in univariate analysis were included in multivariable analyses, and backward and forward selection methods were performed to identify significant variables at $p<0.05$ for any category of each variable. The main focus was to determine whether the outcome of interest (i.e., death or composite severe outcome) was more likely as C_t values decreased (i.e., virus load increased). A variable was considered to be a significant confounder of the C_t association if the parameter estimate for C_t changed by >10% and was included in the model regardless of statistical significance. Separate analyses were run for the C_t values resulting from the upE and ORF1a RT-PCR targets. All analyses were performed by using SAS 9.3 (SAS Institute, Cary NC, USA). No formal sample size was calculated; rather, all eligible patients during the study period were included in the analysis.

Results

During the study period, the clinical and laboratory datasets for 120 (50%) of 239 MERS-CoV–positive patients were able to be linked. Seventy-three percent were men, and the median age of all patients was 50 years (range 8–86 years); for 19 patients, information about age was missing. Thirty-two (26%) patients were health care workers, and 65 (56%) were Saudi. Specimens for MERS-CoV were collected in 18 facilities; 59% of specimens were collected from 4 facilities, and the remaining 14 facilities provided ≤5 samples each. In addition, 15 (13%) specimens were collected at home for asymptomatic or mildly symptomatic contacts.

Mean and median C_t values for the 120 linked MERS-CoV–positive patients for upE were 30.7 and 32.0, respectively (range 15–37). For 118 patients for whom ORF1a results were available, mean and median C_t values were 30.8 and 32.0, respectively (range 15–37). The correlation between upE and ORF1a C_t values was high (r = 0.94, p<0.001, Spearman ρ). The median C_t for the upE target from 106 nasopharyngeal swab samples was 32.0; from 8 sputum samples, 24.5; and from 6 bronchoalveolar lavage samples, was 24.5 (p<0.001, Kruskal-Wallis test) (Figure 1). When restricted to hospitalized patients, the median C_t values for nasopharyngeal swab, sputum, and bronchoalveolar lavage samples were 31.0, 25.0, and 26.0, respectively (p<0.001, Kruskal-Wallis test).

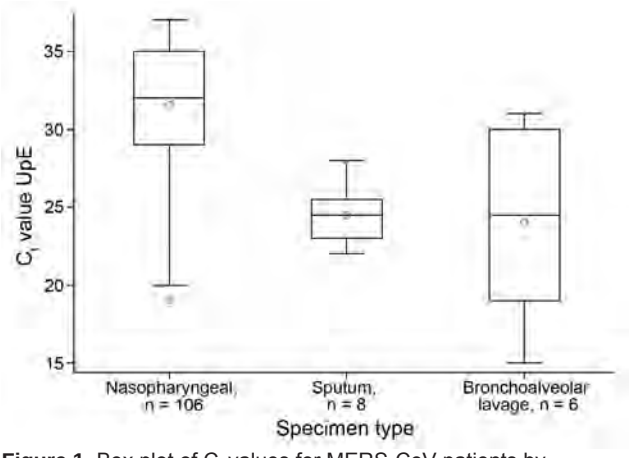

Figure 1. Box plot of C_t values for MERS-CoV patients by specimen type, Kingdom of Saudi Arabia, 2014. Box and whiskers plot features are as follows: central line in box is median, bottom line of box is first quartile (25%), top line of box is third quartile (75%), diamond is mean, bottom of whiskers is first quarter minus 1.5 × interquartile range, top of whiskers is third quarter plus 1.5 × interquartile range, and dots are outliers. Groups were compared by using the Kruskal-Wallis test, p<0.0001. C_t, cycle threshold; MERS-CoV, Middle East respiratory syndrome coronavirus; upE, upstream of E gene.

The remaining analysis is restricted to the 102 patients with nasopharyngeal swab samples and known outcome status. Of these 102 patients, 41 (40%) died. The C_t values for the upE target for 41 patients who died were significantly lower than those for 61 patients who survived (medians 31.0 and 33.0, respectively, p = 0.009, Wilcoxon rank sum test) (Figure 2, panel A). Using the composite severity variable, we categorized 48 (47%) illnesses as severe; for these patients, Ct values were significantly lower than for the 54 patients without severe disease (medians 31.0 and 33.0, respectively, p<0.0036, Wilcoxon rank sum test) (Figure 2, panel b). Sixty-seven (66%) patients were hospitalized. C_t values were significantly lower for the 36 hospitalized patients admitted to the ICU than for the 31 hospitalized patients admitted the general ward (medians 29.5 and 32.0, respectively, p = 0.014, Wilcoxon rank sum test) (Figure 2, panel C). Of the 61 patients who survived, Ct values were not significantly lower for the 30 symptomatic patients than they were for the 31 mildly symptomatic or asymptomatic patients (medians 32.0 and 34.0, respectively, p = 0.08, Wilcoxon rank sum test) (Figure 2, panel D). We found similar associations when we categorized C_t values as low, medium, and high (Table 1). The findings were similar for ORF1a (data not shown).

Age was not associated with C_t when assessed either as a continuous variable (r = –0.07, p = 0.48 Spearman ρ) or as quartiles (p = 0.91, Kruskal-Wallis test). Of the 73 patients for whom information was available on days from illness onset to swab sample collection, we found correlation between the days since onset and C_t as continuous variables (r = 0.26, p = 0.029, Spearman ρ) (Figure 3) with higher virus load found earlier in the course of illness. Among patients for whom the presence (52 patients) or absence (16 patients) of underlying illness was noted, the C_t values did not differ significantly (medians 31.5 and 31.0, respectively, p = 0.48, Wilcoxon rank sum test). For only 13 surviving hospitalized patients was enough information available to calculate the length of hospital stay (median 7 days), and C_t and length of stay were not correlated for these patients (r = –0.02, p = 0.94, Spearman ρ). C_t values did not differ significantly by hospital in which the sample was collected (p = 0.33, Kruskal-Wallis test).

In univariate logistic regression, increased probability of death was predicted by older age, presence of underlying illness, and lower C_t (Table 2). In multivariable analysis, being >60 years of age (odds ratio [OR] 11.7, 95% CI 2.00–67.9) and having an underlying illness (OR 5.19, 95% CI 1.08–25) were the strongest predictors of death. Lower C_t values remained predictive of death when adjusted for age and presence of underlying illness; the odds of death increased 17% for each 1 point drop in C_t (OR 1.17, 95% CI 1.01–1.35). For the composite severe outcome, the same factors were identified in univariate and multivariate analysis; C_t was borderline significant (OR 1.16, 95% CI 1.00–1.34) when adjusted for age and underlying illness (Table 2). We found similar regression results with ORF1a– when

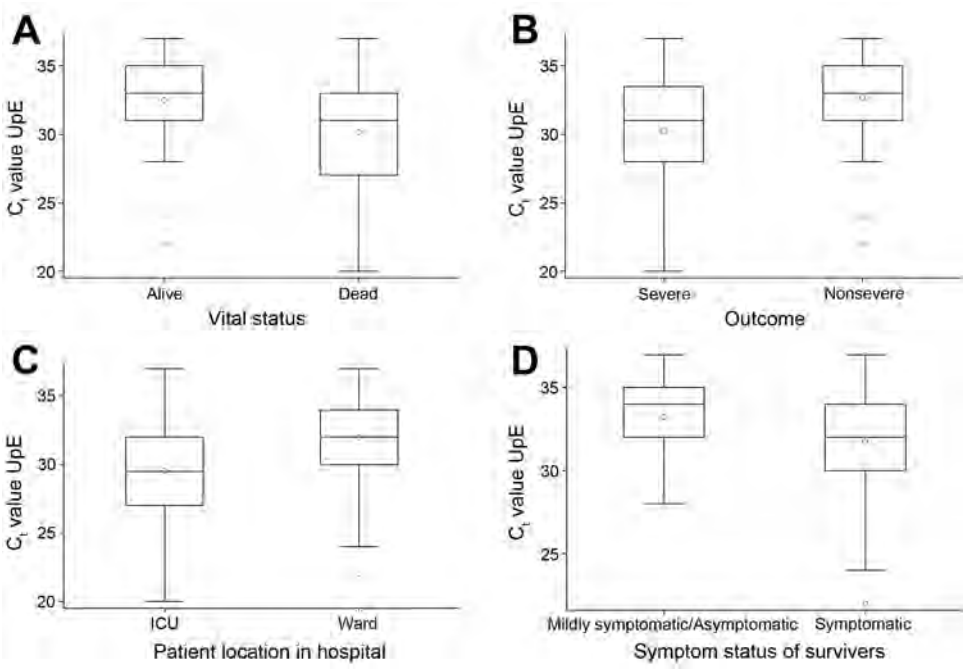

Figure 2. Box plot of C_t values for 102 patients infected with MERS-CoV by severity status. Kingdom of Saudi Arabia, 2014. A) Patients who were alive (n = 61) versus dead (n = 41) at the time of follow-up chart review or phone contact. Wilcoxon rank-sum test, p = 0.0087. B) Patients who had a severe outcome (death or ICU admission, n = 48) versus nonsevere outcome (n = 54). Wilcoxon rank-sum test, p = 0.0036. C) Patients who were admitted to the ICU (n = 36) versus the regular ward (n = 31). Wilcoxon rank-sum test, p = 0.014. D) Among patients who survived, symptomatic (n = 30) versus mildly symptomatic/asymptomatic (n = 31). Wilcoxon rank-sum test, p = 0.08. Box and whiskers plot features are as follows: central line in box is median, bottom line of box is first quartile (25%), top line of box is third quartile (75%), diamond is mean, bottom of whiskers is first quarter minus 1.5 × interquartile range, top of whiskers is third quarter plus 1.5 × interquartile range, and dots are outliers. C_t, cycle threshold; ICU, intensive care unit; MERS-CoV, Middle East respiratory syndrome coronavirus.

we adjusted for underlying illness and age with a positive association between lower ORF1a C_t values and death (OR 1.21, 95% CI 1.04–1.40) and severe disease (OR 1.19, 95% CI 1.01–1.40).

Discussion

We found an association between higher virus load of MERS-CoV detected in the upper respiratory tract, as indicated by lower C_t values, and worse clinical outcome, including death and admission to the ICU. This finding is derived from a large number of MERS-CoV patients from KSA during the upsurge in cases during spring 2014, when MERS-CoV was diagnosed in patients with a spectrum of clinical illness (*17*). Our findings did not change when we adjusted for other risk factors for severe outcome and potential confounders. Although the exact pathophysiology of MERS-CoV infection in the lung is still unknown, more virions could lead to worse lung damage either by direct destruction of respiratory epithelial cells or by triggering a more vigorous inflammatory response (*21*). In severe acute respiratory syndrome coronavirus infections, a similar association was observed between worse outcome and higher virus load, as measured in nasopharyngeal, serum, and fecal samples (*22*). Why some patients have higher MERS-CoV virus load is unclear. Virus load might be related to the inoculum size at the time of infection; anecdotally, primary MERS-CoV infections, which are probably more likely to be acquired from environmental exposure (e.g., camels) than from person-to-person spread, tend to have worse outcomes, even among younger patients, although this factor could reflect other risks or case ascertainment bias (*23*,*24*). Virus load might also be related to host factors, such as the immune response to the virus, which in turn could be affected by intrinsic factors, such as the presence of underlying illness or host genetics.

This association between outcome and virus load in the upper respiratory tract has been demonstrated previously for other viral infections. Children with higher respiratory syncytial virus loads are more likely to be hospitalized and require mechanical ventilation (*10*,*12*,*15*). Some studies have found higher virus load for influenza viruses among patients who were sicker (*14*,*25*). Higher virus loads of human bocavirus in nasopharyngeal aspirate samples were associated with greater severity of

Table 1. C_t for upE gene based on several indicators of severity of illness for 102 patients infected with MERS-CoV, Kingdom of Saudi Arabia, 2014*

Indicator	Virus load			p value†
	Low, $C_t \leq 26$, no. (%)	Medium, C_t 27–33, no. (%)	High, $C_t \geq 34$, no. (%)	
Vital status				
Died, n = 41	8 (20)	23 (56)	10 (24)	0.0044
Survived, n = 61	2 (3)	32 (52)	27 (44)	
Composite severity status				
Severe,‡ n = 48	8 (17)	28 (58)	12 (25)	0.0060
Not severe, n = 54	2 (4)	27 (50)	25 (46)	
Hospital location§				
ICU, n = 36	8 (22)	21 (58)	7 (19)	0.018
General ward, n = 31	2 (6)	16 (52)	13 (42)	
Symptom status¶				
Symptomatic, n = 30	2 (7)	18 (60)	10 (33)	0.064
Asymptomatic, n = 30	0	14 (47)	16 (53)	

*Ct, cycle threshold; ICU, intensive care unit; MERS-CoV, Middle East respiratory syndrome coronavirus.
†Ordinal χ^2 test with 1 degree of freedom.
‡Severe is a composite of patients who died and patients who were admitted to the ICU.
§Among admitted patients.
¶Among surviving patients.

illness among Chinese children (*16*). Other studies, however, have not shown an association between virus load and illness severity (*11–13*).

Clearly the specimen type can affect virus load. A previous study from KSA showed that median C_t values were lower for MERS-CoV from tracheal aspirate and bronchoaveolar lavage samples than for nasopharyngeal swab samples, presumably reflecting higher virus load in the lower respiratory tract (*20*). Moreover, lower respiratory tract samples, including expectorated sputum, are positive for MERS-CoV when upper respiratory tract samples were negative (*26*,*27*). We observed similar findings with bronchoalveolar lavage and sputum yielding higher virus load than nasopharyngeal specimens, although this could have reflected that these specimens were only available from sicker patients or been due to chance with so few bronchoalveolar or sputum specimens.

Because the severity of patients most likely was associated with the available specimen (e.g., only intubated patients have bronchoalveolar lavage), we restricted our analysis to patients with nasopharyngeal swab samples, which is the recommended specimen for initial MERS-CoV diagnosis according to KSA MOH guidelines (*18*). The clinical implication of measuring virus load in upper respiratory tract specimens for MERS-CoV is not clear because the virus load probably reflects virus replication in the nasopharyngeal epithelial cells. We assumed that virus load in the upper respiratory tract correlated with that in the lung, although this assumption is not known definitively. By limiting the analysis to virus load among bronchoalveolar lavage specimens, we would have been able to more directly compare virus load in the lung with clinical severity; however, too few of these specimens were available to conduct this analysis.

We showed that several other risk factors were associated with death and severe outcome on multivariable analysis. The strongest risk factors were being elderly and having an underlying illness. Although several studies have shown underlying illness to be common among MERS-CoV patients, including those who died, few have quantified the risk for underlying illness in severe outcomes (*5*,*8*,*28*). We showed that the presence of underlying illness elevated the odds of severe outcome or death by 7–8-fold. The higher risk for severe outcomes earlier in the course of the Jeddah outbreak probably was due to the increase in contact tracing as the outbreak progressed in Jeddah, leading to detection of a greater spectrum of clinical MERS-CoV disease later in the outbreak.

Our analysis is subject to several possible limitations. C_t values are a semiquantitative measure of virus load and therefore only reflect relative virus loads. Without reference to a known standard curve for each real-time RT-PCR run, we were not able to assign specific virus load thresholds to severity categories. Run-to-run variability could have existed in C_t–virus load relationships, although the extraction method, real-time RT-PCR, and

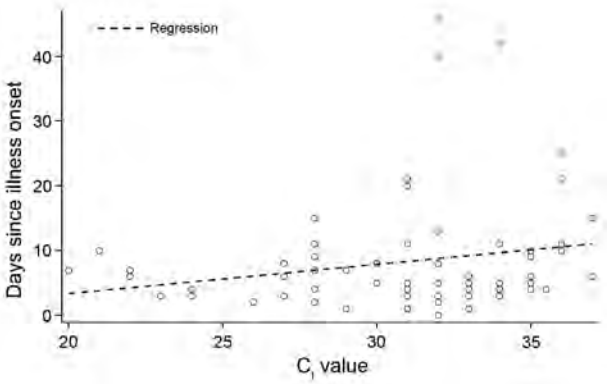

Figure 3. Relationship between days since illness onset and upE C_t values for MERS-CoV from nasopharyngeal swabs, Kingdom of Saudi Arabia, 2014. C_t, cycle threshold; MERS-CoV, Middle East respiratory syndrome coronavirus; upE, upstream of E gene.

Table 2. Results of logistic regression for risk of death and severe outcome (death or ICU admission) for 102 patients with MERS-CoV infection, Kingdom of Saudi Arabia, 2014*

	Died, n = 41, vs. survived, n = 61		Severe, n = 48, vs. not severe, n = 54	
Outcome	Univariate OR (95% CI)	Multivariable OR (95% CI)	Univariate OR (95% CI)	Multivariable OR (95% CI)
Virus load, 1 point decrease in C_t	1.18 (1.06–1.33)	1.17 (1.01–1.35)	1.20 (1.06–1.35)	1.16 (1.00–1.34)
Age, y, grouped in quartiles				
8–28, n = 23	Referent	Referent	Referent	Referent
29–47, n = 21	2.67 (0.57–12.4)	2.79 (0.49–15.8)	4.10 (0.92–18.4)	5.21 (0.88–30.9)
48–60, n = 27	5.33 (1.28–22.3)	3.42 (0.68–17.1)	8.33 (2.00–34.9)	5.13 (0.98–27.0)
>60, n = 19	18.7 (3.82–91.2)	11.7 (2.00–67.9)	25.0 (4.85–129)	14.0 (2.19–89.7)
Age missing, n = 12	6.67 (1.27–35.0)	7.19 (1.17–44.1)	9.33 (1.76–49.6)	12.5 (1.80–86.3)
Male sex, n = 76	2.20 (0.83–5.84)	NA	2.50 (0.97–6.44)	NA
Underlying illness				
No, n = 16	Referent	Referent	Referent	Referent
Yes, n = 52	6.93 (1.76–27.4)	5.19 (1.08–25.0)	8.14 (2.25–29.5)	7.12 (1.55–32.7)
Unknown, n = 34	0.93 (0.20–4.3)	1.43 (0.25–8.29)	0.64 (0.15–2.70)	0.97 (0.18–5.21)
Week of specimen collection				
March 26–April 7, n = 12	Referent	NA	Referent	NA
April 8–21, n = 45	0.57 (0.16–2.07)	NA	0.44 (0.12–1.66)	NA
April 22–May 5, n = 36	0.27 (0.07–1.07)	NA	0.32 (0.08–1.26)	NA
May 6–16, n = 9	0.57 (0.10–3.3)	NA	0.63 (0.11–3.7)	NA

*ORs for C_t for upE target are the odds of having outcome for each 1 point decrease in C_t. C_t, cycle threshold; ICU, intensive care unit; MERS-CoV, Middle East respiratory syndrome coronavirus; NA, not included in multivariate model; OR, odds ratio.

equipment remained the same throughout this period in the Jeddah Regional Laboratory. If real-time RT-PCR is 100% efficient, a 1-unit change in C_t indicates an ≈2-fold difference in concentration of virus copies, although such conditions are rarely met in real-world practice (*12*). C_t values have been used in other reports as an indicator of MERS-CoV virus load (*20*). Second, specimens were collected from multiple hospitals and the home setting for asymptomatic contacts. This could have led to variability in quality of the specimens, and/or handling and shipping practices in getting specimens to the Jeddah Regional Laboratory. However, unless systematic differences were present by site in terms of the severity of illness affecting specimen collection, this variability should have been nondifferential and biased the findings toward the null. Moreover, we observed no difference in the median C_t across hospitals. Third, data for other outcomes indicative of severity of illness were either missing in some patients (e.g., days since onset, length of hospital stay) or not available at all in the current dataset (e.g., mechanical ventilation, oxygen requirements). Fourth, some patients initially identified by the MOH as asymptomatic and not hospitalized did not have medical records for review, and data were collected by phone for these patients; some of these patients were unable to be contacted and so we could not verify the initial data from the MOH, leading to potential misclassification for some variables. Finally, without systematically sampling and testing sequential specimens in the same patient during the course of illness, we are unable to determine the temporal sequence in the association between virus load and outcome, i.e., we cannot determine whether higher virus load causally leads to severe outcomes

Although we present evidence for an epidemiologic association between virus load and severity of MERS-CoV illness, the clinical implications of our findings are unclear. At this time, MERS-CoV has no known treatment, other than supportive care. Several approaches to treatment, including antiviral drugs and immunotherapy are being investigated (*29*). More data are needed on whether modulation of virus load by therapeutic agents can affect clinical outcomes.

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No Geographic Correlation between Lyme Disease and Death Due to 4 Neurodegenerative Disorders, United States, 2001–2010

Joseph D. Forrester,[1] Kiersten J. Kugeler, Anna E. Perea, Daniel M. Pastula, Paul S. Mead

Associations between Lyme disease and certain neurodegenerative diseases have been proposed, but supportive evidence for an association is lacking. Similar geographic distributions would be expected if 2 conditions were etiologically linked. Thus, we compared the distribution of Lyme disease cases in the United States with the distributions of deaths due to Alzheimer disease, amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), and Parkinson disease; no geographic correlations were identified. Lyme disease incidence per US state was not correlated with rates of death due to ALS, MS, or Parkinson disease; however, an inverse correlation was detected between Lyme disease and Alzheimer disease. The absence of a positive correlation between the geographic distribution of Lyme disease and the distribution of deaths due to Alzheimer disease, ALS, MS, and Parkinson disease provides further evidence that Lyme disease is not associated with the development of these neurodegenerative conditions.

Lyme disease is a complex, multisystem tickborne illness caused by the spirochete *Borrelia burgdorferi* (*1*). Each year in the United States, >30,000 cases are reported, but the actual number of infections may be 10-fold higher (*1*,*2*). Lyme disease cases are most commonly reported from the Northeast, mid-Atlantic, and upper Midwest regions of the United States (*3*). Central nervous system infection resulting in early neurologic Lyme disease, and more rarely late neurologic Lyme disease, is well documented (*4*,*5*). Because of the neurotropism of Lyme disease, speculative websites and articles and even peer-reviewed journals have purported causal associations between Lyme disease and several neurodegenerative disorders, including Alzheimer disease, amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), and Parkinson disease (*6*–*11*). Researchers have critically evaluated these proposed biologic associations between Lyme disease and Alzheimer disease, ALS, MS, and Parkinson disease, but none have found evidence of an association (*12*–*21*). We hypothesized that, if there is a link between *B. burgdorferi* infection and subsequent development of Alzheimer disease, ALS, MS, or Parkinson disease, the geographic distribution of these neurodegenerative disorders should correlate with that of Lyme disease. To determine if such a correlation exists, we compared the distribution of confirmed cases of Lyme disease in the United States with the distribution of deaths due to these 4 neurodegenerative disorders.

Methods

We compared Lyme disease incidence rates in each state with death rates for Alzheimer disease, ALS, MS, and Parkinson disease. Reports of confirmed Lyme disease cases submitted to the National Notifiable Diseases Surveillance System during 2001–2010 (*2*) were used to calculate state-specific, age-adjusted incidence rates of Lyme disease. Age-adjusted death rates of Alzheimer disease, ALS, MS, and Parkinson disease during the same time period were obtained from the CDC WONDER (Centers for Disease Control and Prevention Wide-ranging Online Data for Epidemiologic Research) database (http://wonder.cdc.gov/WelcomeT.html). Codes for underlying cause of death from the International Classification of Diseases, Tenth Revision, Clinical Modification (http://www.cdc.gov/nchs/icd/icd10cm.htm), were as follows: G30, Alzheimer disease; G12.2, motor neuron disease (ALS); G35, MS; and G20, Parkinson disease. We standardized the Lyme disease incidence rates and neurodegenerative disease death rates to the 2000 US population by using 10-year age groups (http://www.census.gov/2000census/data/).

We used the Moran's I test for spatial autocorrelation to assess geographic clustering of state incidence rates of Lyme disease and of death rates for the 4 neurologic disorders by using ArcGIS 10.1 (ESRI, Redlands, CA, USA). Geographic correlation between Lyme disease incidence and death rates for each of the other conditions was assessed by using the Spearman rank correlation (r_s) to

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compare pairwise state rates. A subanalysis comparing pairwise state rates by sex was similarly performed. Rates for male patients who died of MS in Hawaii and the District of Columbia were not included in the analysis because of CDC WONDER data use restrictions. Analyses were conducted by using SAS version 9.3 (SAS Institute, Inc., Cary, NC, USA). Because this analysis used publicly available data, human subjects research approval was not sought.

Results

During 2001–2010, a total of 256,373 confirmed Lyme disease cases were reported in the United States. The median age of patients was 42 years; 137,377 (55%) patients were male. As expected, Lyme disease cases were concentrated in the Northeast, mid-Atlantic, and upper Midwest. Standardized state-specific Lyme disease incidence rates ranged from <1 case per 100,000 person-years for 34 states to 19–73 cases per 100,000 person years for 13 high-incidence states in the Northeast, mid-Atlantic, and upper Midwest (overall median incidence 0.4 cases/100,000 person-years) (Figure).

During the study period, 705,735 deaths were attributed to Alzheimer disease (median patient age 86 y, 29% male), 59,769 to ALS (median patient age 69 y, 54% male), 34,298 to MS (median patient age 60 y, 34% male), and 190,428 to Parkinson disease (median patient age 82 y, 58% male). Overall, disease-specific, age-adjusted median death rates ranged from a low of 1.1 deaths per 100,000 person-years for MS (range across states 0.2–2.1) to a high of 24.5 deaths per 100,000 person years for Alzheimer disease (range across states 9.6–41.2) (Figure)

All 5 diseases demonstrated positive spatial autocorrelation by the Moran's I test: Lyme disease I index 0.49 ($p<0.0001$), Alzheimer disease I index 0.21 ($p = 0.01$), ALS I index 0.25 ($p = 0.003$), MS I index 0.56 ($p<0.0001$), and Parkinson disease I index 0.21 ($p = 0.001$). Nevertheless, Lyme disease incidence per state was not correlated with rates of death due to ALS ($r_s = 0.19$, $p = 0.19$), MS ($r_s = 0.20$, $p = 0.17$), or Parkinson disease ($r_s = -0.14$, $p = 0.34$). An inverse correlation was detected between Lyme disease and Alzheimer disease ($r_s = -0.35$, $p = 0.01$). Findings were similar when evaluated with Pearson correlation

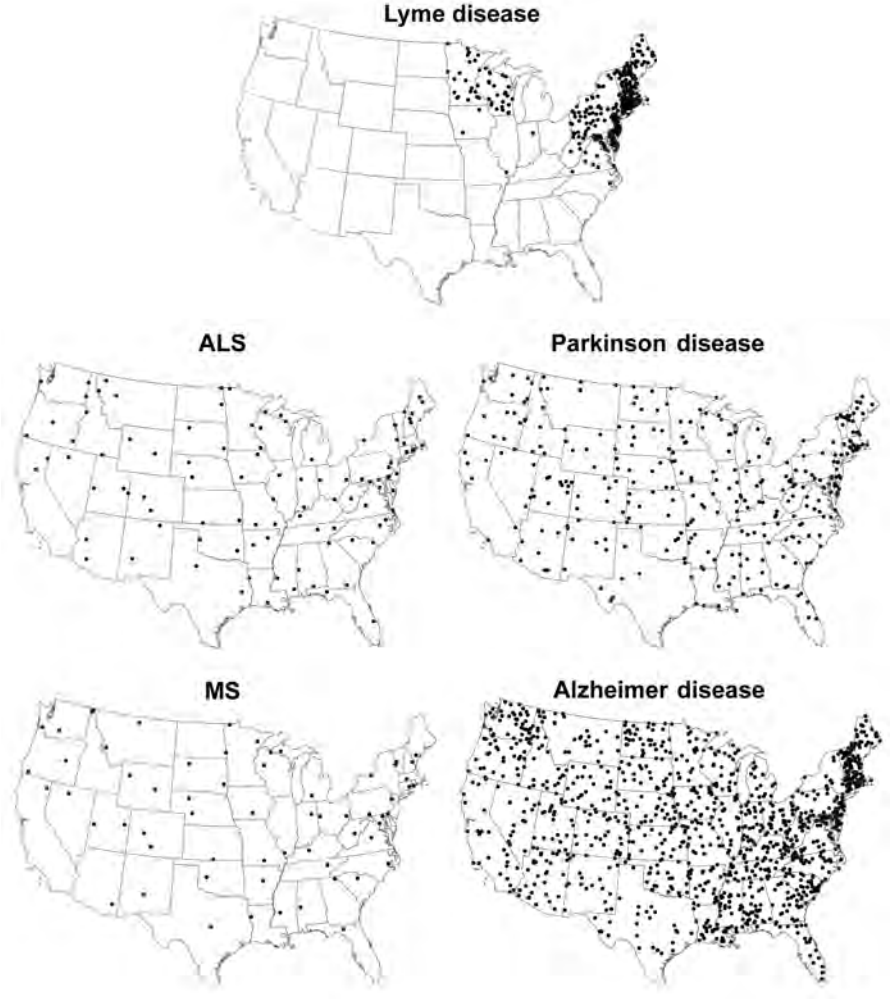

Figure. Geographic distribution of Lyme disease compared with that for deaths due to amyotrophic lateral sclerosis (ALS), Parkinson disease, multiple sclerosis (MS), and Alzheimer disease. One dot represents 1 case (Lyme disease) or 1 death (ALS, Parkinson disease, MS, and Alzheimer disease) per 100,000 person-years; dots are placed randomly within the respective states.

coefficients, when subanalysis by sex was performed, and when analysis was limited to states with >1 Lyme disease case per 100,000 person-years (data not shown).

Discussion

As with other vectorborne diseases, Lyme disease is highly focal in its geographic distribution. If Lyme disease was etiologically linked to Alzheimer disease, ALS, MS, or Parkinson disease, rates of death attributed to these diseases would be expected to correlate geographically with the geographic incidence of Lyme disease. Our findings show, on a coarse geographic scale, no correlation between Lyme disease and these neurodegenerative conditions. The inverse correlation between the rates of Lyme disease and death from Alzheimer disease is in line with findings from a previous report (21); although this finding is of unclear significance, it supports a lack of positive correlation between these conditions.

Positive spatial clustering was demonstrated for each disease. This finding indicates that for each disease, states with high rates are nearer than would be randomly expected to other states that also have high rates of the disease. However, we showed that the clustering of rates of death from the 4 neurodegenerative conditions does not correlate with state-specific Lyme disease incidence, indicating that underlying processes that contribute to geographic clustering of neurodegenerative conditions are unrelated to Lyme disease. Each of the diseases, including Lyme disease, has a unique and nonrandom geographic distribution; the distributions of the neurodegenerative disorders do not mirror that of Lyme disease.

As for ALS and Parkinson disease, Lyme disease patients are more commonly male; MS and Alzheimer disease patients are more commonly female (*3*,*22*–*25*). It is conceivable that variations between states in sex distribution of the population could contribute to the lack of observed correlation between Lyme disease and the 4 neurodegenerative disorders. However, even when we examined sex-specific, age-adjusted rates by state, no correlation was identified between the geographic distribution of Lyme disease and any of the 4 neurodegenerative disorders. This finding suggests that differences in the distribution of the sexes by state did not affect the lack of correlation that we observed.

Our study had several limitations. First, our analysis was based on data from death certificates, which are limited by inconsistent practices in the completion of cause-of-death statements (*26*). Underlying cause of death is defined as the disease that initiated the chain of events resulting in death (*27*). Thus, our analysis did not include the prevalence of neurodegenerative disorders not resulting in death, and could therefore underestimate the incidence of these disorders. Nevertheless, this potential underestimation is unlikely to differentially affect the age-adjusted distribution of the deaths by state, and the death rates should proportionally reflect the prevalence of specific neurodegenerative disorders across states. Second, this cross-sectional ecologic study cannot be used to determine person-level causality. Third, the study did not account for persons who may have acquired Lyme disease in 1 state and subsequently moved to another part of the country before receiving a diagnosis of and dying from a neurodegenerative disorder. In such instances, state classification would be misclassified because of the latent period between exposure and development of disease. This direction of misclassification would reduce any observed correlation between neurodegenerative disorders and Lyme disease. Last, in historically low-incidence areas where Lyme disease has since spread, any potential time lag between infection and development of a neurodegenerative disorder would reduce the observed correlation in these areas. A limitation of the dataset was that we could not assess associations for the time from infection with Lyme disease to development of a neurodegenerative disorder.

In conclusion, although associations between Lyme disease and Alzheimer disease, ALS, MS, and Parkinson disease have been proposed by writers of speculative websites and articles, supportive evidence for such an association is lacking. The absence of a positive correlation in the geographic distributions of these conditions provides further evidence against an association between Lyme disease and deaths from these 4 neurodegenerative conditions.

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Invasive Pneumococcal Disease 3 Years after Introduction of 10-Valent Pneumococcal Conjugate Vaccine, the Netherlands

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Three years after a 7-valent pneumococcal conjugate vaccine was replaced by a 10-valent pneumococcal conjugate vaccine in the Netherlands, we observed a decrease in incidence of invasive pneumococcal disease caused by *Streptococcus pneumoniae* serotypes 1, 5, and 7F. Our data do not support or exclude cross-protection against serotype 19A.

A 7-valent pneumococcal conjugate vaccine (PCV7) was first used in the Netherlands in June 2006 in a 3 + 1 schedule for protection against invasive pneumococcal disease (IPD). A switch to 10-valent pneumococcal conjugate vaccine (PCV10) was made in May 2011. There were no catch-up campaigns; children vaccinated with PCV7 completed their series with PCV7. Vaccination coverage has been 94%–95% since PCV7 introduction (*1*).

After PCV7 introduction, vaccine-type IPD incidence decreased for all age groups (*2*). However, nasopharyngeal carriage and incidence of IPD caused by nonvaccine serotypes increased (*2*,*3*). Nevertheless, a 7% decrease in overall IPD incidence was observed 4 years after PCV7 introduction (*2*).

Studies assessing the effect of PCV10 when used in national vaccination programs are limited. Two studies on PCV10 effectiveness in children suggested cross-protection against vaccine-related serotypes, including 19A (*4*,*5*). No studies have been published on herd effects of PCV10 in unvaccinated persons. We report the effect of switching from PCV7 to PCV10 on IPD incidence in the Netherlands.

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The Study

We used data for June 2004–May 2014 from a sentinel laboratory surveillance system, as described (*2*). These data cover ≈25% of the population of the Netherlands. Cumulative incidence ratios (CIRs) with 95% CIs were calculated for comparisons of pre-PCV7 (June 2004–May 2006), pre-PCV10 (June 2009–May 2011), early post-PCV10 (June 2011–May 2013) and 3-year-post PCV10 (June 2013–May 2014) periods. We discerned IPD caused by PCV7 (4, 6B, 9V, 14, 18C, 19F, 23F), non-PCV7, PCV10–7 (present in PCV10 but not PCV7; 1, 5, 7F), non-PCV10, and PCV10 -related (6A, 6C, 6D, 7A, 7B, 7C, 9A, 9L, 9N, 18A, 18B, 18F, 19A, 19B, 19C, 23A, 23B) serotypes.

We used nationwide laboratory surveillance data for children <5 years of age available from 2006 to compare IPD incidence rates for a PCV7-eligible cohort (children born March 2008–Feb 2011, ≥3 months of age, and given a diagnosis of IPD before June 2011) and a PCV10-eligible cohort (children born March 2011–Feb 2014, ≥3 months of age, and given a diagnosis of IPD before June 2014). Incidence rate ratios (IRRs) with 95% CIs and p values were calculated. Differences between IRRs were tested by calculating p values for interaction between birth cohort and serotype; the IRR for serotypes not related to PCV10 was used as reference.

A total of 6,292 IPD cases were included in sentinel surveillance during June 2004–May 2014. By 2009–2011, overall IPD incidence had decreased by 57% for children <2 years of age, 47% for children 2–4 years of age, and 22% for persons ≥65 years of age (Figure 1; Table 1). No further decrease was observed during 2011–2014 for persons ≥65 years of age. PCV7 IPD incidence decreased for all age groups, and the decrease continued and showed an overall reduction of 90% by 2013–2014 (Figure 2, panel A). Non-PCV7 IPD incidence increased by 38% for all age groups from 2004–2006 to 2009–2011 (Figure 2, panel B; Table 1).

Overall PCV10–7 IPD incidence increased slightly after PCV7 introduction (CIR 1.21, 95% CI 1.02–1.43) (Figure 2 panel C; Table 1). For children <2 years of age, PCV10–7 IPD incidence decreased 2 years after the switch to PCV10, although not significantly, because of a low number of cases (CIR 0.51, 95% CI 0.13–2.02). In the third year (2013–2014) after PCV10 introduction, no IPD cases were caused by serotypes 1, 5, and 7F in children <2 years of age. For other age groups, PCV10–7 IPD incidence did

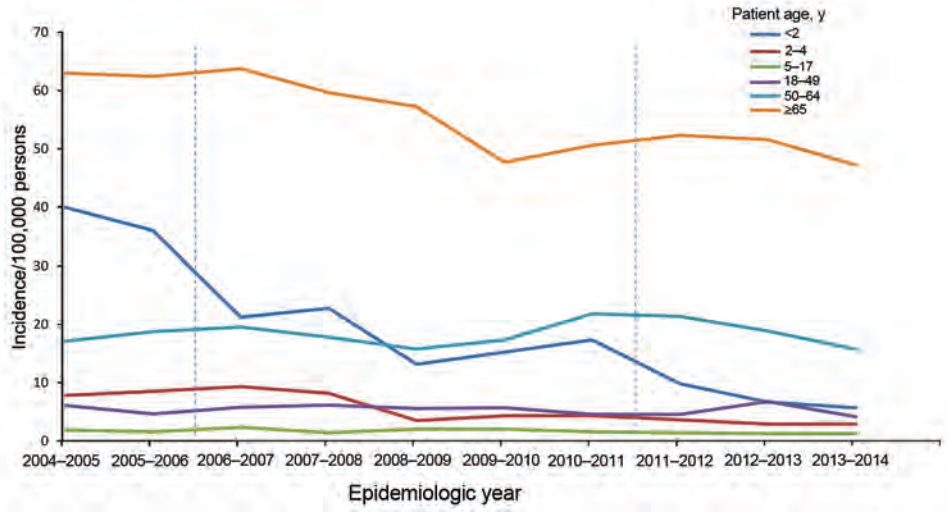

Figure 1. Age-specific incidence of invasive pneumococcal disease caused by any serotype of *Streptococcus pneumoniae* per epidemiologic year (June–May), the Netherlands. Vertical dashed lines indicate introduction of 7-valent pneumococcal conjugate vaccine in June 2006 and 10-valent pneumococcal conjugate vaccine in May 2011. Incidences are based on sentinel surveillance data and extrapolated to the national level.

not change in the 2 years after PCV10 introduction, but in the third year, incidence decreased by 42% and 47% for persons 18–49 years of age and 50–64 years of age, respectively; a 25% decrease was observed for persons ≥65 years of age (Figure 2 panel C; Table 1). Non-PCV10 IPD incidence increased for most age groups in the 2 years after PCV10 introduction (overall CIR 1.25, 95% CI 1.13–1.38) but did not increase further in 2013–2014 (Figure 2, panel D; Table 1), partly because of a decrease in 19A IPD (Figure 2, panel E; Table 1).

The IPD incidence rate for the PCV10-eligible cohort was lower than that for the PCV7-eligible cohort for PCV10–7 serotypes (IRR 0.04, 95% CI 0.01–0.27), PCV7-related serotypes (IRR 0.38, 95% CI 0.19–0.77), and non-PCV10 serotypes (IRR 0.67, 95% CI 0.46–0.99) (Table 2). The decrease in PCV10–7 IPD was greater than that for non-PCV10 serotypes ($p_{interaction}$ = 0.005). However, IRRs for PCV10-related IPD and specifically serotype 19A were not different from the IRR for PCV10-unrelated IPD ($p_{interaction}$ = 0.229/0.165).

Conclusions

We observed a decrease in PCV7-type IPD ≥8 years after PCV7 introduction for all age groups. However, this decrease was lessened by an increase in non–vaccine-type IPD, a finding similar to that reported in other countries (*6*,*7*). There was an overall 80% decrease in IPD incidence for children <5 years of age and a 25% decrease for persons ≥65 years of age.

PCV10 introduction caused a decrease in PCV10–7 IPD incidence in PCV10-eligible children, providing evidence

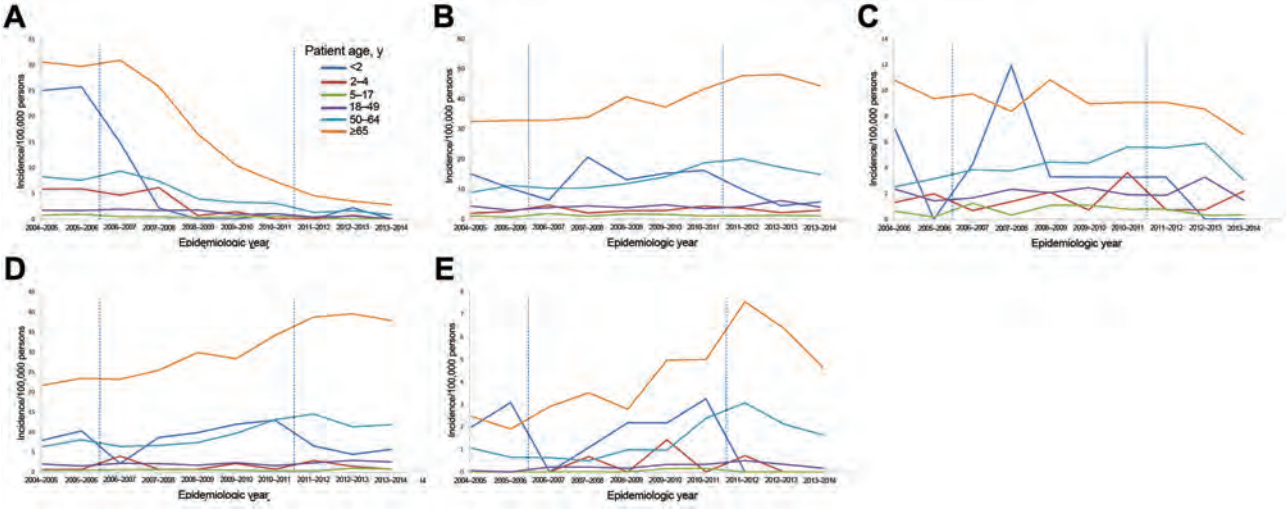

Figure 2. Age-specific incidence of invasive pneumococcal disease caused by different Streptococcus pneumoniae serotypes per epidemiologic year (June–May), the Netherlands. A) 7-valent pneumococcal conjugate vaccine (PCV7) serotypes; B) non-PCV7 serotypes; C) PCV10–7 pneumococcal conjugate vaccine serotypes, D) non-PCV10 serotypes; and E) serotype 19A. Vertical dashed lines indicate introduction of PCV7 in June 2006 and PCV10 in May 2011. Incidences are based on sentinel surveillance data and extrapolated to the national level.

for a direct effect from PCV10. Potential cross-protection of PCV10 against serotype 19A, as corroborated by a case–control study showing 82% effectiveness against 19A IPD (*5*), is still debated. In our study, the incidence rate for 19A IPD was lower in the PCV10-eligible cohort than the PCV7-eligible cohort, but the decrease in 19A IPD was not different from the decrease in PCV10-unrelated IPD, which precludes drawing conclusions about cross-protection against 19A IPD. In addition, 19A carriage had already decreased in toddlers before PCV10 introduction (*8*).

We observed a decrease in non–PCV10 IPD in the PCV10-eligible cohort but have no indication that surveillance sensitivity changed over time. The decrease might be caused by natural fluctuations or different viral seasons (*9*). A study in Canada reported lower incidence

Table 1. Number of IPD cases and cumulative incidence ratios determined on the basis of sentinel surveillance data, the Netherlands, June 2011–May 2013*

Serotype and patient age group, y	No. cases				Comparison, CIR (95% CI)		
	Pre-PCV7, 2004–2006	Pre-PCV10, 2009–2011	Early post-PCV10, 2011–2013	3 years post-PCV10, 2013–2014	Pre-PCV10 vs. pre-PCV7	Early post-PCV10 vs. pre-PCV10	3 years post-PCV10 vs. early post-PCV10
All							
<2	75	30	15	5	0.43 (0.28–0.65)	0.51 (0.27–0.94)	0.69 (0.25–1.90)
2–4	25	12	9	4	0.53 (0.27–1.05)	0.75 (0.32–1.79)	0.89 (0.27–2.89)
5–17	22	23	17	8	1.04 (0.58–1.87)	0.75 (0.40–1.40)	0.95 (0.41–2.20)
18–49	197	184	201	73	0.96 (0.78–1.17)	1.10 (0.90–1.34)	0.73 (0.56–0.96)
50–64	273	326	341	134	1.09 (0.93–1.28)	1.03 (0.88–1.19)	0.78 (0.64–0.95)
≥65	717	622	703	338	0.78 (0.70–0.87)	1.06 (0.95–1.18)	0.91 (0.80–1.03)
Total	1,309	1,197	1,286	562	0.90 (0.83–0.97)	1.06 (0.98–1.15)	0.87 (0.79–0.96)
PCV7							
<2	50	1	2	0	0.02 (0.00–0.16)	2.03 (0.18–22.33)	NC
2–4	18	2	1	0	0.12 (0.03–0.53)	0.50 (0.05–5.55)	NC
5–17	11	6	2	1	0.54 (0.20–1.47)	0.34 (0.07–1.67)	1.01 (0.09–11.1)
18–49	63	35	17	2	0.57 (0.38–0.86)	0.49 (0.27–0.87)	0.24 (0.05–1.03)
50–64	120	53	25	7	0.40 (0.29–0.56)	0.46 (0.29–0.74)	0.56 (0.24–1.29)
≥65	344	112	54	20	0.29 (0.24–0.36)	0.45 (0.33–0.62)	0.70 (0.42–1.17)
Total	606	209	101	30	0.34 (0.29–0.40)	0.48 (0.38–0.61)	0.59 (0.39–0.89)
Non-PCV7							
<2	25	29	13	5	1.24 (0.73–2.12)	0.45 (0.24–0.87)	0.80 (0.28–2.23)
2–4	7	10	8	4	1.57 (0.60–4.13)	0.80 (0.32–2.04)	1.00 (0.30–3.32)
5–17	11	17	15	7	1.54 (0.72–3.30)	0.89 (0.44–1.78)	0.94 (0.38–2.31)
18–49	134	149	184	71	1.14 (0.90–1.44)	1.24 (1.00–1.54)	0.78 (0.59–1.02)
50–64	153	273	316	127	1.63 (1.34–1.99)	1.14 (0.97–1.33)	0.80 (0.65–0.98)
≥65	373	510	649	318	1.24 (1.08–1.41)	1.19 (1.06–1.34)	0.93 (0.81–1.06)
Total	703	988	1,185	532	1.38 (1.26–1.52)	1.19 (1.09–1.29)	0.89 (0.81–0.99)
PCV10–7							
<2	7	6	3	0	0.92 (0.31–2.73)	0.51 (0.13–2.02)	NC
2–4	5	6	2	3	1.32 (0.40–4.33)	0.34 (0.07–1.66)	3.00 (0.50–18.0)
5–17	5	12	7	2	2.40 (0.84–6.81)	0.59 (0.23–1.49)	0.58 (0.12–2.77)
18–49	69	78	91	26	1.16 (0.84–1.60)	1.18 (0.87–1.59)	0.58 (0.37–0.89)
50–64	43	83	97	26	1.77 (1.22–2.55)	1.15 (0.86–1.54)	0.53 (0.35–0.82)
≥65	115	114	119	47	0.90 (0.69–1.16)	0.98 (0.76–1.26)	0.75 (0.53–1.05)
Total	244	299	319	104	1.21 (1.02–1.43)	1.06 (0.90–1.24)	0.65 (0.52–0.81)
Non-PCV10							
<2	18	23	10	5	NA	0.44 (0.21–0.92)	1.03 (0.35–3.03)
2–4	2	4	6	1	NA	1.51 (0.43–5.35)	0.33 (0.04–2.77)
5–17	6	5	8	5	NA	1.61 (0.53–4.93)	1.26 (0.41–3.85)
18–49	65	71	93	45	NA	1.32 (0.97–1.80)	0.98 (0.68–1.39)
50–64	110	190	219	101	NA	1.13 (0.93–1.37)	0.92 (0.72–1.16)
≥65	258	396	530	271	NA	1.25 (1.10–1.43)	0.97 (0.84–1.12)
Total	459	689	866	428	NA	1.25 (1.13–1.38)	0.98 (0.88–1.11)
19A							
<2	5	5	0	0	1.07 (0.31–3.70)	0.00 (NC)	NC
2–4	0	2	1	0	NC	0.50 (0.05–5.55)	NC
5–17	0	2	0	0	NC	0.00 (NC)	NC
18–49	1	12	15	3	12.3 (1.60–94.6)	1.26 (0.59–2.69)	0.40 (0.12–1.39)
50–64	13	28	44	14	1.97 (1.02–3.81)	1.54 (0.96–2.48)	0.63 (0.35–1.15)
≥65	25	63	94	33	2.28 (1.43–3.62)	1.40 (1.02–1.92)	0.66 (0.45–0.99)
Total	44	112	154	50	2.50 (1.77–3.55)	1.36 (1.07–1.74)	0.65 (0.47–0.89)

*PCV7, 7-valent pneumococcal conjugate vaccine; PCV10–7, serotypes present in PCV10 but not PCV7 (3 additional serotypes); PCV10, 10-valent pneumococcal conjugate vaccine; CIR, cumulative incidence ratio; NC, not calculated (numbers too low to be informative or relevant). NA, not applicable.

rates for 19A IPD and other non–vaccine-type IPD in a PCV10-eligible cohort (*4*). It was hypothesized that lower antibody levels induced by PCV10 (*10*,*11*) might lead to smaller disturbances of the nasopharyngeal niche and replacement by new serotypes against which there is no immunity, which might result in a lower incidence of non-PCV10 IPD. However, a randomized controlled trial showed similar carriage rates for non-PCV10 serotypes, including 19A, for infants vaccinated with PCV7 and those vaccinated with PCV10 (*12*).

In the third year after PCV10 introduction, PCV10–7 IPD incidence also decreased in nonvaccinated age groups, which might indicate herd effects. After PCV7 introduction, herd effects appeared after 3 years (*13*). Non-PCV10 IPD incidence did not increase in the second and third years after PCV10 introduction, which was partially caused by a reduction in 19A IPD. Longer follow-up times are needed to distinguish whether these observations were caused by cross-protection against 19A in children through herd effects of PCV10, reduced nonvaccine serotype replacement by PCV10, or temporal fluctuations.

We used data from a stable surveillance system with constant coverage over time; age and serotype data were nearly complete (99.9%). However, a limitation of our

Table 2. Number of IPD cases and incidence rate ratios determined on the basis of sentinel surveillance data, the Netherlands, June 2011–May 2103*

Variable	PCV7-eligible cohort	PCV10-eligible cohort	IRR (95% CI)†	Exact p value	p value for interaction‡
Birth cohort	2008 Mar 1–2011 Feb 28	2011 Mar 1–2014 Feb 28	NA	NA	NA
Observation period	2008 Jun 1–2011 May 31	2011 Jun 1–2014 May 31	NA	NA	NA
Persons at risk	550,297	537,071	NA	NA	NA
Person-years at risk	822,100	814,980	NA	NA	NA
No. (%) IPD cases per 100,000 persons					
Serotypes					
PCV7§	5 (0.6)	0 (0.0)	NC	0.063	NC
6B	2	0	NC	NC	NC
18C	2	0	NC	NC	NC
19F	1	0	NC	NC	NC
PCV10–7	27 (3.3)	1 (0.1)	0.04 (0.01–0.27)	<0.001	0.005
1	2	0	NC	NC	NC
5	2	0	NC	NC	NC
7F	23	1	0.04 (0.01–0.32)	<0.001	0.009
PCV10-related§	33 (4.0)	14 (1.7)	0.43 (0.23–0.80)	0.006	0.229
6A	2	0	NC	NC	NC
6C	0	1	NC	NC	NC
9N	0	1	NC	NC	NC
19A	29	11	0.38 (0.19–0.77)	0.005	0.165
23A	1	1	NC	NC	NC
23B	1	0	NC	NC	NC
PCV10-nonrelated	63 (7.7)	42 (5.2)	0.67 (0.46–0.99)	0.045	Reference
3	4	0	NC	NC	NC
8	3	2	NC	NC	NC
10A	14	16	NC	NC	NC
11A	3	0	NC	NC	NC
12F	2	2	NC	NC	NC
15A	1	0	NC	NC	NC
15B	3	1	NC	NC	NC
15C	1	4	NC	NC	NC
16F	4	1	NC	NC	NC
17F	3	1	NC	NC	NC
22F	5	3	NC	NC	NC
24B	0	1	NC	NC	NC
24F	3	1	NC	NC	NC
27	2	1	NC	NC	NC
33F	11	6	NC	NC	NC
34	1	0	NC	NC	NC
35B	0	1	NC	NC	NC
35F	2	1	NC	NC	NC
38	1	1	NC	NC	NC
Total	128 (15.6)	57 (7.0)	0.45 (0.33–0.61)	<0.001	NA

*IPD, invasive pneumococcal disease; PCV7, 7-valent pneumococcal conjugate vaccine; PCV10–7, serotypes present in PCV10 but not PCV7 (3 additional serotypes); PCV10, 10-valent pneumococcal conjugate vaccine; IRR, incidence rate ratio; NA, not applicable; NC, not calculated (numbers too low to be informative or relevant).
†PCV10-eligible cohort vs. PCV7-eligible cohort.
‡For difference between IRRs.
§serotypes with no IPD cases in both cohorts are not shown.

study was the ecologic design. Thus, one should be cautious in interpreting findings as causally related to vaccination. Also, we have limited data on IPD before PCV7 introduction and after the switch to PCV10.

In conclusion, PCV10 introduction in 2011 decreased vaccine-type IPD incidence in targeted birth cohorts. Three years after introduction, herd effects became apparent. Stabilization of non-PCV10 IPD in the second and third years after PCV10 introduction might indicate reduced serotype replacement by PCV10 or cross-protection against 19A. However, we cannot make firm conclusions on cross-protection of PCV10 against serotype 19A. Continued surveillance of serotype-specific IPD is crucial for evaluating long-term effects of pneumococcal conjugate vaccines in human populations.

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Fosfomycin Resistance in *Escherichia coli*, Pennsylvania, USA

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Fosfomycin resistance in *Escherichia coli* is rare in the United States. An extended-spectrum β-lactamase–producing *E. coli* clinical strain identified in Pennsylvania, USA, showed high-level fosfomycin resistance caused by the *fosA3* gene. The IncFII plasmid carrying this gene had a structure similar to those found in China, where fosfomycin resistance is commonly described.

Fosfomycin is a phosphonic acid derivative with antibacterial activity against a wide range of gram-negative pathogens and some gram-positive pathogens. It inhibits bacterial cell wall synthesis and is bactericidal against most *Escherichia coli* strains, as well as many strains of other members of the family *Enterobacteriaceae.* In the United States, only an oral formulation containing fosfomycin tromethamine is approved for clinical use.

Because of increasing resistance of *E. coli* strains to other commonly used agents, such as ciprofloxacin and trimethoprim/sulfamethoxazole, fosfomycin has become one of the first-line agents recommended for treatment of uncomplicated urinary tract infection. A recent study of *E. coli* strains collected at veterans' hospitals in the United States included ciprofloxacin-resistant and extended-spectrum β-lactamase–producing strains. These strains had 98%–99% susceptibility to fosfomycin (*1*). However, susceptibility data on fosfomycin are relatively limited overall because this agent is not routinely tested in most clinical microbiology laboratories.

Several fosfomycin resistance mechanisms have been described in *E. coli*, including reduced permeability, modification of the *murA* gene target, and modification of fosfomycin (*2*). In *E. coli*, the plasmid-mediated fosfomycin resistance gene *fosA3*, which encodes a glutathione S-transferase, was first identified in a fosfomycin-resistant *E. coli* strain in Japan (*3*). This enzyme modifies fosfomycin, thus inactivating the agent and conferring high-level fosfomycin resistance. The *fosA3* gene has been reported from only countries in eastern Asia, especially China. We report a case of persistent colonization with a *fosA3*-carrying *E. coli* strain in a patient in Pennsylvania, USA.

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The Study

The patient was a woman with multiple hospitalizations related to sickle cell crises and end-stage kidney disease; she was receiving peritoneal dialysis. She produced minimal amounts of urine and had frequent urinary tract infections due to extended-spectrum β-lactamase–producing *E. coli*. She did not have history of travel to eastern Asia, from which all reports of *fosA3* have so far originated. The first available *E. coli* strain from this patient, ECRB1, was isolated from a urine sample in 2007 and was reported as a multidrug-resistant strain harboring $bla_{\text{CTX-M-65}}$ and *rmtB* (*4*).

She came to a hospital in 2010 because of a power outage in her home. During this brief hospitalization, a culture collected from the peritoneal catheter exit site grew *E. coli* strain YD472. This strain was found to be highly resistant to fosfomycin, which initiated the present investigation. She had received multiple antimicrobial agents, including cefazolin, ceftriaxone, ciprofloxacin, moxifloxacin, azithromycin, and trimethoprim/sulfamethoxazole, in the prior 5 years, but she had not received fosfomycin according to available medical records.

E. coli YD472 was highly resistant to fosfomycin (MIC >1,024 μg/mL by Etest), which was confirmed by using the agar dilution method with Mueller-Hinton agar and glucose-6-phosphate (25 μg/mL) as an additive, as endorsed by the Clinical and Laboratory Standards Institute (*5*). Given this high-level resistance, PCR was conducted on YD472 to identify *fosA3* and *fosC2*, which have been reported as acquired fosfomycin resistance genes among recent *E. coli* strains in Japan, China, and South Korea (*3*,*6*,*7*). A PCR result was positive for *fosA3*, which was confirmed by sequencing. The *fosA3* gene was transferable to *E. coli* TOP10 by electroporation of the total plasmid extracted from YD472. The *E. coli* TOP10 transformant strain harboring the *fosA3*-encoding plasmid pYHCC was resistant to cefotaxime, gentamicin, and fosfomycin.

We then sequenced the entire plasmid in pYHCC by using single-molecule real-time sequencing (Pacific Biosciences, Menlo Park, CA, USA) as described (*8*). Sequencing in a single cell resulted in a full-length plasmid that could then be circularized and finished (mean coverage 1,325×). The sequence was manually annotated by using

RAST (http://rast.nmpdr.org/), ORF Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html), and IS Finder (https://www-is.biotoul.fr/), and comparisons were made by using BLAST analysis (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The complete plasmid sequence has been deposited in GenBank under accession no. KR078259.

pYHCC is an 80,206-bp circular plasmid with a G + C content of 51.8% and a typical IncFII replicon, which is classified as F2:A–:B– by replicon sequence typing (*9*). It encodes 121 genes (including hypothetical genes) and harbors a 13-kb multidrug resistance region and a 67-kb R100-like backbone region. The backbone region contains genes for replication, transfer, and maintenance. This backbone of pYHCC contains 24 *tra* genes and 7 *trb* genes. The structure of pYHCC is most closely related to that of pXZ, a 77-kb IncFII, *fosA3*-carrying plasmid reported from *E. coli* strains isolated from diseased chickens and ducks in China (99% identity with 95% coverage) (Figure 1) (*10*). It also shares high similarities (99% identity with 84%–87% coverage) with other *fosA3*-carrying *E. coli* plasmids reported, including pHN7A8 from a dog in China (*11*), pHN3A11 from a cat in China, pHK23a from a pig in Hong Kong (*12*), and pFOS-HK151325 from a human in Hong Kong (*13*).

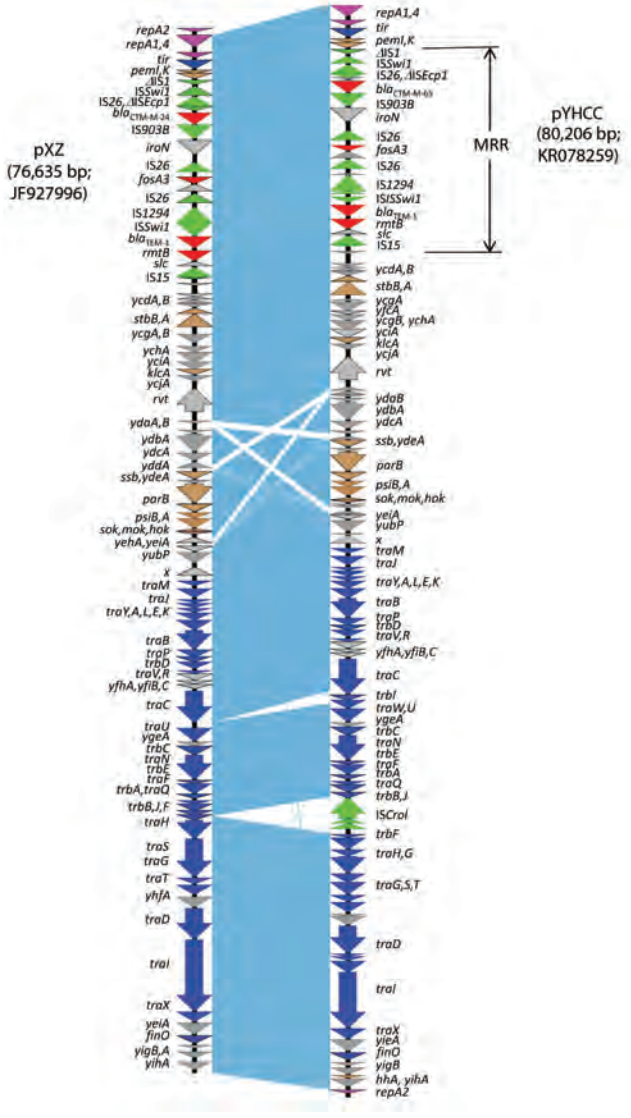

Figure 1. Comparative analysis of *fosA3*-carrying IncFII plasmids pYHCC with pXZ. Open reading frames are indicated by arrows and colored according to their putative functions: magenta arrows indicate genes involved in replication; blue arrows indicate genes associated with plasmid conjugal transfer. Brown arrows indicate genes involved in plasmid stability; red arrows indicate antimicrobial drug resistance genes; green arrows indicate accessory genes of mobile elements; gray arrows indicate other backbone genes and inserted foreign genes. Light blue shading indicates shared backbone regions with a high degree of homology. MRR, multidrug resistance region.

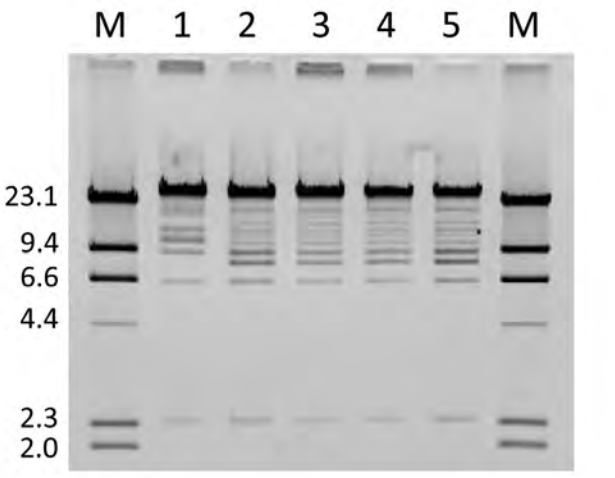

Figure 2. *Eco*RI restriction profile of *fosA3*-carrying plasmids from a woman in Pennsylvania, USA, who was colonized with fosfomycin-resistant *Escherichia coli*. Lanes M, lambda DNA/*Hin*dIII marker; lane 1, February 2011 (YD472); lane 2, March 2008; lane 3, June 2007 (ECRB1); lane 4, April 2008; lane 5, January 2008. Values on the left are in kilobases.

A feature that distinguishes pYHCC from pXZ and contributes to the size difference of the plasmids is insertion of IS*CroI* between *trbJ* and *trbF* in pYHCC. Plasmid pYHCC carries 4 antimicrobial resistance genes, all of which are in the multidrug resistance region. These genes are $bla_{\text{CTX-M-65}}$ (conferring cephalosporin resistance), *fosA3* (conferring fosfomycin resistance), $bla_{\text{TEM-1}}$ (conferring ampicillin resistance), and *rmtB* (conferring aminoglycoside resistance). The *fosA3* gene and its downstream open reading frame encoding a putative 172-aa protein is flanked by 2 tandem copies of IS*26*. This arrangement is identical to that in pXZ and pHN7A8, except that pXZ carries $bla_{\text{CTX-M-24}}$ instead of $bla_{\text{CTX-M-65}}$. The $bla_{\text{CTX-M-24}}$ and $bla_{\text{CTX-M-65}}$ gene products differ by only 1 aa (Val for CTX-M-65 and Ala for CTX-M-24 at position 77).

A total of 6 *E. coli* isolates were collected from the patient during 2007–2010, including the isolates reported

here and previously (*4*). All isolates were obtained from urine samples, except for YD472, which was cultured from the peritoneal catheter exit site. Pulsed-field gel electrophoresis (PFGE) with *Xba*I as the restriction enzyme showed that all isolates had identical banding patterns. Furthermore, 5 of the 6 isolates, including the first isolate from 2007, were resistant to fosfomycin and positive for *fosA3*, *rmtB*, and $bla_{\text{CTX-M-9-group}}$ by PCR. *E. coli* TOP10 transformants harboring *fosA3*-carrying plasmids were generated from each of these 5 isolates.

The plasmids were then extracted and digested with restriction enzyme *Eco*RI. The resulting restriction profile was nearly identical across the plasmids except for pYHCC from YD472, which had a shift of a band from ≈8 kb to ≈11 kb (Figure 2). This shift approximated the size difference between pYHCC and pXZ, and further suggested pXZ as the origin of pYHCC.

Conclusions

The first reported clinical *E. coli* isolate carrying *fosA3* was identified in Japan in 2006 (*3*), but *fosA3*-carrying *E. coli* isolates have also been identified in pigs in China as far back as in 2004 (*14*). Although the patient in Pennsylvania lacked a relevant travel history, the timeline and structural similarities of these *fosA3*-carrying plasmids make it plausible that this multidrug resistance plasmid originated in *E. coli* in Asia and moved to the continental United States by human travel or importation of food products. Emergence of *E. coli* with high-level fosfomycin resistance is a clinically relevant event, especially in the United States, where fosfomycin is increasingly used for empiric treatment of urinary tract infection in the absence of routine drug susceptibility testing.

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Dr. Alrowais is a research trainee at the University of Pittsburgh, Pittsburgh, Pennsylvania. Her research interests include emerging mechanisms of antimicrobial resistance in *E. coli*.

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Epidemiology of Primary Multidrug-Resistant Tuberculosis, Vladimir Region, Russia

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We studied the epidemiology of drug-resistant tuberculosis (TB) in Vladimir Region, Russia, in 2012. Most cases of multidrug-resistant TB (MDR TB) were caused by transmission of drug-resistant strains, and >33% were in patients referred for testing after mass radiographic screening. Early diagnosis of drug resistance is essential for preventing transmission of MDR TB.

Drug-resistant tuberculosis (TB) is a public health problem worldwide (*1*). Compared with drug-susceptible TB, multidrug-resistant TB (MDR TB; i.e., TB with resistance to at least isoniazid and rifampin) requires longer, more expensive treatment and is less likely to be cured (*2*,*3*). Russia has the third highest burden worldwide of MDR TB; ≈41,000 pulmonary cases were notified in 2013 (*1*). The World Health Organization estimated that 19% of new TB cases notified in Russia in 2013 were primary MDR TB (*1*); however, drug-susceptibility testing (DST) coverage varies across the country (*1*,*4*). Prevalence of primary MDR TB varied from 5.4% to 28.3% among the 12 regions in Russia that reported MDR TB data in 2010 (*5*). Understanding the burden of drug-resistant TB and the factors associated with its transmission may help determine who is at risk for infection and develop measures for preventing transmission. We describe the epidemiology of primary MDR TB in Vladimir Region, Russia.

The Study

During February 1–December 31, 2012, we performed a secondary analysis of data collected for a study conducted at the Vladimir Regional TB Dispensary (hereafter referred to as the dispensary), which is located 190 km east of Moscow in Vladimir Region (Figure). The dispensary, a referral center for TB patients in Vladimir Region, serves ≈25% of all TB patients in the region and is the only facility in the region that performs DST.

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All adults referred to the dispensary for suspected TB during the study period were eligible for study enrollment. Study participants underwent clinical examination, chest radiography, and HIV testing. In addition, clinical samples were obtained for culture and DST, which was performed by using Lowenstein-Jensen media and Bactec Mycobacteria Growth Indicator Tube 960 (MGIT; Becton, Dickinson and Company, Franklin Lakes, NJ, USA). In August 2012, the Xpert MTB/RIF (Xpert; Cepheid, Sunnyvale, CA, USA) diagnostic test became available for routine patient care at the dispensary. We used rifampin resistance, which we defined as resistance detected by Lowenstein-Jensen media, MGIT DST, or Xpert, as a marker for MDR TB (*6*). Rifampin susceptibility was defined as susceptibility by all 3 testing methods or by any 1 of the 3 methods when results from all 3 methods were not available. Primary MDR TB was defined as the presence of rifampin resistance in new TB cases.

We described the epidemiology of new TB and MDR TB cases that were diagnosed in the dispensary during the study period. To identify the predictors of primary MDR TB, we compared the characteristics of MDR TB and non–MDR TB case-patients by using bivariate log-binomial regression.

The study was approved by ethics committees at the Centers for Disease Control and Prevention and the Central Tuberculosis Research Institute. All patients provided written informed consent.

During the study period, samples from 402 patients with presumptive TB were tested bacteriologically in the dispensary. Among these samples, 229 (56.9%) were *Mycobaterium tuberculosis*–positive by culture or Xpert. Of these 229 samples, 225 (98.3%; 191 from new TB patients, 34 from previously treated TB patients) were tested for rifampin resistance; 60 (26.7%) showed MDR TB. Forty-four (23.0%) of the 191 samples from new TB patients and 16 (47.1%) of the 34 samples from previously treated TB patients showed MDR TB (prevalence ratio 1.45, $p = 0.004$). The proportion of primary MDR TB cases among all MDR TB cases was 73.3% (44/60).

The median age of the 191 new TB patients at the time of diagnosis was 39 years; 146 (74.4%) were men (Table). Most new TB patients had pulmonary disease (96.3%, 183/191) and positive sputum smear results (73.7%, 140/191); 18 (9.6%) were HIV-positive. Lowenstein-Jensen media or MGIT DST results were available for 91.6% (175/191) of new TB case-patients; isoniazid monoresistance was detected in 16.0% (28/175). No rifampin monoresistance was found.

Figure. Vladimir Region in Russia (red shading). Map source: Google Maps, Vladimir Oblast, Russia.

Among 44 study participants with primary MDR TB, 17 (38.6%) had been referred to the dispensary for TB examination after routine mass radiographic screening in the primary health care setting (Table); 15 (88.2%) of these 17 patients were asymptomatic at the time of diagnosis. Of the 44 patents with primary MDR TB, 27 (61.4%) reported TB symptoms at diagnosis, and 16 (35%) had known contact with someone with TB. All patients with primary MDR TB had pulmonary TB disease. *M. tuberculosis* isolates from 4 (9.1%) of the 44 patients with primary MDR TB were also resistant to at least 1 second-line injectable drug and a fluoroquinolone; therefore, these 4 patients had primary extensively drug-resistant TB.

Among newly diagnosed TB case-patients, a positive sputum smear was the only factor significantly associated with MDR TB (prevalence ratio 2.8, 95% CI 1.2–6.7) (Table). The prevalence ratio for the association of HIV positivity with primary MDR TB suggested an increased, but not statistically significant, risk (prevalence ratio 1.8, 95% CI 0.96–3.50).

Conclusions

Primary MDR TB was prevalent in Vladimir Region during 2012, matching the World Health Organization prevalence estimate for Russia (*1*). Most persons in this study who received a diagnosis of MDR TB (73.3%, 44/60) had not previously received TB treatment, a finding that indicates ongoing transmission of drug-resistant *M. tuberculosis* in the community. This percentage is near the upper end of the range of primary MDR TB prevalence (31%–82%) reported in a review of data for 30 countries (*7*). In settings with a high prevalence of primary MDR TB, not just previously treated TB patients but new TB patients as well should be included in MDR TB case-finding strategies. Russia does not have full DST coverage (*1*); however, in Vladimir Region, all bacteriologically confirmed TB case-patients, including new case-patients, must undergo MGIT DST.

Our findings show that 38.6% of primary MDR TB cases were detected by routine radiographic screening, and most of these patients lacked symptoms at diagnosis. In Russia, mass chest radiographic screening is conducted every 1–2 years. The screening is part of the mandatory occupational health screenings for persons in some high-risk occupations and for patients with certain medical conditions, and it is also included in routine prophylactic health screenings. Active TB case finding in combination with early detection of drug resistance, supported by universally available DST and Xpert diagnostics, may substantially reduce transmission of MDR TB in the community.

A positive sputum smear was the only significant predictor of MDR TB among patients with primary TB. MDR TB was more common among HIV-infected than non–HIV-infected new TB patients; this finding was similar to those in cross-sectional studies conducted in several Eastern European countries, although this association was not statistically significant in our study (*5*,*8*,*9*). A high level (16%) of isoniazid monoresistance detected among previously untreated TB case-patients is concordant with findings from other regions in Russia (*10*).

Table. Association between sociodemographic and clinical characteristics of persons with newly diagnosed cases of non–MDR TB and MDR TB, Vladimir Region, Russia, 2012*

Characteristic	Total, n = 191	MDR TB, n = 44	Non–MDR TB, n = 147	Prevalence ratio	95% CI	p value
Sex						
Male	146 (74.4)	34 (77.3)	112 (76.2)	1.05	0.56–1.95	0.88
Female	45 (23.6)	10 (22.7)	35 (23.8)	Referent		
Median age, y (interquartile range)†	39 (30–49)	36 (30–46)	40 (30–50)			0.34‡
HIV†						
Positive	18 (9.6)	7 (16.3)	11 (7.6)	1.8	0.96–3.51	0.09
Negative	170 (90.4)	36 (83.7)	134 (92.4)	Referent		
Health care worker						
Yes	2 (1.1)	1 (2.3)	1 (0.7)	2.2	0.54–9.01	0.27
No	189 (98.9)	43 (97.7)	146 (99.3)	Referent		
Homelessness						
Yes	11(5.8)	4 (9.1)	7 (4.8)	1.64	0.72–3.75	0.24
No	180 (94.2)	40 (90.9)	140 (95.2)	Referent		
Employment						
Employed	42 (22.0)	10 (22.7)	32 (21.8)	Referent		
Unemployed	114 (59.7)	25 (56.8)	89 (60.5)	0.92	0.48–1.75	0.80
Not working§	35 (18.3)	9 (20.5)	26 (17.7)	1.08	0.49–2.35	0.85
History of imprisonment ¶						
Yes	51(26.8)	10 (22.7)	41 (28.1)	0.8	0.43–1.50	0.49
No	139 (73.2)	34 (77.3)	105 (71.9)	Referent		
Alcohol abuse						
Yes	35 (18.3)	9 (20.5)	26 (17.8)	1.15	0.61–2.16	0.67
No	156 (81.7)	35 (79.5)	121 (82.3)	Referent		
Illicit drugs use#						
Yes	12 (6.3)	3 (7.0)	9 (6.2)	1.11	0.40–3.06	0.85
No	177 (93.7)	40 (3.05)	137 (93.8)	Referent		
Diabetes¶						
Yes	13 (6.8)	3 (7.0)	10(6.8)	1.02	0.36–2.86	0.97
No	177 (93.2)	40 (93.0)	137 (93.2)	Referent		
Contact with TB patient						
Yes	55 (28.8)	16 (36.4)	39 (26.5)	1.45	0.85–2.49	0.17
No	130 (68.1)	26 (59.1)	104 (70.7)	Referent		
Unknown	6 (3.1)	2 (4.5)	4 (2.8)	–		
Cavities on radiograph¶						
Yes	88 (46.3)	24 (54.5)	64 (43.8)	1.39	0.83–2.34	0.21
No	102 (53.7)	20 (45.5)	82 (56.2)	Referent		
Sputum microscopy¶						
Positive	140 (73.7)	39 (88.6)	101(69.2)	2.78	1.16–6.67	0.02
Negative	50 (26.3)	5 (11.4)	45 (30.82)	Referent		
Site of disease¶						
Any pulmonary	189 (99.5)	44 (100)	145 (99.3)			0.59
Extrapulmonary only	1 (0.5)	0	1 (0.7)	Referent		
Symptoms						
Yes	122 (63.9)	27 (61.4)	95 (64.6)	0.9	0.53–1.53	0.69
No	69 (36.1)	17 (38.6)	52 (35.4)	Referent		
Reasons for TB screening						
Symptomatic	110 (57.6)	27 (61.4)	83 (56.4)	1.1	0.62–1.79	0.85
Abnormal finding on radiograph	6 (3.1)	0	6 (4.1)	–		
Contact with TB patient	2 (1.1)	0	2 (1.4)	–		
Routine screening	73 (38.2)	17 (38.6)	56 (38.1)	Referent		

*Values are no. (%) patients except as indicated. Cases were diagnosed at the Vladimir Regional TB Dispensary, Vladimir Region, Russia. MDR TB, multidrug-resistant TB. –, not applicable.
†Age and HIV status missing for 3 patients.
‡Wilcoxon rank sum test.
§Reasons for not working: retired, student, housewife, disabled.
¶Information was missing for 1 patient.
#Drug use data were missing for 2 patients.

Our study had several limitations. The small sample size limited statistical power. Xpert was introduced halfway through the study. Nonavailability of conventional DST for some patients limited our ability to analyze resistance pattern among all enrolled patients. There was a possibility of misclassification of treatment history. We did not include patients from the penitentiary sector or from other diagnostic facilities in the region; thus generalizability of

our results may be limited. The lack of networking data and molecular epidemiology may also present a limitation for the study conclusions.

Despite these limitations, our results show that primary MDR TB was common in the overall burden of MDR TB in Vladimir Region. Active case finding and expansion of DST or Xpert testing to new TB patients are key measures for achieving universal access to MDR TB diagnosis and treatment and for preventing the spread of drug-resistant TB in the community.

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Use of Whole-Genome Sequencing to Link *Burkholderia pseudomallei* from Air Sampling to Mediastinal Melioidosis, Australia

Bart J. Currie, Erin P. Price, Mark Mayo, Mirjam Kaestli, Vanessa Theobald, Ian Harrington, Glenda Harrington, Derek S. Sarovich

The frequency with which melioidosis results from inhalation rather than percutaneous inoculation or ingestion is unknown. We recovered *Burkholderia pseudomallei* from air samples at the residence of a patient with presumptive inhalational melioidosis and used whole-genome sequencing to link the environmental bacteria to *B. pseudomallei* recovered from the patient.

Melioidosis is thought to be caused predominantly by percutaneous inoculation with the bacterium *Burkholderia pseudomallei*; however, inhalation, aspiration, and ingestion of the bacterium can also occur (*1*). Although an evidence-based clinical definition of inhalational melioidosis has recently been published (*2*), the proportion of melioidosis cases resulting from inhalation is unknown, and attempts to culture *B. pseudomallei* from air sampling in melioidosis-endemic regions have been largely unsuccessful (*3*). We recovered *B. pseudomallei* from air samples at the residence of a patient with presumptive inhalational melioidosis, and whole-genome sequencing linked the environmental bacteria to *B. pseudomallei* recovered from the patient.

The Study

A 47-year-old man with poorly controlled type 2 diabetes sought care at the Royal Darwin Hospital in the tropical north of the Northern Territory of Australia in January 2011 after several weeks of increasing lethargy and 1 week of fevers and cough. He was patient 692 in the Darwin Prospective Melioidosis Study (*4*), which is approved by the Human Research Ethics Committee of the Northern Territory Department of Health and the Menzies School of Health Research (approval 02/38). The patient's chest radiograph (Figure 1, panel A) showed patchy pneumonia in the right lung and a large soft-tissue mass on the left side of the chest. A computed tomography scan (Figure 1, panel B) confirmed the mass to be a 7-cm × 6-cm loculated fluid collection in the anterior mediastinum contiguous with patchy bilateral pneumonia and associated with multiple enlarged mediastinal lymph nodes and a pericardial effusion. Blood cultures collected at admission were positive for *B. pseudomallei*. The patient required initial management in the intensive care unit for his pneumonia, severe sepsis, and ketoacidosis. He received 45 days of intravenous antimicrobial drugs (meropenem for 11 days, followed by ceftazidime for 34 days) in conjunction with oral trimethoprim/sulfamethoxazole. After discharge, he received 15 subsequent weeks of eradication therapy with trimethoprim/sulfamethoxazole. At follow up, he has remained well 4 years after completing his melioidosis therapy.

The unusual finding of extensive mediastinal disease raised the possibility that the patient had inhalational melioidosis. The patient described sitting most days outside his urban accommodation on an elevated and exposed mowed grassy area that overlooks ground sloping downhill to a rocky open drain, an environment that prompted the potential for targeted air sampling. The site was visited and environmental samples taken 6 weeks after the patient's hospital admission. During the sampling, squally rain showers occurred, accompanied by wind blowing up the hill and the drain flowing swiftly.

Two air samplings and 3 soil samples were collected. Each air collection entailed passing 1,000 L of air (50 L/min for 20 min) through a portable microbiologic air sampler (MD8 AirPort; Sartorius Stedim, Dandenong, Victoria, Australia) with a disposable gelatin filter (3.0 μm) for sample collection. The air sampler was placed on a tripod at 1.0 m elevation above ground level and was protected by a secured, angled overhead umbrella to prevent direct rain contact. After each air sampling, the gelatin filter was placed in 30 mL of modified Ashdown selective broth and incubated at 37°C, with the broth supernatant plated onto Ashdown agar after 2 and 7 days (*3*). Soil samples were collected at ≈10 cm below the surface. Sterile water (20 mL) was added to 20 g soil and shaken at 220 rpm for 48 h at 37°C. After the soil samples were removed from the shaker and left to stand for 1–2 h, 10 mL of supernatant was placed in 30 mL of modified Ashdown selective broth and

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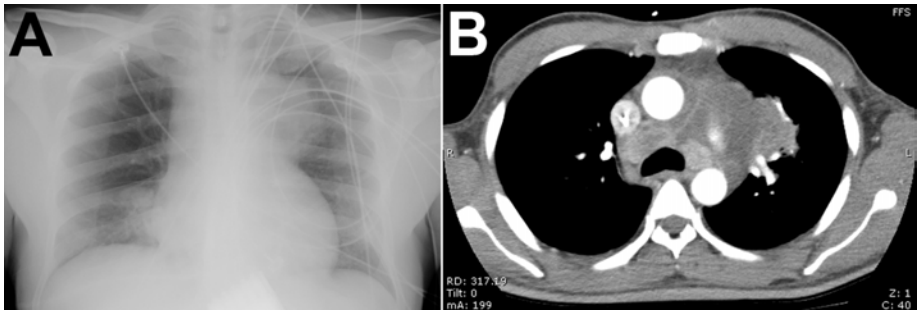

Figure 1. Clinical studies of a patient with melioidosis, Royal Darwin Hospital, Darwin, Northern Territory, Australia. A) Chest radiograph shows a soft-tissue mass associated with the left side of the mediastinum and obscuring the aortic arch. B) Chest computed tomography scan shows a large loculated mass in the anterior mediastinum; the mass is contiguous with multiple enlarged mediastinal lymph nodes and with pulmonary consolidation.

incubated at 37°C; the broth supernatant was plated onto Ashdown agar after 2 and 7 days. Suspected *B. pseudomallei* colonies were confirmed by using the *B. pseudomallei*–specific TTS1 real-time PCR, described previously (*5*).

B. pseudomallei was cultured from 1 of the 2 air samples and 1 of the 3 soil samples. Multilocus-sequence typing, completed by using standard methods (*6*), confirmed that 2 isolates from each of the positive air and soil samples and the isolate from the patient's blood culture were all sequence type (ST) 562. To further resolve the relatedness of ST562 isolates, PCR-based, multilocus variable-number tandem-repeat analysis of 4 loci (MLVA-4) was performed as described (*7*) on the 4 environmental isolates, the patient's blood culture isolate, and isolates from 13 other patients in the Darwin prospective study whose melioidosis was caused by ST562 *B. pseudomallei*. MLVA-4 categorized the 18 ST562 isolates into 3 distinct types: 55 (n = 7), 71 (n = 8), and 168 (n = 3). The isolate from patient 692, all 4 environmental isolates, and isolates from 2 of the 13 other patients were MLVA-4 type 55.

To further define the relatedness of ST562 isolates, whole-genome sequencing was performed on 17 of the 18 ST562 isolates for which MLVA-4 results were available; to reduce duplication and cost, 1 of the 2 *B. pseudomallei* isolates from air samples was not sequenced because it was clonal with the other air sample. Genomic DNA was extracted by using the QIAGEN DNeasy blood and tissue kit (QIAGEN, Chadstone, Victoria, Australia), as described (*7*). Samples were sequenced at Macrogen Inc. (Gasan-dong, Seoul, South Korea) by using HiSeq 2000 (Illumina, San Diego, CA, USA). Genome analysis was performed with SPANDx version 2.3 (*8*) by using the ST562 strain MSHR4388 from the MLST database (http://bpseudomallei.mlst.net/) as the reference genome.

Whole-genome identification of single-nucleotide polymorphisms (SNPs), followed by phylogenetic reconstruction

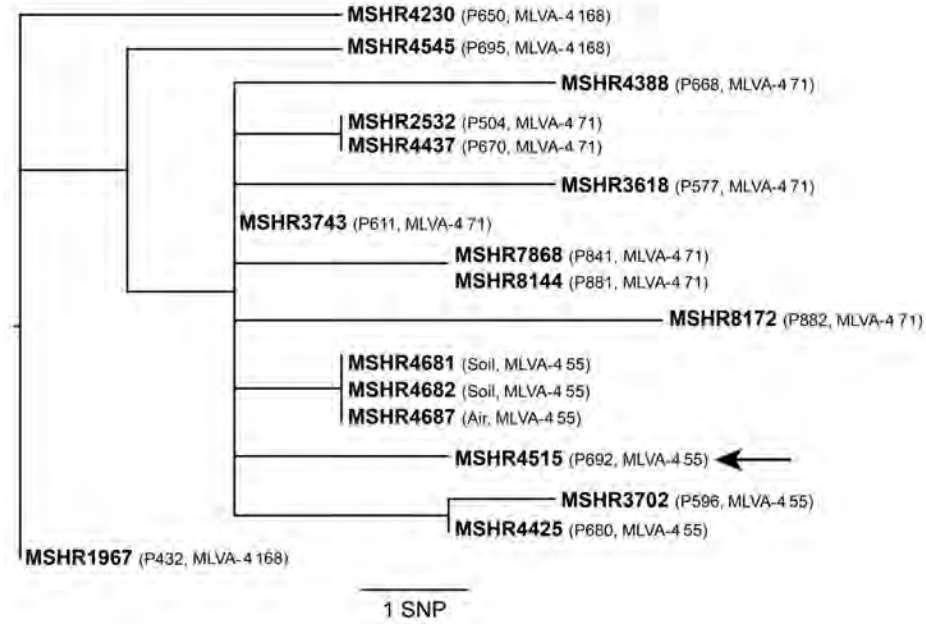

Figure 2. Whole-genome core orthologous single-nucleotide polymorphism (SNP) phylogeny of sequence type 562 *Burkholderia pseudomallei* isolates from a patient with melioidosis and from environmental sampling at the patient's residence, Darwin, Northern Territory, Australia. MSHR4515 (MLST database identifier, http://bpseudomallei.mlst.net/) was a blood culture isolate from the index patient, identified as patient (P) 692 (P692, arrow). Analysis of isolates from 13 other patients with sequence type 562 are also shown (identifiers begin with P). Comparison of data for SNPs and for multilocus variable-number tandem-repeat analysis of 4 loci (MLVA-4) types (shown in parentheses) supports the hypothesis that P692 was infected from environmental *B. pseudomallei* at his residence. Consistency index = 1.

by using maximum parsimony in PAUP 4.0b10 (*9*), showed that all ST562 isolates were closely related; only 26 SNPs were observed among all 17 genomes (Figure 2). The air isolate (added to the MLST database as MSHR4687) and the 2 soil isolates (MSHR4681 and MSHR4682) obtained from the environment outside the residence of patient 692 were identical by whole-genome sequencing and differed from the blood culture isolate of patient 692 (MSHR4515) by only 3 SNPs. These genetic similarities support the epidemiologic link between the air and soil *B. pseudomallei* and the patient's infection.

Epidemiologic data from Australia, Singapore, and Taiwan support the hypothesis that inhalation may replace inoculation as the predominant route of *B. pseudomallei* transmission during severe weather events (e.g., tropical monsoonal storms, cyclones, and typhoons) (*10*–*12*). Similar clinical distinctions between percutaneous and inhalational infections are observed for anthrax, plague, and tularemia (*4*). Animal studies have also shown the potential importance of aerosol inhalation of *B. pseudomallei* with high lethality (*13*,*14*). Inhalational melioidosis is supported by the increasing recognition from computed tomography scanning that enlarged mediastinal lymph nodes are not uncommon in patients with severe melioidosis pneumonia (*4*,*10*). Nevertheless, no direct evidence exists to confirm occurrence of inhalation of *B. pseudomallei* in melioidosis-endemic regions. A recent report from Taiwan documented an air sampling technique that uses a filtration real-time quantitative PCR method to quantify ambient *B. pseudomallei* DNA; high positive rates were found during typhoons (*12*).

Conclusions

B. pseudomallei was recovered from air samples taken outside the residence of a patient with clinical features consistent with inhalational melioidosis. Whole-genome sequencing linked the environmental *B. pseudomallei* to an isolate from the patient's blood culture. These data provide evidence of aerosolization of *B. pseudomallei* during stormy conditions in an endemic location and strong circumstantial evidence for inhalation of *B. pseudomallei*. The proportion of melioidosis cases resulting from inhalation rather than percutaneous inoculation or ingestion requires further study and is likely to vary substantially by location and season.

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Rotavirus P[8] Infections in Persons with Secretor and Nonsecretor Phenotypes, Tunisia

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To determine whether rotavirus infections are linked to secretor status, we studied samples from children in Tunisia with gastroenteritis. We phenotyped saliva for human blood group antigens and tested feces for rotavirus. Rotavirus was detected in 32/114 patients. Secretor genotyping showed that P[8] rotavirus infected secretors and nonsecretors, and infection correlated with presence of Lewis antigen.

Each year, millions of persons worldwide suffer from acute gastroenteritis. Group A rotavirus is the leading cause of acute gastroenteritis in children <5 years of age. The disease causes ≈453,000 deaths annually, mostly in developing countries (*1*); however, the number of cases has declined in industrialized countries where vaccines have been recommended (*2*).

Recent findings showed that human blood group antigens (HBGAs) might be involved in rotavirus attachment to intestinal cells (*3*,*4*,*5*,*6*). Expression of the HBGAs (A, B, H, and Lewis antigens) in saliva and on the surface of intestinal cells is driven by the *FUT2* (A, B, and H antigens [secretor]) and *FUT3* (Lewis antigens) genes, which express type 2 and type 3 fucosyltransferases, respectively. Approximately 20% of the white population is homozygous for a recessive point mutation of the *FUT2* gene, which leads to the absence of A, B, and H antigen expression, also called the nonsecretor phenotype. There is also a Lewis-negative phenotype resulting from various mutations of the *FUT3* gene (*7*).

The entry of rotavirus into cells involves several factors. Human and porcine rotaviruses could specifically interact with H antigen type 1, Lewis b antigen, or Lewis a antigen through their viral protein (VP) 8 and VP5 during the attachment phase (*3*,*4*,*8*). Of note, the HBGA binding profile is P genotype–dependent (*4*), and rotavirus infection correlates with the secretor and partial secretor phenotype (i.e., with active *FUT2* gene status) (*5*,*6*,*9*). However, in some studies, no association has been observed between HBGAs from blood cells (*10*), including Lewis antigens (*11*), and rotavirus infection. A recent epidemiologic survey of children in the region of Monastir, Tunisia, gave us the opportunity to determine whether rotavirus infections in children could be linked to secretor status and HBGAs.

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The Study

During November 2011–February 2012, feces and saliva samples were collected from 114 children <6 years of age who were seen for acute gastroenteritis at the Fattouma Bourguiba children's hospital (Monastir). For 98 of these patients, blood samples were also collected at symptom onset for *FUT2* genotyping by sequencing for the A385T and G428A nonsense mutations from total blood DNA (*9*). The study was approved by the Ethics Committee of the Fattouma Bourguiba University Hospital in Monastir, and informed consent was obtained from the parents of the 114 study participants.

The feces were first screened for the presence of group A rotavirus antigen by using the Premier Rotaclone detection kit (Meridian Bioscience, Inc., Paris, France). The remainder of the suspension was used for the extraction of nucleic acids by using a Nuclisens easyMAG system (bioMérieux, Marcy l'Étoile, France) according to the manufacturer's instructions. RNA was eluted in a final volume of 110 mL and used for the molecular detection and typing of rotavirus. Norovirus PCR detection is described elsewhere (*12*).

Samples positive for rotavirus by ELISA were further confirmed and genotyped by PCR as described previously (online Technical Appendix Table, http://wwwnc.cdc.gov/EID/article/21/11/14-1901-Techapp1.pdf). Of the 114 patients, 32 had confirmed rotavirus infections by ELISA and PCR. Of the 32 confirmed cases, 24 (75%) occurred during the cold season, and 26 (80%) occurred in children <14 months of age; the mean age for infected persons was 8.1 months. We used ELISAs to screen the saliva of rotavirus-positive patients for A and B antigens (anti-A and anti-B mouse IgG from DIAGAST, Loos, France), H antigen (anti-H specific IgM from Thermo Fisher Scientific, Villebon sur Yvette, France), and Lewis antigens (anti-Lewis a [clone 7LE] and anti-Lewis b [clone 2–25LE] hybridoma

Table 1. Distribution of rotavirus and norovirus cases among 114 children <6 years of age by ABO blood group, Lewis phenotype status, and nonsecretor status, Tunisia, November 2011–February 2012*

Phenotype	No. rotavirus-positive patients	No. norovirus-positive patients
ABO and Lewis antigen distribution in patients, n = 90		
O		
Le+, n = 37	12	21
Le–, n = 1	0	0
A		
Le+, n = 26	10	7
Le–, n = 1	0	0
B		
Le+, n = 16	4	4
Le–, n = 3	0	2
AB		
Le+, n = 6	2	1
Le–, n = 0	0	0
Nonsecretor group, Lewis antigen status, n = 24		
Positive, n = 24	4	7
Negative, n = 0	0	0
Total	32	42

*All children had gastroenteritis. Paired saliva and feces specimens were collected from each patient (N = 114) and screened for the presence of ABO and Lewis antigens (saliva) and rotavirus and norovirus (feces). Le+, positive for Lewis antigen; Le–, negative for Lewis antigen.

supernatants; gift from Jacques Bara, INSERM U673). Among the secretor phenotype–positive rotavirus patients, no blood group antigen nor P or G genotypes (in feces specimens) were significantly overrepresented. For comparison, we assessed the distribution of ABO blood groups and Lewis antigens among patients with norovirus and rotavirus; no statistical difference was found (Table 1). Rotavirus infection was observed only in Lewis antigen–positive patients (p = 0.017, exact logistic regression); however, the prevalence of the Lewis antigen–negative phenotype in the population was low.

Among the 32 rotavirus isolates, 30 were genotype P[8] and 2 were genotype P[4] (Table 2). G9, G3, and G1 were the most common genotypes and were detected in 13 (40%), 8 (25%), and 7 (21%) of the cases, respectively. The G genotype could not be determined for 1 P[8] genotype isolate. Genotypes G1, G3, G4, and G9 were all associated with the P[8] genotype, and genotype G2 was associated with the P[4] genotype. Rotavirus G9P[8] strains were predominant (n = 12), followed by G3P[8] (n = 8) and G1P[8] (n = 7) strains.

For 3 G9P[8] and 1 G3P[8] rotavirus-positive patients, saliva samples were negative for Lewis b antigen

Table 2. Distribution of ABO blood groups and Lewis antigens among 32 children <6 years of age infected with various rotavirus strains in Monastir, Tunisia, November 2011–February 2012*

Isolated rotavirus strain, patient *FUT2* genotype	No. patients by ABO blood group and Lewis antigen status†								No. nonsecretor patients	
	O		A		B		AB			
	Le+	Le–	Le+	Le–	Le+	Le–	Le+	Le–	Le+	Le–
G9P[8]	4	0	1	0	2	0	2	0	3	0
Se/Se	0	0	1	0	1	0	1	0	0	0
Se/se	4	0	0	0	1	0	1	0	0	0
se/se	0	0	0	0	0	0	0	0	3	0
G3P[8]	1	0	4	0	2	0	0	0	1	0
Se/Se	0	0	2	0	1	0	0	0	0	0
Se/se	1	0	1	0	1	0	0	0	0	0
se/se	0	0	0	0	0	0	0	0	1	0
G1P[8]	4	0	3	0	0	0	0	0	0	0
Se/Se	0	0	0	0	0	0	0	0	0	0
Se/se	4	0	2	0	0	0	0	0	0	0
G4P[8]	1	0	1	0	0	0	0	0	0	0
Se/se	0	0	1	0	0	0	0	0	0	0
G2P[4]	2	0	0	0	0	0	0	0	0	0
Se/Se	1	0	0	0	0	0	0	0	0	0
P[8]	0	0	1	0	0	0	0	0	0	0
Se/Se	0	0	1	0	0	0	0	0	0	0
Total	12	0	10	0	4	0	2	0	4	0
Se/Se	1	0	4	0	2	0	1	0	0	0
Se/se	9	0	4	0	2	0	1	0	0	0
se/se	0	0	0	0	0	0	0	0	4	0

*Le+, positive for Lewis antigen; Le–, negative for Lewis antigen; Se/Se, homozygous secretor; Se/se, heterozygous secretor; se/se, nonsecretor.
†Human blood group antigen distribution was determined by using a typing assay with saliva samples.

(mean absorbance at 450 nm was 0.25) and positive for the presence of Lewis a antigen (mean absorbance at 450 nm was 3.67), suggesting that the patients were Lewis-positive and nonsecretors. A total of 98 blood samples were genotyped by sequencing of the *FUT2* gene. Homozygous secretor, heterozygous secretor, and nonsecretor genotypes represented 23.47%, 54.08%, and 22.45% of the cohort, respectively. All nonsecretors (n = 22) harbored the G428A mutation. The A385T mutation was absent. Blood samples were available for 28 of the 32 rotavirus-positive patients. Of these 28 patients, 24 were homozygous and heterozygous secretors and 4 were nonsecretors. Because rotaviruses were P- and G-typed by PCR using VP4- and VP7-specific primers, we further confirmed the presence of rotavirus in samples from nonsecretor patients. For 3 of the samples, we used a TaqMan-based quantitative reverse transcription PCR with VP2-specific primers to detect rotavirus (*13*), and for 2 rotavirus isolates (GenBank accession nos. KP862856 and KP862857) for which feces samples were still available, we confirmed the P[8] genotype by sequencing.

Conclusions

Our findings show that rotaviruses can infect secretor and nonsecretor Lewis antigen–positive persons, which suggests that rotavirus infection is not associated with the secretor phenotype or HBGA type. However, it should be noted that one limitation of our study was the small size of our cohort. A larger number of cases might provide new insights about the affinity of rotavirus toward certain types of HBGAs; a larger study with a more robust statistical analysis might confirm that rotavirus infections only occur in Lewis antigen–positive persons. In addition, we detected genotype P[8] rotavirus infection in both secretor and nonsecretor patients; this finding was not observed in previous studies (*5*,*6*,*9*). P[8] infection of nonsecretors might be associated with preexisting health conditions, and healthy nonsecretors might never be infected by P[8] rotavirus.

We and others (*3*,*4*,6) have characterized the secretor and Lewis phenotypes related to infection by rotaviruses. The interaction between rotavirus particles and HBGAs might constitute the first step in the attachment to the cell before internalization of the virus particle, after binding with integrins (*4*,5). However, other types of ligand, such as non-HBGA ligands and bacteria from intestinal flora, might also play a role during the infection process, as recently shown for noroviruses (*14*,*15*).

In conclusion, our data and that of others show that rotavirus infection might be correlated with genetic factors, such as HBGAs. Further studies will be required to determine the exact role of HBGA ligands and other ligands in rotavirus infection.

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RmtC and RmtF 16S rRNA Methyltransferase in NDM-1–Producing *Pseudomonas aeruginosa*

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We investigated 16S rRNA methyltransferases in 38 bla_{NDM-1}–positive *Pseudomonas aeruginosa* isolates and found RmtC in 3 isolates, 1 of which also harbored RmtF. The isolates were clonally unrelated; *rmtC* and *rmtF* genes were located on a chromosome with the bla_{NDM-1} gene. Strategies are needed to limit the spread of such isolates.

Pseudomonas aeruginosa causes severe and chronic invasive infections in critically ill patients. Aminoglycosides are used either alone or in combination with β-lactams as effective agents for treating such infections (*1*). Aminoglycosides block protein synthesis by binding to bacterial 16S rRNA of the 30S ribosomal subunit. Methylation of 16S rRNA makes bacteria highly resistant to aminoglycosides (*2*). Increasing instances are reported of 16S rRNA methyltransferase (16S RMTase)–producing, Gram-negative bacteria that confer high levels of resistance to aminoglycosides. Eleven types of 16S RMTases (ArmA, RmtA–RmtH, and NpmA) have so far been reported in several nosocomially transmitted pathogens, including *P. aeruginosa* (*2*–*5*). Recently, 16S RMTases have been reported in association with the New Delhi metallo-β lactamase-1 (NDM-1) in *Enterobacteriaceae* (*3*). However, such association has not been reported in *P. aeruginosa*. Therefore, we investigated the presence of 16S RMTases in NDM-1–positive *P. aeruginosa* isolates recovered from different clinical specimens.

The Study

A total of 130 consecutive *P. aeruginosa* isolates recovered from different clinical specimens at Sanjay Gandhi Postgraduate Institute of Medical Sciences in Lucknow, Uttar Pradesh, India, during November 2013–April 2014 were included in the study; all specimens were collected from within the state (Figure 1). *P. aeruginosa* isolates were identified by standard microbiological techniques (*6*) and further confirmed by Phoenix automated identification and sensitivity systems (BD Biosciences, San Jose, CA, USA). The drug susceptibility profile was interpreted by using Clinical and Laboratory Standards Institute breakpoints (*7*). A total of 38 (29.23%) isolates were resistant to meropenem and imipenem. These isolates were subjected to PCR by using bla_{NDM}–specific primers (*8*) followed by amplicon sequencing. Sequencing identified bla_{NDM-1} in all 38 isolates. The isolates were further screened for high-level aminoglycoside resistance by their ability to grow on Muller Hinton agar containing amikacin and gentamicin 256 mg/L each as a marker for 16S RMTase (*3*). A total of 33 (86.84%) isolates were positive for high-level aminoglycoside resistance. Geographic locations of patients infected with these isolates are provided in Figure 1. Each of these isolates were further subjected to PCR for detection of 16S RMTases (ArmA and RmtA–RmtH) by using primers and conditions described previously (*2*–*5*); 17 (51%) isolates were positive for 16S RMTases. Their distributions were as follows: ArmA in 6 (18%); RmtB in 4 (12%); ArmA + RmtB in 4 (12%); RmtC in 2 (KnPa1A and KnPa1C) (6%); and RmtC + RmtF in 1 (KnPa1B) (3%). RmtC and RmtF had not previously been reported in *P. aeruginosa*. KnPa1A, KnPa1B, and KnPa1C were further characterized; sequence analysis of amplicons confirmed *rmtC* with 100% nucleotide identity originally described in *Proteus mirabilis* strain ARS68 from Japan (*9*) and assigned EMBL/GenBank nucleotide accession nos. KJ476816, KJ476817, and KJ476818. MICs of the 3 isolates for different aminoglycosides, β-lactams, β-lactam/β-lactamase inhibitor combinations, carbapenems, and colistin are provided in the Table.

KnPa1A was isolated from surgical drainage from a woman, 59 years of age, who had hypertension and underwent an abdominal hysterectomy for cervical carcinoma, followed by external beam radiotherapy. Eventually, a vesicovaginal fistula developed, and pelvic fluid was collected. *P. aeruginosa* (KnPa1A) was isolated from pelvic drainage. Her condition stabilized, and she was discharged with advice for repair of the fistula, but she did not return for further treatment. During hospitalization, she received multiple antimicrobial drugs.

KnPa1B was isolated from endoscopic nasobiliary drainage (ENBD) collected from a man, 57 years of age, who had extrahepatic biliary obstruction as a complication of hilar cholangiocarcinoma. Endoscopic retrograde

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Figure 1. Location of Uttar Pradesh state, India, showing geographic location of patients infected with 16S rRNA methyl transferase–positive *Pseudomonas aeruginosa* (gray shading) and RmtC-positive isolates KnPa1A, KnPa1B, and KnPa1C (black dots); KnPa1B was also positive for RmtF. Inset shows location of Uttar Pradesh within India.

cholangiopancreatography with stenting was performed. Stent block and fever occurred, necessitating a repeat of the procedure and drainage of fluid. He was discharged with the drainage tube in situ and was advised to return for surgery. *P. aeruginosa* (KnPa1B) was isolated from ENBD. The patient did not report for follow-up treatment.

KnPa1C was isolated from a man, 68 years of age, who had diabetes mellitus and hypertension. He had stricture of the urethra and meatal narrowing after having a transurethral prostate resection. He underwent urethral dilatations and placement of a urinary catheter. A urinary tract infection was diagnosed, and *P. aeruginosa* (KnPa1C) was recovered from urine. The patient received piperacillin/tazobactam and colistin combination therapy; urine culture was sterile on day 3 posttreatment.

Resistance genes such as metallo-β-lactamases (e.g., IMP, VIM, SIM, GIM, SPM) and extended-spectrum β-lactamases such as TEM, SHV, CTX-M, and AmpC were detected by using PCR (*8*) (Table). To study genetic relatedness among the 3 isolates, genomic DNA in agarose blocks was separated on 1.0% agarose gels in 0.5 × tris-borate-EDTA buffer with the CHEF II D-Mapper XA pulsed-field gel electrophoresis system (Bio-Rad, Hercules, CA, USA) following standard conditions (*10*). All 3 isolates had different PFGE patterns (Figure 2). Multilocus sequence typing (MLST) was done according to protocols described in the *Pseudomonas aeruginosa* MLST Database (http://pubmlst.org/paeruginosa). Seven chromosomal genes were PCR amplified and sequenced; the sequences were compared with those on

Table. MICs of different antimicrobial drugs, resistance genes, and association of IS*Ecp1* with *rmtC* in 3 *Pseudomonas aeruginosa* clinical isolates, India, 2013–2014*

Isolate	MIC, mg/L											Antimicrobial drug resistance genes	Association of IS*Ecp1* with *rmtC*
	CAZ	CTX	FEP	ATM	CPS	TZP	IPM	MER	COL	AK	G		
KnPa1A	>512	>512	512	8	256	64	16	32	2	>512	>512	bla_{NDM-1}, *rmtC*	Intact
KnPa1B	256	>512	>512	16	128	32	16	64	2	>512	>512	bla_{NDM-1}, *rmtC,rmtF,* bla_{CMY-2}	Truncated
KnPa1C	>512	>512	512	16	128	64	32	128	2	>512	>512	bla_{NDM-1}, *rmtC*, bla_{TEM-1}, $bla_{CTX-M-15}$	Truncated

*CAZ, ceftazidime; CTX, cefotaxime; FEP, cefepime; ATM, aztreonam; CPS, cefoperazone/sulbactam; TZP, piperacillin/tazobactam; IPM, imipenem; MER, meropenem; COL, colistin; AK, amikacin; G, gentamicin.

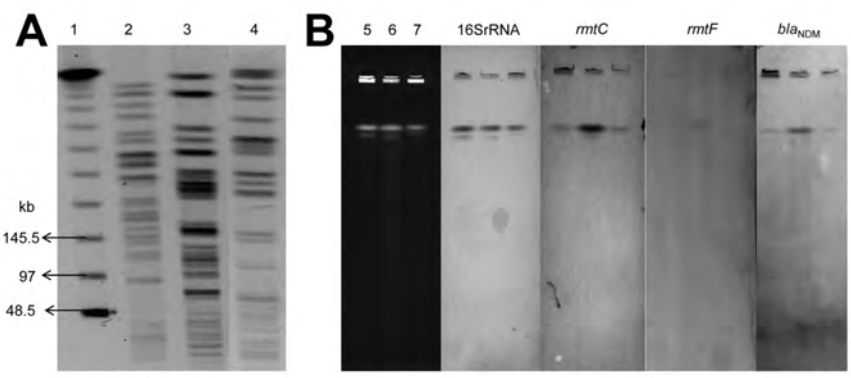

Figure 2. A) Pulsed-field gel electrophoresis patterns of *rmtC*-positive *Pseudomonas aeruginosa*. Lane 1, λ ladder; 2, KnPa1A; 3, KnPa1B; 4, KnPa1C. B) Chromosomal location of *rmtC, rmtF*, and bla_{NDM-1} genes by I-CeuI-digested genomic DNA of *P. aeruginosa* isolates. Lane 5, KnPa1A; 6, KnPa1B; 7, KnPa1C; smears show Southern blot analysis of genomic DNA with probes specific to 16S rRNA, RmtC, RmtF, and NDM-1 genes.

the MLST database to determine allele numbers and sequence types (STs). KnPa1A, KnPa1B, and KnPa1C belonged to ST764, ST902, and ST880, respectively.

*ISEcp*1 was previously shown to promote both expression and transposition of *rmtC* (*11*); hence, to assess association of *ISEcp*1 with *rmtC*, PCR was performed on the 3 isolates with primer pairs ISEcpIR-F and *rmtC*-down and *ISEcp*1–5′ and RMTC-R, as described (*12*). Sequence analysis of amplicons revealed association of an intact IS*Ecp*1 element with *rmtC* in KnPa1A; however, complete *ISEcp*1 could not be amplified in KnPa1B and KnPa1C, corroborating earlier observations of either partial deletion of this element or role of a different IS*Ecp1*-like element in the spread of *rmtC* in gram-negative bacteria (*13*). Attempts to transfer *rmtC* from all 3 isolates to rifampin-resistant *Escherichia coli* 20R764 and ciprofloxacin-resistant *P. aeruginosa* of strain 105 through conjugation were unsuccessful (*1*,*13*). Repeated attempts to obtain amikacin-resistant (MIC ≥16 g/mL) transformants of *E. coli* DH5α and *P. aeruginosa* PA01 by electroporation with plasmid preparation by using the Kado and Liu method (*14*) were also unsuccessful, despite successful transfer of control plasmids. To determine the location of *rmtC, rmtF*, and bla_{NDM-1}, genomic DNA from the 3 isolates was digested separately with restriction enzyme I-Ceu-1 (New England Biolabs, Beverly, MA, USA), separated by PFGE, and subsequently assayed with 16S rRNA, *rmtC, rmtF*, and bla_{NDM-1} probes (*13*). All these probes were hybridized with chromosomal DNA (Figure 2) and not with plasmid extract. This result shows that *rmtC, rmtF*, and bla_{NDM-1} were located and stabilized on the chromosome of *P. aeruginosa*.

Conclusions

We describe an occurrence of 16S RMTases RmtC and RmtF in clinical isolates of *P. aeruginosa* co-producing bla_{NDM-1}. The *rmtC* and *rmtF* genes might have been acquired from plasmids as part of mobile genetic elements and finally integrated and stabilized on the chromosome, but the underlying mechanism of transmission needs to be elucidated. Further, spread of multidrug-resistant *P. aeruginosa* strains expressing RmtC with and without an intact IS*Ecp1* element and NDM-1 is of major clinical concern and calls for further studies to limit the spread of such strains.

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Serogroup W Meningitis Outbreak at the Subdistrict Level, Burkina Faso, 2012

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In 2012, *Neisseria meningitidis* serogroup W caused a widespread meningitis epidemic in Burkina Faso. We describe the dynamic of the epidemic at the subdistrict level. Disease detection at this scale allows for a timelier response, which is critical in the new epidemiologic landscape created in Africa by the *N. meningitidis* A conjugate vaccine.

Since 2010 in sub-Saharan Africa, a meningococcal A conjugate vaccine (MenAfriVac; http://www.meningvax.org/) has been widely introduced to at-risk areas in the meningitis belt, resulting in a change in the epidemiology of meningococcal meningitis in the region (*1*,*2*). Fewer meningitis cases are now diagnosed, and large outbreaks of *Neisseria meningitidis* serogroup A are diminishing. Proportionally, more epidemics are caused by other meningococcal serogroups (e.g., serogroup W) that have less salient epidemic patterns, limiting detection through district-level surveillance and delaying intervention measures (*3*). World Health Organization guidelines for outbreak response in sub-Saharan Africa were revised in 2014 partly to address this issue (*4*). To ensure timelier intervention, the guidelines recommend that epidemic risk be assessed for populations of 30,000–100,000.

In 2010, MenAfriVac was introduced in Burkina Faso (*5*), and meningitis incidence was low in 2011; however, during February–April 2012, several epidemic foci occurred at the district level (*6*). This epidemic was the first in Burkina Faso since introduction of MenAfriVac and the second serogroup W epidemic in the country, occurring 10 years after the initial outbreak (*7*,*8*).

Meningitis outbreak dynamics are seldom studied on a small scale (e.g., at the subdistrict level). However, key information for improving detection of and response to epidemics can be learned from such analyses. To add to the current knowledge, we studied meningitis outbreak dynamics in areas of Kombissiri district, Burkina Faso, that were most severely affected by the 2012 epidemic.

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The Study

The study was a collaborative effort of the Disease Control Department of the Burkina Faso Ministry of Health and the World Health Organization. District-level aggregated surveillance data (weeks 1–17, 2012) and official population data were used. At the subdistrict level, population and surveillance data (weeks 1–16, 2012) were collected from the Kombissiri district surveillance unit. Standards for meningitis surveillance were used (*9*). The weekly alert and epidemic status for Kombissiri and its subdistricts were determined by using the 2012 meningitis incidence thresholds and the 2014 revised guidelines, which have a lower alert threshold (*4*,*9*).

In 2005, subdistrict zones were created in Burkina Faso to improve disease surveillance accuracy and timeliness; districts were subdivided into zones of ≈30,000 persons (Table). Within these zones, population numbers are similar, unlike numbers in health facility (HF) catchment areas, which in Kombissiri range from 440 to 25,000 persons. In Burkina Faso, zone data are infrequently analyzed, except in Kombissiri (Figure 1).

On a national level, 13 (21%) of the 63 districts in Burkina Faso reached meningitis epidemic status in 2012 (Table; online Technical Appendix, http://wwwnc.cdc.gov/EID/article/21/11/15-0304-Techapp1.pdf). *N. meningitidis* serogroup W caused 82.6% (384/465) of confirmed cases in these districts, in which no reactive vaccination against this serogroup had been recently conducted. Kombissiri had the highest cumulative attack rate (CAR) and was affected longer than other districts. Of 136 suspected meningitis cases in Kombissiri, 44 (32%) were confirmed: 36 (82%) of those were caused by *N. meningitidis* serogroup W, 1 (2%) was caused by *N. meningitidis* serogroup X, and 7 (16%) were caused by *Streptococcus pneumoniae*. District- and subdistrict-level distributions of these pathogens were comparable.

The outbreak in Kombissiri reached alert and epidemic thresholds during weeks 7 and 11, respectively; a total of 9 weeks were spent in these phases (Table). At the subdistrict level, alert and epidemic thresholds were reached at weeks 1 and 3, respectively, 6 and 8 weeks, respectively, earlier than at the district level. Alert and epidemic thresholds were first crossed in zone 2, and contiguous zones were gradually

Table. Details of the 2012 outbreak of *Neisseria meningitidis* serogroup W at the district and subdistrict (Kombissiri district) levels, Burkina Faso

Outbreak level	Population	Duration, wk (starting wk)			Time, wk, to peak**††	Attack rate, no. cases/100,000 population	
		Alert plus epidemic*†	Preepidemic‡§	Epidemic¶#		Weekly maximum‡‡	Cumulative§§¶¶
District, epidemiologic wks 1–17							
Banfora	312,923	4 (11)	1	1 (12)	0	10.5	62.0
Bittou	116,080	8 (8)	5	3 (13)	1	20.7	92.2
Dafra	285,184	8 (8)	4	3 (12)	2	16.8	96.4
Dande	225,917	6 (9)	3	3 (12)	1	12.0	70.8
Gourcy##	196,686	5 (12)	3	1 (15)	0	10.2	62.5
Kombissiri	173,885	9 (7)	4	3 (11)	1	14.4	105.2
Orodara	346,319	7 (9)	2	4 (11)	2	16.2	91.2
Pama	98,308	7 (9)	4	1 (13)	0	15.3	89.5
Po	185,632	6 (10)	3	1 (13)	0	10.2	51.2
Sapone	96,020	6 (9)	5	1 (14)	0	11.5	67.7
Seguenega##	189,363	7 (11)	3	1 (14)	0	12.1	60.7
Sindou	147,477	7 (9)	1	4 (10)	2	15.6	89.5
Solenzo	314,593	7 (8)	0	5 (8)	2	16.2	101.7
Kombissiri District, epidemiologic wks 1–16							
Zone 1	39,163	10 (6)	2	7 (8)	3	38.3	153.2
Zone 2	32,037	15 (1)	2	13 (3)	10	46.8	293.4
Zone 3	34,591	1 (12)	Not applicable	Not applicable	Not applicable	5.8	14.5
Zone 4	30,541	2 (12)	0	1 (12)	0	13.1	39.3
Zone 5	37,553	9 (6)	5	1 (11)	0	10.7	39.9

*Defined as time between weekly attack rate crossed at least the alert threshold (5 cases/week/100,000 population) and descended below the alert threshold, (i.e., from alert to alert).
†Mean 6.7; median 7; SD 1.32.
‡Defined as time between weekly attack rate crossed the alert threshold and reached the epidemic threshold (10 cases/week/100,000 population) (i.e., from alert to epidemic). Not applicable if only the alert threshold was crossed.
§Mean 2.9; median 3; SD 1.55.
¶Defined as time between weekly attack rate crossed the epidemic threshold and descended below the epidemic threshold again (i.e., from epidemic to epidemic). Not applicable if only the alert threshold was crossed.
#Mean 2.4; median 3; SD 1.45.
**Defined as time between weekly attack rate crossed the epidemic threshold and reached maximum incidence (i.e., from epidemic to peak). A zero value indicates peak was reached when the epidemic threshold was crossed. Not applicable if only the alert threshold was crossed.
††Mean 0.8; median 1; SD 0.9.
‡‡Mean 14.0; median 14.4; SD 3.9.
§§Over the study period.
¶¶Mean 80.0; median 89.5; SD 18.0.
##A reactive immunization campaign with ACWY polysaccharide vaccine was conducted during week 18, 2012.

affected from week 6 onward (Figure 2). Zones 1 and 2 (outbreak epicenters) were the only zones in the epidemic phase for >2 continuous weeks. In these zones, the average alert and epidemic phases were longer than those at the district level (12.5 vs. 9.0 weeks), the preepidemic phase was shorter (2.0 vs. 4.0 weeks), the time to peak was longer (6.5 vs. 1.0 weeks), and the epidemic phase started earlier (week 3 [zone 2] and week 8 [zone 1] vs. week 11) and was longer (10 vs. 3 weeks) (Table). The average CAR and peak incidence in zones 1 and 2 were higher than those for Kombissiri (CAR 223.3 vs. 105.2 cases/100,000 population; peak incidence 42.6 vs. 14.4 cases/week/100,000 population) and other districts (Table). The levels were also higher than those in 2012 serogroup W epidemics with district-level documentation in The Gambia (CAR 111 cases/100,000 population) and Benin (CAR 123.7 cases/100,000 population; peak incidence 16.7 cases/week/100,000 population) (*10*,*11*). If the 2014 recommendations for epidemic detection had been used in Kombissiri, the district-level preepidemic phase would have been 4 weeks longer, reaching the alert threshold in week 3 rather than 7 (13 weeks total in alert and epidemic phases). At the zone level, in the outbreak epicenter, the alert phase would have begun 1 week earlier in zone 1; no change would have been seen for zone 2, which was in the alert phase since week 1.

Conclusions

During the 2012 serogroup W meningitis epidemic in Burkina Faso, localized subdistrict epidemics occurred before those identified at the district level. Subdistrict epidemics were also of longer duration and greater intensity. At the subdistrict level, the epidemic spread from the 2 epicenter zones to other contiguous zones; several zones were affected before the district reached epidemic status.

If a subdistrict-level epidemic risk assessment had triggered district-level interventions in Kombissiri, meningitis surveillance and microbiologic testing could have been intensified (alert phase) and epidemic control measures could have been implemented (epidemic phase) up to 6 and 8 weeks earlier, respectively, by using

Figure 1. Zones within Kombissiri district, Burkina Faso.

epidemic response guidelines in use at the time and up to 2 weeks earlier by using 2014 guidelines. At the district level, the short time between crossing the epidemic threshold and attaining the epidemic peak (average <1 week) leaves a limited window for effective intervention because reactive immunization has a moderate effect on natural epidemic evolution after an epidemic has peaked (*12*). In this epidemic, the time between crossing the epidemic threshold and reaching peak was much longer when characterized at the zone level (3 and 10 weeks, respectively, in the 2 outbreak epicenters). Characterizing the epidemic risk at the subdistrict level with the 2014 alert threshold for a population of ≈30,000 yielded critical time gains, particularly during the epidemic preparedness phase. Early interventions with longer implementation windows improve response efficiency and might have halted this epidemic before it spread throughout Kombissiri. Timelier, targeted interventions could potentially be mounted at district or even subdistrict levels if the epidemic risk is localized. Too little evidence is available now to consider modifying the epidemic threshold; lowering the threshold might trigger unnecessary resource-intensive interventions, using limited vaccine supplies. However, modifications should be reconsidered once dynamics of non–serogroup A meningitis epidemics are better understood at the subdistrict level (*4*).

The advantages of reducing the spatial scale of epidemic risk assessment were recognized when *N. meningitidis* A was driving the epidemiology of meningitis in Africa and phenomena detected at HF level developed into large epidemics (*13*,*14*). However, outbreaks caused by non-A meningococcal meningitis serogroups have less resonant patterns, and analysis of the epidemic risk at a level within the subdistrict (e.g., HF level) may lack sensitivity (*4*). Nevertheless, it is essential that good quality data be readily available at that level for finer analysis when needed. The infrequent use of subdistrict-level data is partly due to their limited routine availability. Collection of subdistrict-level data was difficult when enhanced surveillance was the predominant approach for surveillance in the meningitis belt, but the transition toward case-based strategies (according to which meningitis cases are individually described at the HF level) should help fill this gap (*9*,*15*).

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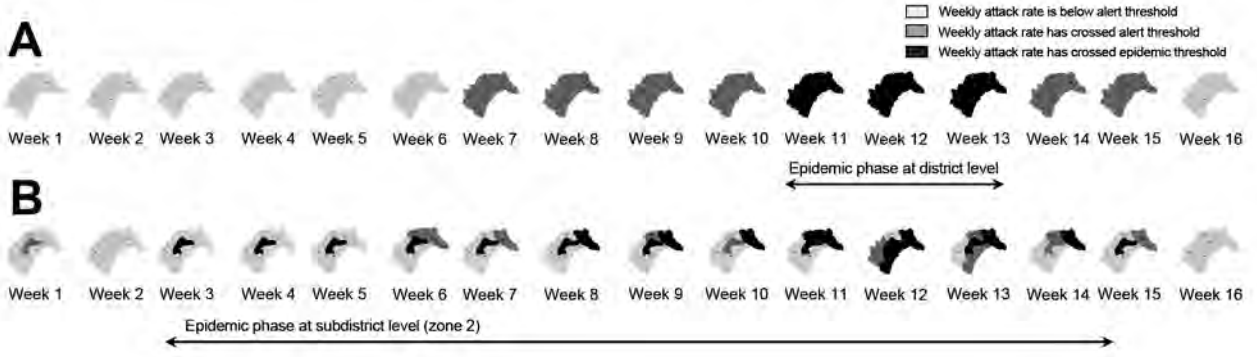

Figure 2. Weekly meningitis alert status and epidemic status at the district (A) and subdistrict (zone) (B) level in Kombissiri district, Burkina Faso, during epidemiologic weeks 1–16, 2012. The alert threshold was 5 cases per week per 100,000 population. The epidemic threshold was 10 cases per week per 100,000 population.

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Economic Costs of Measles Outbreak in the Netherlands, 2013–2014

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In 2013 and 2014, the Netherlands experienced a measles outbreak in orthodox Protestant communities with low measles–mumps–rubella vaccination coverage. Assessing total outbreak costs is needed for public health outbreak preparedness and control. Total costs of this outbreak were an estimated $4.7 million.

During May 2013–March 2014, the Netherlands was affected by a large measles outbreak (*1*). The outbreak began in the center of the country in an orthodox Protestant community and spread mainly to regions with low vaccination coverage. Overall, the Netherlands has high measles–mumps–rubella (MMR) vaccination coverage, with >95% coverage for the first dose of MMR for children. However, some orthodox Protestant and anthroposophic communities opt out of childhood vaccination programs on religious grounds or on the basis of personal beliefs (*2*). In addition to the effects of disease on a society, measles outbreaks have economic consequences, including direct medical costs and productivity losses. Moreover, a measles outbreak demands a range of responses from the National Institute for Public Health and the Environment (RIVM) and municipal public health services (MHS). Assessing outbreak costs, including costs of response activities by public health authorities, can help in planning for future outbreaks and in optimizing allocation of public resources. Recent research on measles outbreak costs in industrialized countries is scarce and has addressed hospitalizations costs (*3*), costs of imported cases of measles (*4*–*7*) or small outbreaks (*8*,*9*). We assessed the economic costs of a large measles outbreak in the Netherlands.

The Study

All physicians and laboratories are mandated to report measles to MHSs in the Netherlands. Each MHS records patient information in a national database, which includes information on age, postal code, date of symptoms, complications, hospitalization, and source of infection. Notifications of measles cases were used to assess medical costs and productivity losses (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/21/11/15-0410-Techapp.pdf). Information on additional serologic tests and extra vaccinations among health care workers in hospitals were obtained from a study on the implementation of measles guidelines for hospitals (online Technical Appendix). Information about vaccinations of infants and older unvaccinated children in response to the outbreak was retrieved from the national immunization register. We interviewed staff at MHSs and the RIVM to assess the amount of personnel time related to outbreak response activities (online Technical Appendix).

During the epidemic, 2,700 measles cases were reported, mostly among children 5–14 years of age (Table 1). In 329 patients, complications such as otitis media, pneumonia, and encephalitis developed. One child died from measles complications, and 181 patients were hospitalized. One patient with encephalitis spent 8 months in a rehabilitation clinic. Of patients who consulted a physician but were not hospitalized, 199 experienced complications, mostly otitis media (104 patients) or pneumonia (75 patients). Total estimated cost for direct health care was $1,255,718 (mean $465/case). An additional $365,885 ($136/case) was attributed to productivity losses and informal child care losses (online Technical Appendix Table 1). In 2013, most (85%) responding hospitals in the Netherlands offered a serologic test to employees to ensure that they were sufficiently protected against measles (online Technical Appendix). Employees identified as being at risk for measles infection were offered an MMR vaccination. On average, 80 serologic tests led to 63 vaccinations per hospital for a total estimated cost of $222,203 (online Technical Appendix Table 2).

At the start of the outbreak, RIVM convened a national outbreak management team to discuss a strategy regarding targeted vaccination campaigns for infants living in communities with low vaccination coverage and for previously unvaccinated persons. A total of 6,652 infants received a complimentary MMR vaccination. Among children 18 months–19 years of age, 6,948 received an MMR vaccination during July 2013–March 2014. Costs for these vaccinations were $299,840. During this outbreak, the RIVM also coordinated outbreak control, conducted enhanced surveillance, and responded to extensive media attention

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Table 1. Estimated direct health care costs during measles outbreak, the Netherlands, 2013–2014*

Type of cost	Total no. patients	Unit cost, $	Average health care utilization	Total cost, $
Physician consultation				
Uncomplicated measles, no. visits	2,320	37.35	0.2	17,330
Uncomplicated measles, no. phone calls	2,320	18.07	0.1	4,192
Hospitalizations, no. cases	181	37.35	1.0	6,760
Other complicated measles, no. cases	199	37.35	2.0	14,865
Treatment for pneumonia in general practice, no. cases†	75	16.02	1.0	1,202
Length of hospitalization, d				
General ward	174	600	4.6	480,240
Intensive care unit	7	2,866	13.1	262,812
Rehabilitation	1	447	245	109,515
Serologic test results, no. cases†			.	
Positive	139	21.37	1.0	2,970
Negative	854	21.37	1.0	18,250
DNA/RNA amplification, no. cases‡				
Positive	765	251.55	1.0	192,436
Negative	577	251.55	1.0	145,144
Total				1,255,718

*Costs are calculated in 2013 US dollars ($). Total number of measles cases = 2,700. Total cost differs from sum of category costs because of rounding.
†IgM.
‡PCR.

(online Technical Appendix). Total costs for outbreak response activities by the RIVM were an estimated $698,280 ($259/case). In addition, we collected information from 6 MHSs that together had recorded more than half of all notified measles cases nationally. Their response activities included registration and processing of cases, vaccination activities, and advising of local authorities, professionals, and the general population (online Technical Appendix). Total estimated costs for all MHSs was $1,852,470 ($686/case).

The MHSs incurred most of the costs of the outbreak, followed by costs for hospitalizations (Table 2). Costs of outbreak response activities by the RIVM were also considerable. Costs classified as other medical costs (i.e., consultations with general practitioners), productivity losses, and costs for vaccination campaigns were among the lowest (Table 2; online Technical Appendix Table 3).

Conclusions

The measles outbreak occurring in the Netherlands during 2013–2014 was associated with substantial costs of ≈$4.7 million (€3.9 million), or 0.0042% of overall health care costs ($113 billion in 2013) in the Netherlands. The 2,700 reported measles cases during this outbreak resulted in an estimated $1,739 per case. Outbreak management costs were the primary cost, probably because of demands for expert advice, response to extensive media attention, registration of notified cases, and more surveillance activities than usual.

Despite being substantial, the outbreak costs in our study are underestimated. Because of data limitations, we were unable to estimate normal human immunoglobulin costs, patients' traveling costs, or costs of vaccinations of adults or of long-term complications of disease. Also, reported cases in other countries have been linked to this outbreak, including Canada, United States, and Belgium; associated costs for cases exported to other countries are not included in our calculations. Furthermore, surveillance systems are affected by a degree of underreporting; therefore, uncertainty exists about the "true" economic costs of disease (*10*). In a previous measles outbreak in the Netherlands, the estimated true number of measles cases was ≈10 times the number of cases reported in the surveillance system (*11*). Moreover, only 47% of hospitalized cases in the previous outbreak were reported (*12*). Applying these data to our results, the estimated total outbreak costs would be ≈$0.9 million higher. Further research into the extent of underreporting in this outbreak is planned.

In Australia, the public health unit cost for responding to a single case of measles was $1,701 (*7*), a similar amount to our results. In the United States, costs of containing an outbreak were estimated at $6,180 per case. Additional US studies report that containment of a single imported measles case resulted in even higher costs per case (*5*,*6*). Explanations for the higher costs in the United States

Table 2. Distribution of costs of measles outbreak, the Netherlands, 2013–2014*

Category	Costs, $	% of total costs
MHS	1,852,470	39.5
Hospitalization	852,567	18.2
RIVM	698,280	14.9
Production losses	365,885	7.8
Laboratory tests	358,801	7.6
Vaccination of children	299,840	6.4
Vaccination of health care workers	222,203	4.7
General practitioner consultation	44,350	0.9
Total	4,694,395	100

*Costs are calculated in 2013 US dollars ($). Total cost differs slightly from sum of category costs because of rounding. MHS, municipal public health services; RIVM, National Institute for Public Health and the Environment, the Netherlands.

include more extensive contact tracing and higher medical care expenses.

The 2013–2014 measles outbreak posed considerable logistical challenges for MHS staff. Registration of reported cases contributed especially to the increased workload and costs created by this measles outbreak. To reduce this workload during a large outbreak, information considered to be critical for review could be collected for most patients, who usually recover within a few days or weeks, while more detailed information should continue to be collected for patients with complications or serious illness.

Measles substantially affects patients' quality of life (*13*) and their ability to perform their usual daily activities. Complications resulting from measles, such as pneumonia, encephalitis, and subacute sclerosing panencephalitis, sometimes occur a few years after the illness (*14*). Complications from measles also affect quality of life and incur high financial costs, as shown in the extensive rehabilitation care needed by a patient with encephalitis that resulted from this outbreak. In the Netherlands, because religious arguments affect vaccination rates (*15*), elimination of measles will be challenging. Measles outbreaks are expected to continue to cause substantial effects from disease and economic costs. To prepare for new outbreaks, medical costs, productivity losses, and containment costs should be considered.

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Chikungunya Virus as Cause of Febrile Illness Outbreak, Chiapas, Mexico, 2014

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Since chikungunya virus (CHIKV) was introduced into the Americas in 2013, its geographic distribution has rapidly expanded. Of 119 serum samples collected in 2014 from febrile patients in southern Mexico, 79% were positive for CHIKV or IgM against CHIKV. Sequencing results confirmed CHIKV strains closely related to Caribbean isolates.

Chikungunya virus (CHIKV), an arbovirus in genus *Alphavirus*, family *Togaviridae*, has undergone a rapid geographic expansion during the past decade (*1*). CHIKV is the causative agent of chikungunya fever, which may be accompanied by severe, debilitating, and even chronic arthralgia. During urban outbreaks, CHIKV uses the highly susceptible and anthropophilic *Aedes aegypti* and *A. albopictus* mosquitoes as vectors, which results in high attack rates (*1*).

The West African and East/Central/South African (ECSA) CHIKV lineages, the 2 most ancient enzootic lineages, primarily circulate in sub-Saharan Africa. Over the past century, the ECSA lineage has given rise to the Asian lineage, found in urban cycles in India and Southeast Asia, and later to the Indian Ocean lineage, which emerged from Kenya into the Indian Ocean Basin in 2004 (*1*). In late 2013, an outbreak of chikungunya fever caused by an Asian lineage strain began in the Caribbean island of St. Martin (*2*,*3*). During 2014, CHIKV spread throughout the Caribbean and into Latin America, causing epidemics in South and Central America, while also causing sporadic autochthonous cases in North America (*1*). The importation from Angola and local circulation of an ECSA strain was also recently reported in Brazil (*4*), which now has 2 CHIKV lineages circulating. The total number of suspected cases in the Americas now exceeds 1.6 million and is steadily rising (*3*,*5*).

In October 2014, physicians in Chiapas State, Mexico, noticed large numbers of patients reporting febrile illness accompanied by rash and an unusual arthralgia, and chikungunya fever was suspected. Here we report a chikungunya fever outbreak in southern Mexico, involving CHIKV of Asian lineage as the etiologic agent.

The Study

To ascertain the etiologic agent causing an outbreak of febrile illness with symptoms similar to chikungunya fever, we selected 3 sites in Chiapas State, Mexico, for sampling: Tapachula, La Libertad, and Ciudad Hidalgo (Figure 1, panel A). After patients' informed consent was obtained, blood samples were collected from persons whose condition met the following case definition for possible chikungunya fever: acute onset of fever >38.5°C, accompanied by severe arthralgia not explained by other medical conditions (*6*). Samples from Tapachula were collected from patients who sought treatment at the Centro Regional de Investigacion en Salud Publica, whereas in La Libertad and Ciudad Hidalgo, researchers surveyed houses to identify potential case-patients. In total, 119 blood samples were collected, and serum was isolated by centrifugation. Six samples were stored in MagnaPure LC buffer (Roche, Nutley, NJ, USA), which inactivates virus particles but preserves the genomic RNA.

Viral RNA was extracted from serum samples using the ZR-96 Viral RNA Kit (Zymo Research, Orange, CA, USA) according to the manufacturer's protocol. One-step quantitative reverse transcription PCR (qRT-PCR) (*7*) was performed by using the TaqMan RNA-to-C_t 1-step Kit (Applied Biosystems, San Francisco, CA, USA). Standard plaque assays were performed for the samples positive by qRT-PCR with Vero cells. Anti-CHIKV IgM-capture enzyme-linked immunosorbent assays (ELISAs) (*8*) were performed by using a chimeric Eilat-CHIKV (*9*) that contained the nonstructural proteins from Eilat virus and

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Figure 1. Map of Mexico showing the 3 sites where serum samples were obtained to test for chikungunya virus in Chiapas, Mexico, 2014: Tapachula, La Libertad, and Ciudad Hidalgo.

structural proteins from CHIKV, resulting in a structure indistinguishable from that of CHIKV. Plaque-reduction neutralization tests were used to confirm ELISA results. A sample was considered to be CHIKV negative if the sample was not positive by qRT-PCR or IgM ELISA.

Viral RNA from 5 serum samples was sent for Illumina deep sequencing and assembled by using the virus-specific HIVE-Hexagon algorithm (*10*) and the NGen module in Lasergene Suite version 10 (Bioinformatics Pioneer DNA-Star, Inc., Madison, WI, USA). Single nucleotide polymorphisms were analyzed by using the sequencing profiling tool in the HIVE suite of programs (*10*). Sequences were aligned in SeaView (*11*) by using translated proteins for the open reading frames and using nucleotides for the untranslated genome regions, and all gaps were removed. Bayesian phylogenetic inference was performed by using the general time reversible plus invariant sites plus gamma distribution 4 model in MrBayes (*12*) with 1.5 million iterations to reach congruence. Partial genome sequencing of the E2 and E1 glycoproteins was performed by using traditional Sanger methods on PCR amplicons on an additional 8 samples.

Over 100 serum samples were obtained from persons seeking treatment for chikungunya fever–like illness during October–December 2014 in 3 locations in Chiapas, Mexico (Tapachula, La Libertad, Ciudad Hidalgo) (Figure 1). These samples were analyzed by qRT-PCR and IgM-capture ELISA to detect viremia and IgM, respectively. No overlap occurred between the samples that were positive for CHIKV by qRT-PCR or those positive by IgM, demonstrating the importance of the humoral response to viral clearance. With few exceptions, viremia was detectable up to 3 days after fever onset (Figure 2, panel A), after which most samples were IgM positive. All age groups were equally likely to be infected, as expected during infectious disease outbreaks involving a naive population (*13*).

Plaque assays were performed to determine serum virus titers (Table); 3 qRT-PCR samples from Tapachula could not be assayed because of sample limitations. Mean

Table. Proportions of CHIKV viremia and IgM in 119 serum samples collected at 3 collection sites in Chiapas, Mexico, October–December 2014*

Collection site	No. serum samples	% qRT-PCR positive serum samples (no.)	Mean serum virus titer, $\log_{10}$ PFU/mL (±SD)	% IgM-positive serum samples† (no.)
La Libertad	43	20 (9)	3.26 (0.57)	51.2 (22)
Ciudad Hidalgo	63	23.8 (15)	3.36 (0.56)	68.2 (43)
Tapachula	13	30.8 (4)	3.66	7.7 (1)

*CHIKV, chikungunya virus; qRT-PCR, quantitative reverse transcription PCR.
†Six patient samples that were negative for CHIKV by qRT-PCR were unable to be used for plaque assays or ELISAs because they were stored in MagnaPure Buffer (Roche, Nutley, NJ, USA).

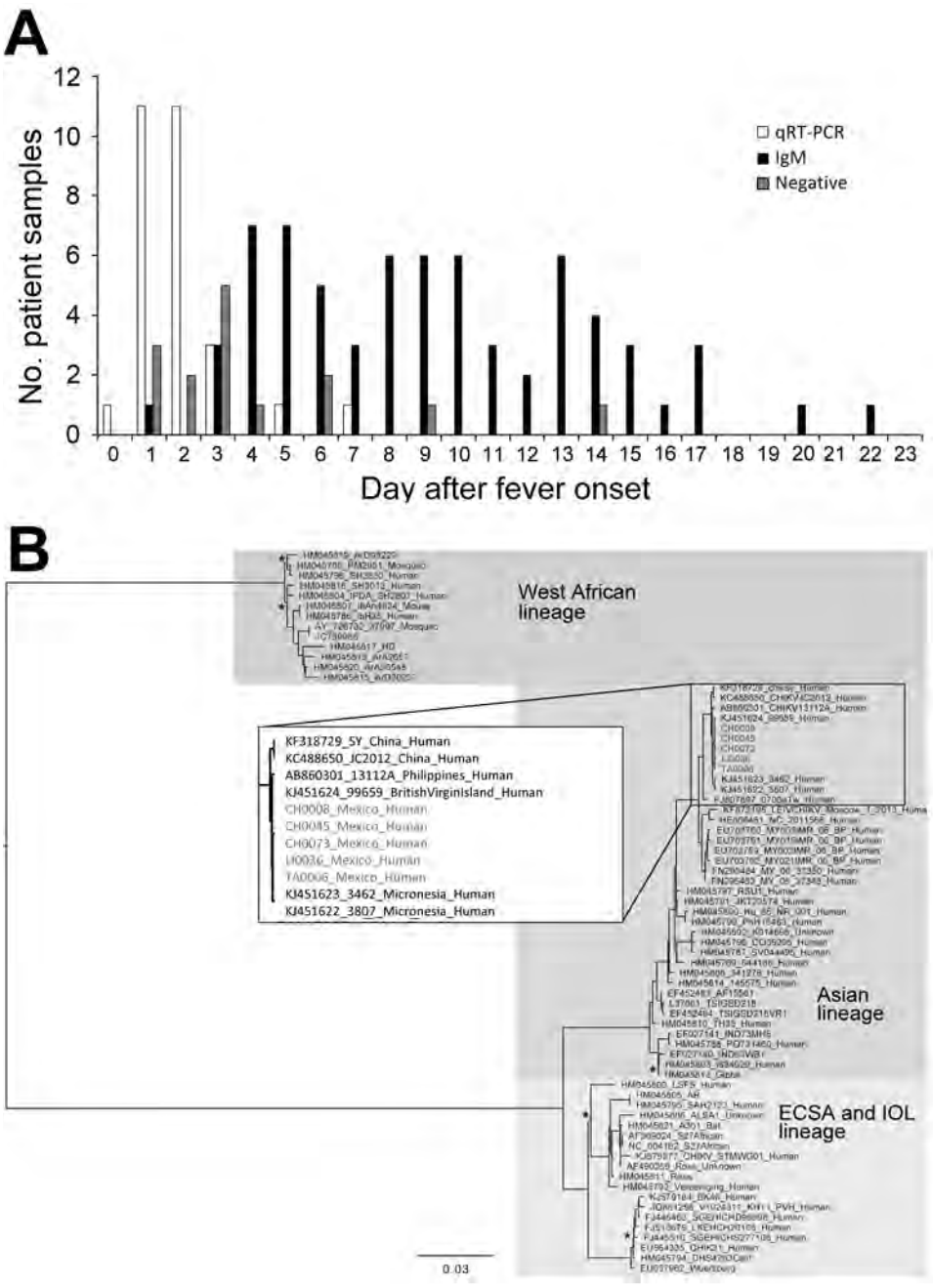

Figure 2. A) Number of serum samples positive for chikungunya virus (CHIKV) by reverse transcription quantitative PCR (qRT-PCR), for CHIKV IgM by ELISA, and negative for CHIKV by both methods, arranged according to day after fever onset. B) Phylogenetic tree generated by Bayesian analysis showing the relationship of the complete genomic sequences of 5 chikungunya virus isolates from Mexico and representative sequences from the GenBank library. All nodes showed posterior probabilities of >0.9, except those indicated with a star. The inset shows the closest relatives of the 5 isolates. ECSA, East/Central/South African, IOL, Indian Ocean lineage. Scale bar indicates nucleotide substitutions per site.

viremia level was similar among the 3 sampled locations and ranged from <2 $\log_{10}$ to 4.2 $\log_{10}$ PFU/mL.

Five serum samples from diverse locations and collection dates were selected for Illumina sequencing (GenBank accession nos. KT327163–KT327167). The strains circulating in Chiapas were most closely related to Asian lineage strains first detected in the Caribbean (represented by a British Virgin Islands isolate) and now presumed to be circulating in much of Latin America (Figure 2, panel B). Curiously, no novel mutations appear to have been fixed in the year since the British Virgin Islands isolate was collected.

Although the genomic sequences confirmed that the circulating virus in Chiapas belonged to the Asian lineage, which is primarily transmitted by *A. aegypti* mosquitoes (*1*), we nevertheless examined the sequences for mutations known to adapt CHIKV for transmission by *A. albopictus* mosquitoes. Because both mosquito species are found in Chiapas (*14*), adaptation of the CHIKV strain circulating in Mexico to *A. albopictus* mosquitoes could place temperate regions of the eastern United States and millions of naive persons at risk for infection. None of the E2 or E1 substitutions previously reported to increase

fitness in *A. albopictus* mosquitoes was observed in 8 additional samples analyzed by Sanger sequencing (GenBank accession nos. KT247378–KT247385) (*15*). One sample had a nonsynonymous mutation, in comparison to the January 2014 British Virgin Islands isolate (GenBank accession no. KJ451624) that encoded E1-V7M, which had not previously been described.

Conclusions

We found that 79% of febrile illness cases with polyarthralgia in Chiapas State during late 2014 were caused by CHIKV. Our sequencing of CHIKV genomes confirmed spread of an Asian lineage strain from the Caribbean and suggested that although CHIKV has circulated in the Americas since 2013, no adaptive mutations have occurred. However, continued screening for vector-adaptive mutations will be critical, especially now that strains of the ECSA lineage, which gave rise to the Indian Ocean lineage, have been introduced into Brazil (*4*).

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Encephalitis-Associated Human Metapneumovirus Pneumonia in Adult, Australia

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Human metapneumovirus pneumonia, most commonly found in children, was diagnosed in an adult with encephalitis. This case suggests that testing for human metapneumovirus RNA in nasopharyngeal aspirate and cerebrospinal fluid samples should be considered in adults with encephalitis who have a preceding respiratory infection,

Human metapneumovirus (HMPV) was first described in 2001 (*1*). HMPV is a member of the *Paramyxoviridae* family, the same family as respiratory syncytial, Nipah, Hendra, mumps, and measles viruses. HMPV has been reported worldwide and causes upper and lower respiratory tract infections, most commonly in children. A study in the Netherlands showed that, by 5 years of age, all children had been infected by HMPV (*1*). However, infection does not confer lifelong immunity; reinfection has been observed in adults and immunocompromised persons (*2*). During the past decade, HMPV respiratory infection associated with encephalitis has been documented in children (*3*–*7*). We present a case of encephalitis-associated HMPV infection in an adult in Australia.

The Case

During the winter months of 2014 a 47-year-old man was found unconscious by his family at his home in Victoria, Australia. He had a 2-day history of upper respiratory tract symptoms (cough, dyspnea, rhinorrhea, myalgia, and headache). He had not traveled overseas recently. Emergency services personnel determined he had a Glasgow coma scale score of 10; in the emergency department, he was intubated when his Glasgow coma scale score dropped to 8. Examination showed blood pressure of 135/83 mm Hg, heart rate of 105 beats/min, and temperature of 37.2°C. He had no cranial nerve palsies; limb examination showed normal tone and reflexes; and he was moving all 4 limbs. He had mild neutrophilia (8.2×10^9 cells/L [reference range 2.0–8.0]), mild lymphopenia (0.9×10^9 cells/L [reference range 1.0–4.0]), and elevated C-reactive protein (30 mg/L [reference range <5]). Electrolytes, liver function, coagulation screen, thyroid function, ammonia, creatinine kinase, ethanol level, and paracetamol level were normal. Electrocardiogram demonstrated sinus tachycardia, and chest radiograph showed right basal pneumonia (Figure 1). At day 0, cerebrospinal fluid (CSF) showed glucose 4.2 mmol/L (reference range 2.0–3.9), protein 0.77 g/L (reference range 0.15–0.45), erythrocytes 43×10^6 cells/L, and no leukocytes. Gram stain and culture were negative for microorganisms.

The patient was begun on acyclovir, ceftriaxone, vancomycin, and benzylpenicillin for suspected meningoencephalitis. CSF viral PCR was negative for *Mycoplasma pneumoniae*, herpes simplex viruses I and II, varicella zoster virus, and enterovirus. Tests for cytomegalovirus, *M. pneumoniae*, Epstein-Barr virus, and paraneoplastic antibodies and cytology were negative. Urine microscopy was unremarkable, and urine testing for *Legionella pneumophilia* serogroup 1 antigen, *Streptococcus pneumoniae* antigen, and *Chlamydia pneumoniae* antibody were negative. Repeat CSF examination on day 2 demonstrated glucose 4.2 mmol/L, protein 0.90 g/L, no erythrocytes, leukocytes 3×10^6 cells/L, polymorphs 2×10^6 cells/L, and mononuclear cells 1×10^6/L. Gram stain and repeat viral testing results were unremarkable. Blood cultures did not grow microorganisms. Magnetic resonance imaging (MRI) with contrast showed bilateral subcortical and external capsule fluid-attenuated inversion recovery (FLAIR) and diffusion weighted imaging (DWI) hyperintensities, with perirolandic predominance (Figure 2, panels A, B) without leptomeningeal or parenchymal enhancement. Results of magnetic resonance angiogram were normal; there was no hemorrhage.

Given that CSF viral PCR was negative and no clinical improvement occurred, the patient was treated with a 5-day course of intravenous 1-g methylprednisolone for suspected autoimmune encephalitis/cerebral vasculitis. He was extubated on day 2 but had persistent confusion and agitation and was reintubated. He was subsequently extubated on day 4 when his confusion resolved. On day 4, clinical examination and electroencephalography were normal, and his nasopharyngeal aspirate (NPA) was positive for HMPV and negative for respiratory syncytial virus; influenza A and B viruses; parainfluenza viruses 1, 2, and 3; adenovirus; and picornaviruses. We had insufficient CSF for HMPV testing. Follow-up 3 months later indicated no residual deficits, and MRI demonstrated resolution of the acute changes (Figure 2, panel C).

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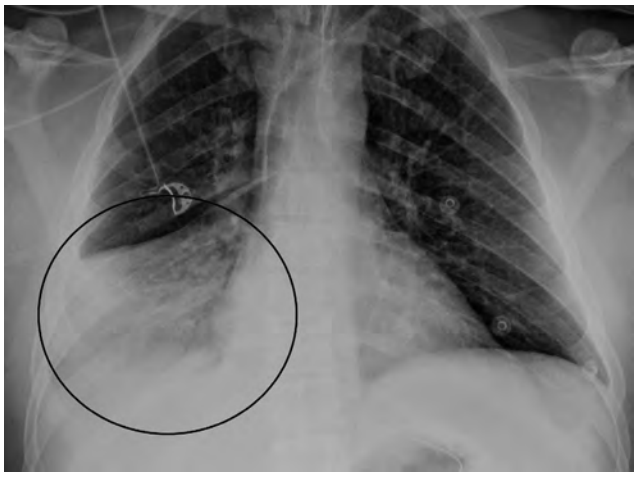

Figure 1. Frontal chest radiograph of a 47-year-old man with encephalitis-associated human metapneumovirus, Australia. Consolidation in the right middle lobe (circle) is compatible with pneumonia.

Conclusions

The clinical presentation, CSF results, and radiologic findings supported the diagnosis of encephalitis. CSF examinations showed elevated protein with no marked pleocytosis typically seen in viral encephalitis and similar to a case of respiratory HMPV with central nervous system (CNS) involvement in a child (*7*). Normal or near-normal CSF leukocyte counts have been reported in encephalitis-associated HMPV infection (*3*–*5*,*7*) and might reflect the lack of meningeal inflammation, which suggests that primary infection is through the respiratory tract and not through CNS invasion. The patient's NPA PCR was negative for other viruses, except HMPV. Other viral encephalitides are less likely because common encephalitic viruses were not found on 2 occasions in the CSF. CSF samples were not blood stained and were unlikely to be falsely negative from heme products inhibition of PCR processes (*8*).

MRI findings were similar to those in 2 other encephalitis-associated HMPV cases (*6*,*7*). In a 10-year-old girl with encephalitis, HMPV was detected in NPA and CSF, and MRI showed cortical and subcortical T2 FLAIR hyperintensities with evolving DWI hyperintensities (*6*). Another case described cortical and subcortical FLAIR hyperintensities (*7*). In the case reported here, given the MRI DWI abnormalities, cerebral vasculitis and autoimmune encephalitis were differential diagnoses. However, the acute clinical presentation and benign course did not support either of these diagnoses. In addition, the resolution of DWI lesions and radiologic lack of disease activity at 3 months with 1 course of methylprednisolone rarely occurs in either of these diseases. MRI findings were not typical of herpes simplex virus–associated encephalitis. In Hendra virus–associated encephalitis, MRI shows similar microinfarcts with T2/FLAIR and DWI lesions (*9*). We did not test for Hendra virus because it has never been reported in Victoria and the patient had no contact with horses, the

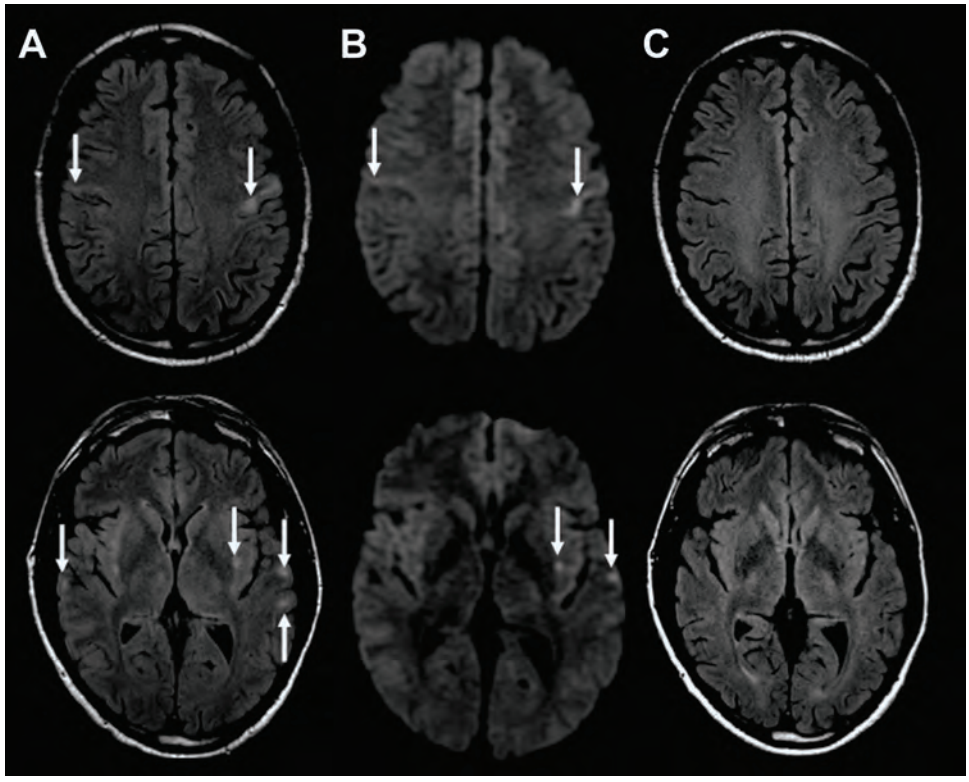

Figure 2. MRI findings from a 47-year-old man with encephalitis-associated human metapneumovirus pneumonia, Australia. A) Axial MRI FLAIR at presentation. Arrows indicate multiple areas of bilateral subcortical and external capsule FLAIR hyperintensities and perirolandic predominance (top image). B) Axial MRI DWI at presentation. Arrows indicate corresponding increase in DWI signal in the affected areas. C) Axial FLAIR MRI after 3 months. The MRI changes have all resolved. DWI, diffusion weighted imaging; FLAIR, fluid-attenuated inversion recovery; MRI, magnetic resonance imaging.

primary vector in Australia. The clinical course of Hendra encephalitis is longer and the outcome more severe. MRI findings in this patient suggested micro-infarcts involving the small vessels at the corticosubcortical junctions and deep white matter.

The limitation of this case was that we did not test for HMPV in CSF. HMPV encephalitis in adults has not been documented in the literature and at the time was not considered as a cause. We learned later in the patient's admission about the positive HMPV NPA and did not have enough saved CSF for HMPV PCR testing. This limitation emphasizes an important teaching point: for all patients with encephalitis, a portion of CSF should be saved for future analyses and, if an etiology is not found in the initial CSF analysis, then less common but possible etiologies, such as HMPV, should be tested for with saved CSF. HMPV RNA was detected in NPA and CSF in 1 child with encephalitis (*6*). In other cases with NPA positive for HMPV, HMPV PCR has yielded negative results in CSF (*3*–*5*). In the case reported here, the negative test results for other infectious agents and positive NPA by HMPV PCR conclude that HMPV is the most likely causative agent for encephalitis. Asymptomatic carriage of HMPV is uncommon, and positive NPA by HMPV PCR in asymptomatic persons without any respiratory symptoms is rare (*1*,*10*).

Previously reported cases of HMPV with CNS involvement have occurred in children. Most of these reports describe respiratory symptoms before CNS involvement (*3*–*7*); in 1 case, HMPV RNA was found in brain and lung tissue on autopsy (*7*). This finding supports an etiologic agent with predilection for lung and brain infection but requires initial respiratory tract inoculation. The case reported here raises the possibility of HMPV causing encephalitis in adults with preceding respiratory infection. Testing for HMPV RNA in NPA and CSF should be considered not only in children with encephalitis but also in adults with encephalitis who have a preceding respiratory infection and CSF and radiologic abnormalities suggestive of a viral infectious agent.

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Workplace Safety Concerns among Co-workers of Responder Returning from Ebola-Affected Country

Benjamin P. Chan, Elizabeth R. Daly, Elizabeth A. Talbot

We surveyed public health co-workers regarding attitudes toward a physician who returned to New Hampshire after volunteering in the West African Ebola outbreak. An unexpectedly large (18.0%) proportion of staff expressed discomfort with the Ebola responder returning to work. Employers should take proactive steps to address employee fears and concerns.

The largest *Zaire ebolavirus* epidemic reported began in West Africa in December 2013 (*1*) and spread to at least 10 countries, resulting in >27,000 suspected, probable, or confirmed cases of Ebola virus disease (EVD) as of June 2015 (*2*). Arrival of the first case of EVD in the United States resulted in intense public fear and concern, exacerbated by continuous news coverage and misinformation (*3*). A New Hampshire physician, who worked at the New Hampshire Division of Public Health Services (DPHS), volunteered in West Africa; she provided EVD case management training for health care workers in Ebola treatment centers in Sierra Leone but did not provide direct patient care. When she returned to New Hampshire, in accordance with guidelines of the Centers for Disease Control and Prevention and DPHS, she was considered low risk for EVD development and instructed to self-monitor with daily phone checks from DPHS; home quarantine and movement restrictions were not required (*4*,*5*). Given the heightened fear surrounding Ebola, we conducted an assessment of attitudes and intended practices in presumably EVD-educated state public health employees in relation to their Ebola responder co-worker.

The Study

We developed a Web-based questionnaire (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/21/11/15-0780-Techapp1.pdf) and distributed it through workplace email prior to the Ebola responder's return. The questionnaire provided background information about the returning Ebola responder and asked 26 questions related to 13 hypothetical scenarios and 5 demographic questions. Respondents were asked to predict their reaction, depending on whether the Ebola responder had direct contact with Ebola patients while using appropriate personal protective equipment.

We grouped responses into dichotomous categories of "comfortable" and "uncomfortable" on the basis of the reported level of comfort or anticipated actions to avoid contact; data were analyzed with SAS v. 9.3 (SAS Institute Inc., Cary, NC, USA). We performed a univariate analysis using the Mantel-Haenszel χ^2 and McNemar tests to evaluate response comparisons for demographic groups and paired response proportions, respectively. Two-tailed Fisher exact p values were used to assess statistical significance with $p<0.05$ considered significant. We performed multivariate analyses using logistic regression to assess for associations between the multiple demographic variables and reported comfort level; odds ratios (ORs) were evaluated and considered significant if $p<0.05$.

A total of 178 (71.2%) of 250 staff members completed the questionnaire. Respondent characteristics are shown in Table 1; scenarios are listed in the first column of Table 2.

Even when the Ebola responder had had no contact with an EVD patient, 18.0% of respondents were uncomfortable with the person returning to the workplace; 7.9% reported that they might not come to work if the Ebola responder was present. The proportion of respondents indicating discomfort generally increased in scenarios that described closer and/or more prolonged contact, from walking in the same hallway (14.7%) to attending a holiday party at the Ebola responder's home (46.9%). When the Ebola responder had direct contact with an EVD patient, the proportion of respondents uncomfortable with each scenario was significantly higher (Table 2), and the percentage of persons who were uncomfortable with a particular scenario increased by an average of 18.3%.

In a univariate analysis, staff members who reported being uncomfortable with the return of an Ebola responder co-worker who had had no contact to EVD patients were 11 times more likely to work in noninfectious disease program areas (OR = 10.7, p = 0.003). No staff person working in the infectious disease program reported discomfort with the Ebola responder returning to work, despite the fact that these employees would have the most contact with the Ebola responder. Because of this strong

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Table 1. Respondent characteristics for survey assessing workplace comfort levels with co-worker travel to an Ebola-affected country

Characteristic	No. (%) respondents, n = 178*
Sex	
F	133 (78.7)
M	34 (20.1)
Other	2 (1.2)
Age, y; range 23–68, median 51	
20–49	67 (40.6)
50–69	98 (59.4)
Education level	
High school	15 (8.9)
Some college	19 (11.2)
Bachelor's degree	60 (35.5)
Graduate degree	75 (44.4)
Program area	
Infectious disease	38 (22.8)
Other	129 (77.2)
Clinician	26 (15.1)

*Nonresponders for each demographic question were excluded from the respective proportion calculations. Reponses were missing for program area (n = 11), sex (n = 9), age (n = 13), education level (n = 9), and clinical background (n = 6).

association, we conducted a subgroup analysis of personnel working outside the infectious disease program, which showed that staff who reported being uncomfortable around the Ebola responder were 3 times more likely to have education below a bachelor's degree (OR = 2.7, p = 0.039). In univariate or multivariate analyses, no other respondent characteristics were significantly associated with comfort or discomfort.

Conclusions

Without a doubt, Ebola virus is transmitted through direct contact with infectious body fluids from a symptomatic person (*6–10*), but this study suggests that even health department staff may not fully understand this concept. A substantial number of public health staff—with presumably excellent access to accurate EVD information—were uncomfortable with having an asymptomatic Ebola responder return to the workplace. Almost 15% of surveyed staff reported discomfort even walking in the same hallway as an Ebola responder who had had no contact with any EVD patients. Discomfort increased to 35.6% in scenarios describing closer proximity to or physical contact with the returning Ebola responder. Duration of contact also appeared to be a critical factor in perceived risk; more staff reported being uncomfortable sitting in a chair next to the Ebola responder (35.6%) than with shaking the person's hand (29.6%). This finding may reflect a residual, albeit incorrect, concern over airborne transmission.

Our survey did find that infectious disease staff demonstrated less discomfort. We surmise that these persons had better knowledge of EVD than noninfectious disease staff because they were directly involved in EVD response activities. When we excluded infectious disease staff, having an education level below a bachelor's degree was the only characteristic significantly associated with increased discomfort. These results are consistent with findings of several public polls: up to two thirds of persons believed that EVD spreads "easily" by multiple routes of transmission, with more than a third concerned that they or a family member could be exposed and get sick from Ebola virus; these beliefs were more common among those with less education (*11–13*).

The fear and concern expressed by public health staff are not unique to the United States. Fear and stigmatization in West Africa EVD-epidemic countries, fueled by lack of information and deeply engrained misperceptions, have hindered efforts to control the epidemic and have led to survivor discrimination. Likewise, although state policy allowed this Ebola responder to return to the workplace, the concern expressed by staff created an environment in which she felt she could not work, and she opted to telework.

Table 2. Respondent comfort level with co-worker travel to an Ebola-affected country and co-worker contact with Ebola patients

Scenario	No. paired responses, n = 178	No. (%) respondents		% Change (ratio)	p value*
		Travel with contact with Ebola patients	Travel with no contact with Ebola patients		
Uncomfortable with					
Co-worker return to work	178	76 (42.7)	32 (18.0)	--24.7 (2.4)	<0.001
Walking in same hallway as co-worker	177	57 (32.2)	26 (14.7)	–17.5 (2.2)	<0.001
Being in same room as co-worker for meeting	176	65 (36.7)	30 (17.1)	–19.6 (2.1)	<0.001
Sitting in chair next to co-worker	177	88 (49.7)	63 (35.6)	–14.1 (1.4)	<0.001
Standing in line next to co-worker	177	78 (44.1)	47 (26.6)	–17.5 (1.7)	<0.001
Using same restroom as co-worker	176	77 (43.8)	40 (22.7)	–21.1 (1.9)	<0.001
Shaking hands with co-worker	176	85 (48.3)	52 (29.6)	–18.7 (1.6)	<0.001
Hugging co-worker	175	87 (49.7)	59 (33.7)	–16.0 (1.5)	<0.001
Riding in co-worker's car	177	81 (45.5)	51 (28.8)	–16.7 (1.6)	<0.001
Assisting co-worker if he/she fainted	177	95 (53.7)	62 (35.0)	–18.7 (1.5)	<0.001
Eating homemade food made by co-worker	176	95 (54.0)	67 (37.9)	–16.1 (1.4)	<0.001
Would consider not coming to work if co-worker returned	178	37 (20.8)	14 (7.9)	–12.9 (2.6)	<0.001
Would not attend party at co-worker's home	176	125 (70.6)	82 (46.9)	–23.7 (1.5)	<0.001

*McNemar's test for paired proportions.

During this period, we conducted outreach to staff through staff meeting presentations, a small group question-and-answer session, and individual meetings to allow persons to ask questions and express concerns. When the monitoring period was over, the Ebola responder returned to work without incident.

Our survey has several limitations. First, scenarios cannot assess the source of discomfort evoked by interacting with a returning traveler; baseline discomfort from a handshake is predictably less than is assisting a co-worker who fainted, regardless of EVD risk. In addition, some responses appear inconsistent. Although riding in a car with an Ebola responder and sitting in an adjacent chair are not different in terms of proximity and contact, the responses differed substantially. Finally, the survey may have drawn attention to the Ebola responder and created concern that would not naturally have occurred if the Ebola responder had returned unannounced to the workplace.

Travelers returning from countries with widespread Ebola virus transmission can elicit strong reactions within their communities. Employers should consider taking active steps to address fears and concerns because workplace reactions and discrimination may have substantial effects on returning Ebola responders. Staff education remains the best approach to alleviating concerns and maintaining a functional workplace, while facilitating the needed humanitarian response to the historic EVD disaster.

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Pneumococcal Infection among Children before Introduction of 13-Valent Pneumococcal Conjugate Vaccine, Cambodia

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Vaccination of children with pneumococcal conjugate vaccine (PCV13) was initiated in Cambodia in 2015. To determine baseline data, we collected samples from children in 2013 and 2014. PCV13 serotypes accounted for 62.7% of colonizing organisms in outpatients and 88.4% of invasive pneumococci overall; multidrug resistance was common. Thus, effectiveness of vaccination should be high.

Infection with *Streptococcus pneumoniae* remains a substantial cause of death among children (*1*). In high-income countries, introduction of pneumococcal conjugate vaccine (PCV) has substantially decreased incidence of invasive pneumococcal disease (IPD) (*2*). Data for PCV effect in low-income countries are less robust (*2*). We therefore studied the characteristics of pneumococci responsible for colonization and invasive disease among children in Cambodia before the early 2015 introduction of 13-valent PCV (PCV13).

The Study

The study was conducted at Angkor Hospital for Children, Siem Reap, Cambodia. Before enrollment of a child, written consent was obtained from the parent/guardian. Ethical approval was granted by the hospital institutional review board and the Oxford Tropical Research Ethics Committee. For the colonization study, which was conducted in January (cool/dry season) and August (hot/wet season) 2014, colonization surveys were conducted in the outpatient department. Nasopharyngeal swab samples were collected from children 1 month to 15 years of age who had minor illnesses, excluding nonsevere pneumonia, not requiring hospital admission. Children were eligible for enrollment 1 time per survey. For the invasive disease study, which was conducted during August 1, 2013–July 31, 2014, samples were collected from hospitalized children 1 month to 15 years of age who met World Health Organization (WHO) clinical case definitions for pneumonia, meningitis, or sepsis (*3*). Children readmitted within 14 days were excluded from reenrollment. Samples were processed according to the WHO pneumococcal colonization detection protocol (*4*). Pneumococci were confirmed by optochin susceptibility and/or bile solubility and were serotyped by latex agglutination (*5*). Antimicrobial drug susceptibilities were determined according to Clinical and Laboratory Standards Institute guidelines (*6*). Serotype and antimicrobial drug susceptibilities were also determined for all invasive pneumococcal isolates cultured from patients during January 1, 2013–December 1, 2014. Pneumococci were grouped into vaccine serotypes (PCV13: 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19F, 19A, 23F), nonvaccine serotypes (all others), and nontypeable isolates. Multidrug resistance was defined as resistance to ≥3 agents (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/21/11/15-0914-Techapp1.pdf) (*7*).

The outpatient colonization study included 974 children (Table 1; online Technical Appendix Figure 1). None were known to be HIV infected. Pneumococcal colonization was detected in 601 (61.7%) of children (online Technical Appendix Table 1). Colonization prevalence declined with age: 78.6% (206/262) in those 1–11 months, 61.9% (284/459) in those 12–59 months, and 43.9% (111/253) in those ≥5 years of age. The proportion colonized were 75.2% (342/455) in the cool/dry season and 49.9% (259/519) in the hot/wet season ($p<0.001$). The adjusted odds ratio for colonization in the hot/wet season was 0.38 (95% CI 0.28–0.51, $p<0.001$) after controlling for age, household size, cohabitation with other young children, current upper respiratory tract symptoms, and recent antimicrobial use. A total of 667 pneumococci were isolated (Figure 1). Among 601 colonized children, >1 serotype was identified in 11.0% (66/601). PCV13 serotypes accounted for 62.7% (418/667), nonvaccine serotypes for 29.5% (197/667), and nontypeable isolates for 7.8% (52/667) of isolates. The proportion of children colonized by PCV13 serotypes was greater among those <5 years of age (70.2% [344/490]) than among older children (48.6% [54/111]); $p<0.001$; whereas the opposite was true for colonization with nonvaccine serotypes (27.8% [136/490] vs. 48.6% [54/111]; $p<0.001$). Colonization with nontypeable isolates did not vary by age (data not

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Table 1. Characteristics of 974 children enrolled in outpatient pneumococcal colonization surveys at Angkor Hospital for Children, Siem Reap, Cambodia, January and August 2014*

Characteristic	Overall	January 2014	August 2014	p value†
Total no. enrolled	974	455	519	
Age, median (IQR)	2.5 (0.9–5.1)	1.9 (0.9–4.1)	2.9 (1.1–6.0)	<0.001
Age category, no. (%)				
1–11 mo	262 (26.9)	142 (31.2)	120 (23.1)	0.005
12–59 mo	459 (47.1)	231 (50.8)	228 (43.9)	0.03
5–15 y	253 (26.0)	82 (18.0)	171 (33.0)	<0.001
Male sex, no. (%)	499 (51.2)	241 (53.0)	258 (49.7)	0.3
Reason for outpatient visit, no. (%)				
Upper respiratory tract infection	794 (81.5)	394 (86.6)	400 (77.1)	<0.001
Gastroenteritis	92 (9.5)	47 (10.3)	45 (8.7)	0.4
Other	88 (9.0)	14 (3.1)	74 (14.2)	<0.001
Antimicrobial drug use in preceding month, no. (%)‡	453/967 (46.8)	149/453 (32.9)	175/514 (34.0)	0.7
Household size, median (IQR)	5 (4–6)	5 (4–7)	5 (4–6)	0.01
Other children <5 y of age in household, no. (%)	841/973 (86.4)	422/454 (93.0)	419 (80.7)	<0.001
Attendance at school or daycare, no. (%)	293/973 (30.1)	105/454 (23.1)	188 (36.2)	<0.001

*Weather conditions were cool and dry in January and hot and wet in August. Where data were missing, an alternate denominator is included in the affected cell. IQR, interquartile range.
†For proportions, comparisons were made by using the χ^2 test. For continuous variables, comparisons were made by using the Wilcoxon rank-sum test.
‡Includes definite and possible (unknown systemic medication) consumption in the community before outpatient visit.

shown). Overall, 68.8% (459/667) of pneumococci were multidrug resistant: 85.4% of PCV13 isolates, 50.0% of nontypeable isolates, and 38.6% of nonvaccine serotypes (p<0.001). Among colonized children, multidrug-resistant pneumococci were more commonly cultured from children <5 years of age (75.1% [368/490]) than from older children (53.2% [59/111]); p<0.001.

From August 1, 2013, through July 1, 2014, a total of 2,613 cases of medical admissions were screened; of these, 1,009 were included in the analysis (online Technical Appendix Figure 1). Median patient age at admission was 1.2 years (interquartile range 0.5–2.4), 56.5% (570/1,009) of patients were male, and 1.4% (14/1,006) were HIV positive. Most cases met the WHO category of severe pneumonia (online Technical Appendix Table 2). Pneumococcal colonization was identified in 29.1% (293/1,008) of children from whom a swab sample was obtained (online Technical Appendix Table 3). Colonization was less frequent in those who had received ≥1 dose of an antimicrobial drug (most frequently ceftriaxone) in hospital before the swab sample collection (23.8% [187/785]) than among those who had not (48.5% [95/196]); p<0.001. Colonization was identified in 31.3% (175/559) of children during the dry seasons (hot: March–May; cool: November–February) and in 26.3% (118/449) during the wet season (June–October); p = 0.08. A total of 305 pneumococci were isolated, comprising 27 serotypes plus nontypeable isolates (Figure 2). PCV13 serotypes accounted for 71.1% (217/305) of isolates, nonvaccine serotypes for 15.4% (47/305), and nontypeable isolates for 13.4% (41/305). Multidrug resistance was found in 79.3% (242/305) of isolates.

During 2013–2014, a total of 43 cases of IPD were culture proven (online Technical Appendix). Median patient age was 2.5 years (interquartile range 1.4–8.6). Overall, PCV13 serotypes accounted for 38 (88.4%, 95% CI 74.9–96.1) infections (Table 2). Multidrug resistance was identified in 55.8% (24/43): 22/38 (57.9%) of PCV13 serotypes and 2 (40.0%) of 5 nonvaccine serotypes;

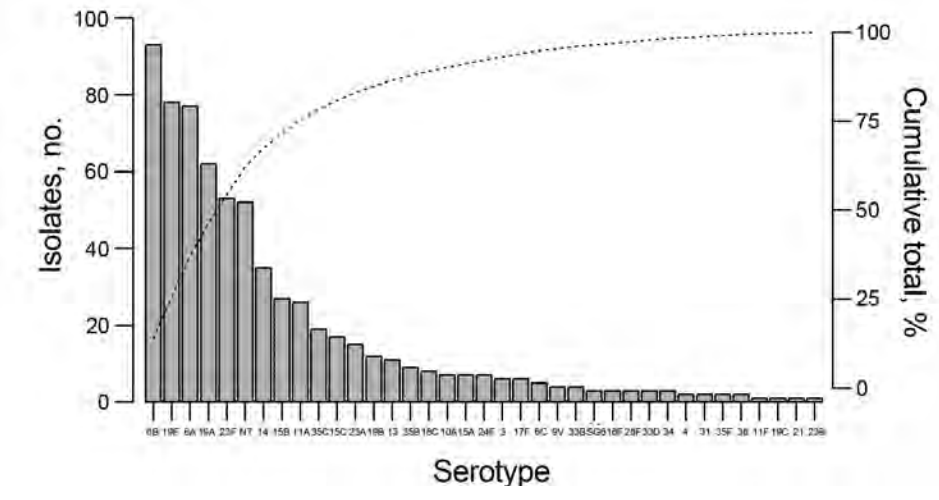

Figure 1. Serotype distribution of 667 pneumococcal isolates cultured from nasopharyngeal swab samples collected from 974 outpatients 1 month–15 years of age, at Angkor Hospital for Children, Cambodia, Siem Reap, January and August 2014. Bars indicate number of isolates; dotted line indicates cumulative total percentage of isolates.

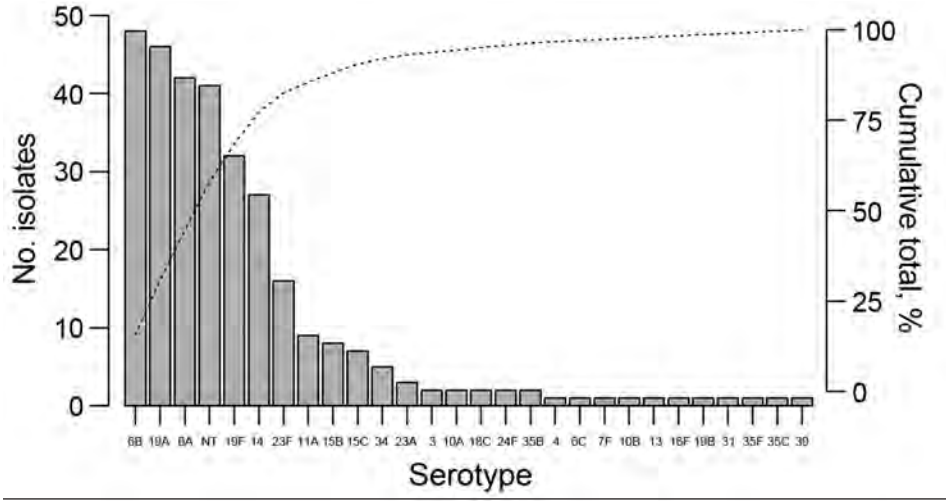

Figure 2. Serotype distribution of 305 pneumococcal isolates cultured from nasopharyngeal swab samples collected from 1,008 hospitalized patients 1 month–15 years of age at Angkor Hospital for Children, Siem Reap, Cambodia, August 2013–July 2014. Bars indicate number of isolates; dotted line indicates cumulative total percentage of isolates.

p = 0.6. Full resistance profiles are provided in online Technical Appendix Table 4.

Conclusions

This study highlights the high potential for reduction of IPD among children after introduction of PCV13 in Cambodia; 88.4% (95% CI 74.9–96.1) of invasive isolates from this 1 surveillance site were serotypes covered by the vaccine. Vaccination should result in decreased drug-resistant pneumococcal infections, although the substantial reservoir of resistance in nonvaccine type and nontypeable pneumococci will probably erode any reduction over time (*8–10*).

Colonization was high among outpatients and similar to that in other Southeast Asia locations (*5*,*11*). Multidrug resistance was common, probably the result of poor regulation of antimicrobial drug use in Cambodia (*12*); 72.1% of colonizing isolates and 55.8% of invasive isolates were multidrug resistant. For comparison, a recent study of children in Thailand found 31.6% of colonizing pneumococci to be multidrug resistant (*13*).

The range of serotypes detected in the colonization study was broad but slightly more restricted than that detected in other low-income country studies. In a longitudinal colonization study of refugee infants on the Thailand–Myanmar border, 67 serotypes were identified (*5*). This finding may reflect the high prevalence of antimicrobial drug use in the community, which would reduce the colonization prevalence of less resistant nonvaccine serotypes. However, the identification of several serotypes emerging as causes of IPD in South Africa, the United Kingdom, and the United States after introduction of PCV13 (e.g., serotypes 15A, 15B/C, 23B, 24F; which accounted for 7.8% of colonizing pneumococci in our study) is noteworthy, indicating the need for close monitoring for changes in colonization and IPD serotype distribution after PCV13 introduction (*7*,*14*,*15*).

The study has several limitations. The absolute number of IPD cases was small, and it was not possible to calculate disease incidence rates. The high prevalence of prehospitalization antimicrobial drug use hampered accurate IPD surveillance. Failure to detect more antimicrobial-drug susceptible nonvaccine type infections as a result of prehospitalization antimicrobial drug use may have falsely elevated the proportion of disease covered by PCV13. The low prevalence of colonization among hospitalized children highlights the need for swab sample collection before in-hospital antimicrobial drug administration for accurate evaluation of colonization in unwell children. Because the study was conducted at 1 site, caution is required when extrapolating the results to the general population of Cambodia. These data provide a baseline against which to monitor effectiveness of vaccinating children with PCV13 in Cambodia.

Table 2. Serotypes of invasive pneumococcal isolates from hospitalized children, Angkor Hospital for Children, Siem Reap, Cambodia, 2013–2014*

Specimen type	No.	PCV13 serotype, no. (%)	Serotypes, (no.)
Blood	35	31 (88.6)	1 (10), 6B (9), 14 (4), 23F (3), 6A (2), 12F† (1), 16F† (1), 19F (1), 19A (1), 18C (1), 28F† (1), nontypeable† (1)
Cerebrospinal fluid‡	3	3 (100)	1 (1), 6B (1), 19A (1)
Pleural fluid‡	4	4 (100)	1 (2), 5 (1), 19A (1)
Vitreous fluid	1	0 (0)	Nontypeable (1)†
Total	43	38 (88.4)	1 (13), 6B (10), 14 (4), 19A (3), 23F (3), 6A (2), NT† (2), 5 (1), 12F† (1), 16F† (1), 19F (1), 18C (1), 28F† (1)

*PCV13, 13-valent pneumococcal conjugate vaccine.
†Non-PCV13 serotypes.
‡Identical pneumococci were isolated from pleural fluid for 3 patients and from cerebrospinal fluid for 2 patients.

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Middle East Respiratory Syndrome in 3 Persons, South Korea, 2015

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In May 2015, Middle East respiratory syndrome coronavirus infection was laboratory confirmed in South Korea. Patients were a man who had visited the Middle East, his wife, and a man who shared a hospital room with the index patient. Rapid laboratory confirmation will facilitate subsequent prevention and control for imported cases.

Middle East respiratory syndrome (MERS) is characterized by mild-to-severe respiratory distress and is caused by a novel coronavirus (MERS-CoV) (*1*). Since its first identification in 2012, MERS-CoV infection has been reported for 1,413 persons from 26 countries; case-fatality rate is 40.92% (*2*). We describe an outbreak comprising 3 laboratory-confirmed cases of MERS-CoV infection in South Korea (*3*–*5*).

The Study

In accordance with national MERS control guidelines in South Korea (*6*), specimens are collected from hospitalized patients suspected of having MERS on the basis of epidemiologic history linked to the Middle East; these specimens are then transferred to the Korea National Institute of Health for examination. The index patient, a 68-year-old man engaged in farming-related business, reported that he had traveled to Bahrain on April 24, 2015, the United Arab Emirates on April 29–30, Saudi Arabia on May 1–2, and Qatar on May 2–3 before returning to South Korea on May 4 (Figure 1). While in these countries, he was not exposed to any patients, health care facilities, or animals (including camels and bats) or their excreta. On May 11, the patient experienced chills and a fever (>37°C), and an allopathic medicine was prescribed when he first visited a local clinic. However, his symptoms worsened (temperature >38°C, myalgia, cough, and dyspnea), and after he had visited 4 hospitals (hospitals A–D, in or around Seoul), he was admitted to a general hospital (hospital D, Seoul, South Korea) on May 18. A nasopharyngeal aspiration specimen was collected for MERS-CoV laboratory testing on May 19.

Sputum samples from 2 persons who had been in contact with the index patient were also tested for MERS-CoV. Patient 2 was the 63-year-old wife of the index patient; she had had physical contact with him while caring for him during the 3 days of hospitalization. Fever (38°C) and slight oliguria developed in patient 2 on May 19. The other contact, patient 3, was a 78-year-old man who had chronic obstructive pulmonary disease, asthma, and cholangiocarcinoma and who had shared a hospital room with the index patient and had been within 2 meters from him for 4 hours on May 16. Fever (37.8°C) and respiratory symptoms developed in patient 3 on May 20. In the hospital room, the index patient did not undergo any aerosol-generating procedures, but a severe cough developed. The same health care workers cared for the index patient and patient 3.

Laboratory diagnostic methods were performed according to World Health Organization guidelines for molecular detection of MERS-CoV (*7*–*9*). To check for contamination derived from the positive control, we designed and synthesized the MERS-CoV real-time reverse transcription PCR (rRT-PCR)–positive transcripts for an upstream MERS-CoV envelope protein gene (upE) and the open reading frame 1a (ORF1a) gene containing 50 bp of a foreign gene (centipede).

Initially, nasopharyngeal samples from the index patient were positive for MERS-CoV by multiplex rRT-PCR. Sputum samples from patients 2 and 3 were also positive, supporting a diagnosis of MERS-CoV infection. Multiplex rRT-PCR results for upE and ORF1a were positive (Table 1). According to rRT-PCR, the respiratory samples from the 3 patients were negative for 5 other human coronaviruses (SARS-CoV and human CoV-229E, -OC43, -NL63, and -HKU1) and 7 viruses that cause acute respiratory infection (influenza virus A and B; human adenovirus; bocavirus; human parainfluenza virus types 1, 2, and 3; respiratory syncytial virus A and B; human rhinovirus; human metapneumovirus).

For the index patient, MERS-CoV RNA was detectable in sputum, throat swab, and serum samples but not in a urine sample collected 9 days after symptom onset (Table 1). The viral load, indicated by cycle threshold values, was high in the lower respiratory tract sample but almost undetectable in the throat swab and serum samples. After sequential sampling repeated every 2–5 days, MERS-CoV RNA was detected in sputum until 44 days after symptom onset, although viral RNA was inconsistently detected and patterns of viral load fluctuated (Table

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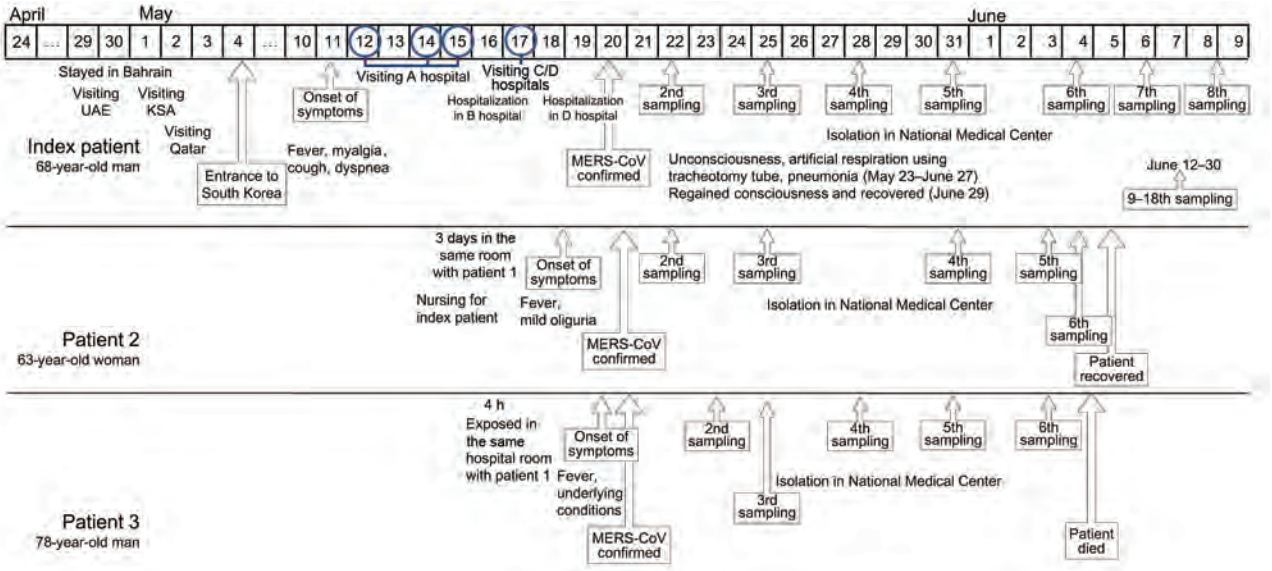

Figure 1. Timeline of events for patients infected with Middle East respiratory syndrome coronavirus (MERS-CoV). The laboratory diagnostic methods used for molecular detection of MERS-CoV RNA were multiplex MERS-CoV real-time reverse transcription PCRs targeting an upstream MERS-CoV envelope protein gene and an open reading frame 1a gene (*8*,*9*). KSA, Kingdom of Saudi Arabia; UAE, United Arab Emirates.

2). Other than initial fever (>37°C), clinical features differed for all 3 patients. The index patient had respiratory symptoms with cough, dyspnea, and myalgia. Patient 2 did not have a relevant medical history and showed mild symptoms. Patient 3 had underlying concurrent conditions and died 16 days after confirmation of MERS-CoV infection (Figure 1).

Virus isolation on Vero cells was attempted for each respiratory specimen from the 3 patients. The culture supernatant after inoculation was serially assessed for virus growth by using rRT-PCR and was used for blind passages every 3–7 days after inoculation. After 3 blind passages, cytopathic effect was observed, and we isolated the MERS-CoV strain from South Korea (KOR/KNIH/002_05_2015) from the Vero cells after inoculation by using the sputum from patient 2. We constructed a phylogenetic tree by using the general time reversible plus gamma model of the RAxML version 8.8.0 software (*10*) and FigTree version 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree) and by using complete genomes of the MERS-CoV isolate from South Korea (GenBank accession no. KT029139) and 67 reference MERS-CoVs (Figure 2).

Conclusions

Because, to our knowledge, cases of MERS-CoV infection in South Korea have not been reported, we had to establish laboratory testing protocols to overcome vulnerabilities in the absence of appropriate epidemiologic support (i.e., generate positive controls to check for contamination and repeat testing). Positive controls containing foreign genes have been generated to check for laboratory contamination. For patients with an unclear exposure history (such as the index patient) and for patients with short exposure durations and unusual clinical symptoms (such as patients 2 and 3), it would be useful if the positive results of rRT-PCR could be confirmed through agarose gel electrophoresis to exclude contamination from the positive control (*3*,*4*).

The index patient had no history of potential exposure to camels, bats, or their excreta; to symptomatic persons; or to health care workers during his trip to the Middle East, including Saudi Arabia. Although the source of infection for the index patient is unclear, phylogenetic analysis of the whole viral genome showed that the isolate from South Korea was closely related to the MERS-CoV strains isolated in Saudi Arabia in 2015.

Table 1. Real-time reverse transcription PCR results for 2 patients infected with Middle East respiratory syndrome coronavirus, South Korea, 2015*

	Index patient (day 9 after illness onset)		Wife of index patient (day 2 after illness onset)	
Sample	upE, C_t	ORF1a, C_t	upE, C_t	ORF1a, C_t
Sputum	18.61	19.32	26.23	26.63
Nasal swab	29.34	29.58	ND	36.64
Nasopharyngeal aspirate	29.35	31.45	NT	NT
Serum	34.81	35.50	ND	ND
Urine	ND	ND	34.34	ND

*C_t, cycle threshold; ND, not detected; NT, not tested; ORF1a, open reading frame 1a gene; upE, upstream envelope protein gene.

Table 2. Real-time reverse transcription PCR results for sputum samples serially collected from 3 patients infected with Middle East respiratory syndrome coronavirus, South Korea, 2015*

	Patient 1 (index patient)			Patient 2†			Patient 3‡		
Date	Days after illness onset	upE, C_t	ORF1a, C_t	Days after illness onset	upE, C_t	ORF1a, C_t	Days after illness onset	upE, C_t	ORF1a, C_t
May 20	9	18.61	19.32	2	26.23	26.63	1	28.10	28.65
May 22	11	25.24	25.67	5	35.53	35.10	3	24.67	25.04
May 25	14	26.48	27.99	11	25.20	26.10	5	26.37	25.53
May 28	17	31.23	32.05	14	32.58	34.80	8	ND	35.94
May 31	20	36.94	ND	17	ND	ND	11	26.30	28.01
Jun 4	24	ND	36.27	18	ND	ND	14	ND	ND
Jun 6	26	33.92	36.70	19	Discharged		15	Died	
Jun 8	29	36.50	ND						
Jun 9	30	36.90	37.46						
Jun 12	33	ND	ND						
Jun15	36	ND	ND						
Jun 16	37	35.46	ND						
Jun 17	38	ND	ND						
Jun 22	43	ND	ND						
Jun 23	44	32.97	36.31						
Jun 26	47	ND	ND						
Jun 29	50	ND	ND						
Jun 30	51	ND	ND						

*C_t, cycle threshold; ND, not detected; ORF1a, open reading frame 1a gene; upE, upstream envelope protein gene. Blank cells indicate not applicable.
†Wife of index patient.
‡Shared hospital room with index patient.

Because the index patient initially concealed his travel history to Saudi Arabia, United Arab Emirates, and Qatar, MERS-CoV infection was not considered and the patient was not isolated until MERS-CoV infection was suspected 7 days after symptom onset. Meanwhile, other patients and health care workers had multiple opportunities for exposure to the index patient (*3*–*5*). The 2 contacts reported here had each been exposed to the index patient. Patient 3 was probably infected via droplet transmission in the hospital room. The hospital room, originally built for 6 persons, had been divided into 2 rooms and lacked ventilation. Furthermore, an air conditioning unit cycled the air in the room with the door and window closed. Thus, poor ventilation might have played a major role in droplet transmission. Detection of MERS-CoV RNA in the respiratory tract varies up to day 33 (*11*–*13*). In this study, virus was detected in the respiratory tract, inconsistently, for up to 44 days.

Development of effective preventive measures for the MERS-CoV prevention will require systemic and prospective studies associated with viral shedding and use of specimens in addition to those obtained from the respiratory tract to define the kinetics of MERS-CoV. Rapid detection of MERS-CoV, using multiplex rRT-PCR to detect upE and ORF1a genes, would be helpful for countries outside the Arabian Peninsula.

Acknowledgments

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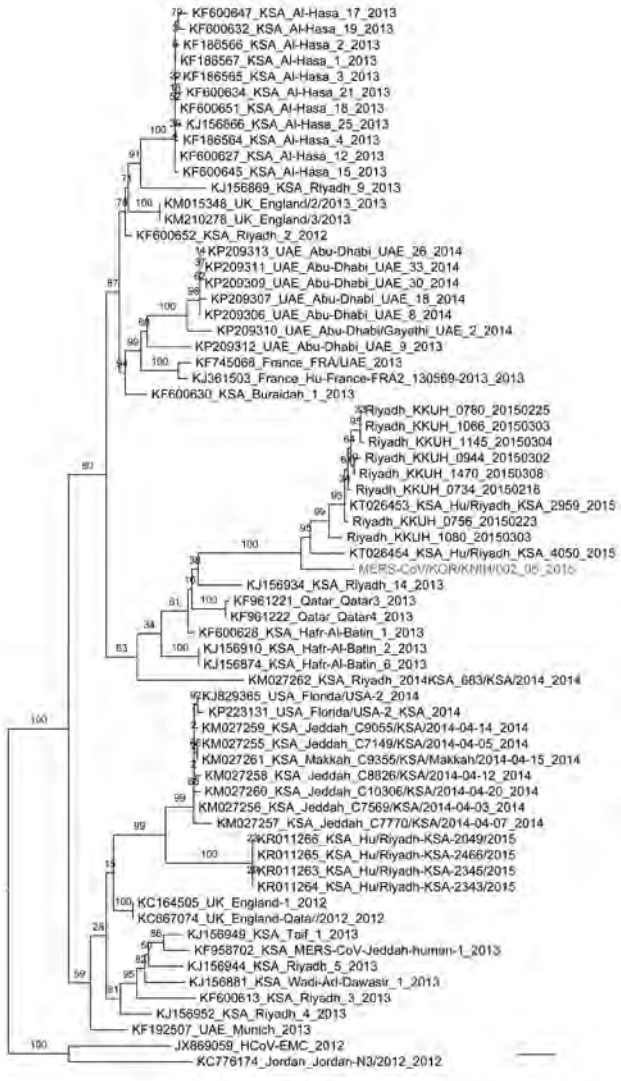

Figure 2. Phylogenetic tree comparing complete genome nucleotide sequences of Middle East respiratory syndrome coronavirus (MERS-CoV) isolate from South Korea (KOR/KNIH/002_05_2015) with those of 67 reference MERS-CoVs (GenBank database). The tree was constructed by using the general time reversible plus gamma model of RAxML version 8.8.0 software (*10*) and visualized by using FigTree version 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree). RAxML bootstrap values (1,000 replicates) are shown above the branches. Bootstrap values >75 are shown on the branches. The MERS-CoV strain from South Korea is shown in gray. National Center for Biotechnology Information accession numbers are shown before each taxon name. The unit of branch length is the number of substitutions per site. Scale bar indicates 4 × 10^{-4} nucleotide substitutions per site.

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Mortality Risk Factors for Middle East Respiratory Syndrome Outbreak, South Korea, 2015

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As of July 15, 2015, the South Korean Ministry of Health and Welfare had reported 186 case-patients with Middle East respiratory syndrome in South Korea. For 159 case-patients with known outcomes and complete case histories, we found that older age and preexisting concurrent health conditions were risk factors for death.

The ongoing outbreak of Middle East respiratory syndrome (MERS) in South Korea is the largest outside Saudi Arabia. As of July 15, the South Korean Ministry of Health and Welfare (MOHW) has reported 186 case-patients (*1*). Of these case-patients, 131 have recovered, 19 remained are hospitalized, and 36 had died (Figure 1). We conducted a preliminary mortality risk factor analysis for case-patients with MERS in South Korea who had known outcomes and covariates. We then compared our findings with those of previous investigations of case-patients in Saudi Arabia.

The Study

Case identification numbers were matched between the June 26, 2015, World Health Organization (WHO) line list (*2*) and daily text-based MERS reports from the South Korean MOHW (*1*). Matching between the 2 data sources was conducted by using age, sex, and date of reporting. The WHO line list included additional risk factor data, which were cross-validated against meta data from the MOHW. The MOHW daily MERS reports provided real-time outcome information.

As of July 15, outcomes and covariates were publicly available for 159 of 186 case-patients, all of whom became ill during weeks 2–7 of the outbreak. We used this subset to describe the patient population, evaluate risk factors for death by using logistic regression models, and assess predictors of time from onset to diagnosis and onset to discharge by using Cox proportional hazards models. Five potential covariates were analyzed: sex, age, concurrent health condition status, health care worker status, and time from onset to diagnosis. For time-to-event analyses, patients were categorized into outbreak weeks by date of onset. We tested the Cox proportional hazards assumption by using Schoenfeld residuals and included an interaction term for predictor and follow-up time.

Of the 159 case-patients analyzed, 94 (59%) were men. Per WHO definitions, 25 (16%) had concurrent health conditions and 22 (14%) were health care workers. Age was normally distributed (range 16–87 years, mean [SD] 55 [15.9] years). All deaths occurred in patients >48 years of age. Time from onset to diagnosis was positively skewed: median 4 days (interquartile range [IQR] 2–7 days). Median time from diagnosis to death and from diagnosis to discharge were 13 (IQR 17–25.3) and 22 (IQR 9–16.5) days, respectively.

As of July 15, a total of 35/159 cases analyzed were considered fatal, which yielded an estimated case-fatality rate (CFR) of 22%. Univariate logistic regression models for each risk factor showed that older age and having a concurrent health condition were associated with death (both $p<0.001$); both variables remained significant after we adjusted for all 5 variables in a multivariate logistic regression model (Table). The model estimated that odds of dying were 7 times higher for persons with concurrent health conditions than for persons without these conditions (odds ratio 7.14, 95% CI 2.27–22.41). Furthermore, for every 1-year increase in age, odds of dying increased by 12% (odds ratio 1.12, 95% CI 1.07–1.17).

Time from onset to diagnosis decreased from a median of 10 days during outbreak week 2 (IQR 8.0–12.0 days) to 2 days during week 7 (IQR 1.0–2.0 days). There was a 43.7% average increase in hazard of diagnosis per week by a univariate Cox proportional hazards model ($p<0.001$). Separate univariate Cox models showed that no recorded risk factors were associated with this change.

Time from onset to discharge for patients who survived decreased from a median of 27 days during outbreak week 2 (IQR 22.0–32.0 days) to 19 days during week 7 (IQR 17.0–23.0). Univariate Cox proportional hazards analyses estimated a 34% average increase in the hazard of discharge per week ($p<0.001$), a 63% increase for health care workers ($p = 0.046$), and an 8% decrease for every 1-day increase in time-to-diagnosis. Multivariate analysis controlling for all risk factors showed that the increase in

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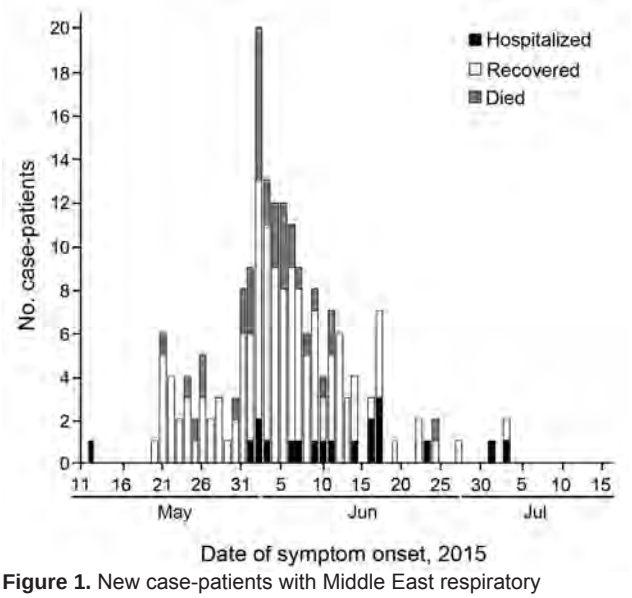

Figure 1. New case-patients with Middle East respiratory syndrome, South Korea, by date of symptom onset and patient status, as of July 15, 2015. When date of symptom onset was unavailable, date of reporting was used. Although all 186 reported case-patients are included in this plot, only case-patients with known outcomes (e.g., recovered, died) and dates of onset were included in the analyses (n = 159).

hazard by week decreased to 26% (p = 0.032); no other covariates remained significant.

Conclusions

We found that older age and preexisting concurrent health conditions were associated with an increase in odds of death from MERS. Although being a health care worker appears to be protective, the association is not significant, probably because only 1 health care worker had died of MERS as of July 15, 2015. Time from onset to diagnosis was not an indicator for death, which suggests that the rapidity with which a patient receives supportive care may be of marginal consequence. Similarly, although being a male patient seems to increase odds of death, this relationship was not significant.

On the basis of case-patients who had known outcomes through July 15, the ongoing MERS outbreak in South Korea had an estimated CFR (22%) that was half the CFR (44%) for all known case-patients with MERS in Saudi Arabia (*3*), but a CFR similar to that calculated for patients with only nonsporadic illness (21%) (*4*). Because 19 (10%) of 186 case-patients reported remain hospitalized, the final CFR of the outbreak might be higher than our current estimate. However, the proportion of patients who died (18%–19%) has been fairly stable since June 27 (Figure 2), which might indicate an asymptotic approach toward the final outbreak-specified CFR (*5*–*7*). If so, the final CFR associated with the MERS outbreak in South Korea during 2015 might be <22%. If all remaining hospitalized case-patients died, the final outbreak CFR would be 29%, which provides an upper limit for our current estimate of 22% excluding additional cases.

A total of 16 (64%) case-patients with MERS in South Korea who had concurrent health conditions died, compared with 19 (14%) case-patients without concurrent health conditions. This finding is comparable to that in a MERS study in Saudi Arabia, which reported a 60% CFR for a study population in which 45 (96%) patients had concurrent health conditions (*8*). Although only 25 (16%) case-patients had documented concurrent health conditions, the MERS outbreak in South Korean during 2015 has been largely nosocomial in nature. This finding suggests that observed differences between average CFRs in South Korea and Saudi Arabia might be driven in part by differential rates of concurrent health conditions for susceptible persons.

Use of publicly available data poses unique challenges. Although such data enable preliminary epidemiologic research during an ongoing outbreak, case information is stringently restricted to protect patient privacy. Because of this limitation, a follow-up analysis will be conducted pending availability of additional covariate data on potentially relevant biometrics (e.g., blood pressure) and behaviors (e.g., tobacco use), as well as outcomes for patients still hospitalized.

Despite these limitations, we found that risk factors for death among patients with MERS in South Korea who had known outcomes (age and concurrent health conditions) were similar to those identified for MERS case-patients in Saudi Arabia (*8*–*10*). Given these epidemiologic similarities and assuming that inherent virulence of MERS coronavirus is not context specific, the CFR difference might be caused not only by differential prevalence of risk factors but also by treatment or surveillance disparities.

Table. Multivariate logistic regression model assessing odds ratios of risk for death for 159 case-patients with Middle East respiratory syndrome and known outcomes and covariates, South Korea*

Variable	Value	Odds ratio (95% CI)	p value
Male sex, no. (%)	94 (59)	2.85 (0.98–8.20)	0.052
Mean (SD) age, y	55 (15.9)	1.12 (1.07–1.17)	<0.001
Concurrent health condition, no. (%)	25 (16)	7.14 (2.27–22.41)	<0.001
Health care worker, no. (%)†	22 (14)	0.88 (0.09–8.93)	0.915
Median time-to-diagnosis, mo (IQR)	4 (2–7)	1.00 (0.89–1.14)	0.957

*IQR, interquartile range.
†Only 1 health care worker died during the study period.

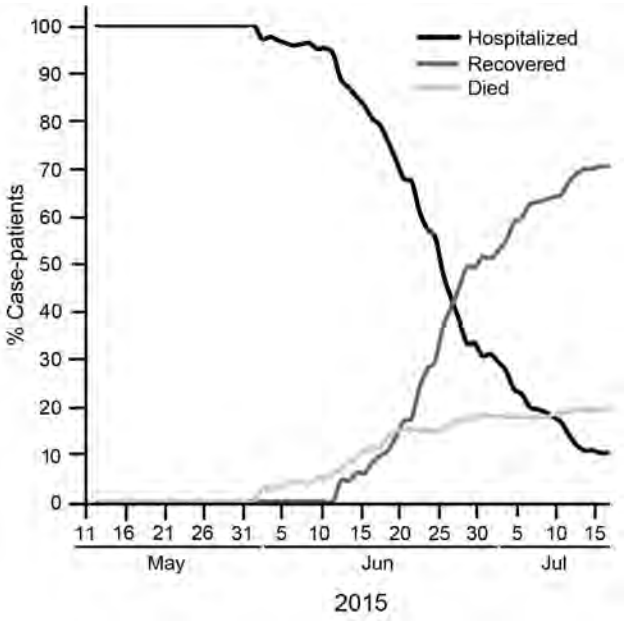

Figure 2. Cumulative proportion of case-patients with Middle East respiratory syndrome who were hospitalized, recovered, and died, South Korea, as of July 15, 2015. Total cumulative cases over time were calculated by date of symptom onset. When date of onset was unavailable, date of reporting was used. Cumulative recoveries and deaths over time were calculated by date of outcome; when date of outcome was unavailable, date of reporting was used. Although all 186 reported case-patients are included in this plot, only case-patients with known outcomes (e.g., recovered, died) and dates of onset were included in the analyses (n = 159).

Time to diagnosis decreased during the first 7 outbreak weeks, which probably contributed to the reduced length of hospitalization for patients who recovered and indicates that supportive care in South Korea might be highly adaptive. Furthermore, as reported by Cowling et al. (*11*), intensive case-finding activities might have produced more comprehensive diagnosis and reporting, thereby capturing less severe cases. In either event, given the frequency of importation events (*12*) and propensity for super-spreading (*13*), these findings provide information about MERS and MERS coronavirus in South Korea that might be useful in improving early case detection and preventing death.

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Liberia—Moving Beyond "Ebola Free"

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Although the ongoing Ebola epidemic has brought much attention to Liberia, diseases of poverty, such as malaria, tuberculosis (TB), and maternal–newborn complications, rarely make the headlines. Along with the other West African countries that bore the brunt of the epidemic, Liberia ranks near the bottom of the Human Development Index, a composite measure that assesses whether persons enjoy a long and healthy life, can acquire knowledge, and have an adequate standard of living (*1*). In Liberia, before the Ebola outbreak, ≈50 doctors attempted to care for ≈4 million persons (*2*). In an already fragile health-care setting, Ebola took a terrible toll: >8% of the health care workforce in Liberia died from the virus (*3*). The consequences of such a dramatic loss will be felt for years to come, especially in the areas of infectious disease and maternal and infant mortality (*3*). As we renew our commitment to make Liberia "Ebola free," we should remind ourselves that in the 21st century, Liberians still die from 19th century diseases. The focus must go beyond "getting to zero." As concerned clinicians, we argue that much more work needs to be done.

In early 2015, we went to Grand Gedeh County as short-term clinicians, working with a nongovernmental organization (NGO) to support Liberia's existing health care infrastructure. Unlike emergency response NGOs, our NGO turned its attention to assisting local hospitals, clinics, and communities in their routine, day-to-day health care activities. Tucked away in Liberia's remote southeastern corner, Grand Gedeh County had largely been spared from the epidemic; at that point, only 1 case had been reported since the epidemic's onset. We would not wear the protective space suits so familiar in the public eye. Instead, we would work in surrounding communities, meeting with local health workers and psychosocial officers, or on the wards with Liberian nurses and doctors, tending to persons who suffered, and at times died, from easily preventable diseases.

The public hospital offered a glimpse into the state of Liberian health care facilities in the wake of Ebola. The building itself lacked electricity most hours of the day; it had no running water, and there was a severe shortage of medications and basic supplies. The only available pain medication at the hospital was oral acetaminophen tablets. We witnessed 5 neonatal deaths in 10 days. In the community, we listened to first-hand experiences about the peak of the epidemic from those who had relocated to Grand Gedeh County. They recounted how entire families died in the span of weeks and how fear and stigma rent communities apart. Nonetheless, working alongside and learning from our colleagues and friends filled us with deep admiration and humility. Despite their country's history of war, poverty, and disease, the Liberians we met believe they can create something better.

Indeed, the inner strength and commitment of our Liberian colleagues prompted much self-reflection. In the throes of the epidemic, Liberian health care workers provided care at the expense of their own safety, and many died. Even before Ebola, however, these same health care workers were risking their own health and well-being, and that of their families, by treating patients with TB without respiratory masks or going without basic vaccinations—all fundamental, common-place measures taken in American hospitals. Our contribution as short-term health care providers seemed minuscule compared with the reality that our Liberian colleagues continue to face. This personal struggle was made all the more disturbing by knowing that an exit was already planned for us. Our Liberian colleagues and friends remain.

As we reflect on our brief time in Liberia, we revisit the central issue that compelled us to go: do we, in our position of comfort and relative ease, have a responsibility to help impoverished persons who have an exotic and frightening disease? We still emphatically believe that the answer is yes. But shouldn't we go further and ask whether that responsibility includes diseases and deaths that have become routine, accepted, or rendered invisible?

The story of Ebola confirmed that we are all connected in a complex sphere of exchange—of resources, technologies, ideas—and that the arrangement benefits some more than others. It was not coincidental that so many persons in that part of the world fell ill from the virus. Decades of conflict, economic impoverishment, and a near-nonexistent health care system created a tangle of injustices that set the stage for the epidemic. We share the conviction that these injustices—unfair social, political, and economic

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arrangements—are the real culprits behind the Ebola epidemic and, in broader terms, today's health disparities. Our participation reminds us that the Ebola experience is but one example of how politics and poverty cause death and suffering on a grand scale.

We should examine how the Ebola epidemic, and the global response to it, fit into this larger picture of health disparities and injustice. Ebola linked Monrovia, Liberia, to Dallas, Texas, USA, and in so doing exposed how interconnected our global society has become. While infected international health workers were evacuated to specialized centers with experimental drugs, infected Africans hoped for supportive care in crowded Ebola treatment units, circumstances that forced us to grapple with the unfairness of today's health care inequities. Despite years of bureaucratic research, financing, and planning, the epidemic's unprecedented scale demanded major reexamination of what is meant by the concept of global preparedness. Thus, in terms of understanding how health disparities should be addressed, Ebola overturned key assumptions: that rich countries can ensure the health of their populations in isolation, that fundamental ethical issues regarding the role of global health agencies and their actors are settled, and that a single, global authority can marshal and coordinate resources effectively (*4*).

Why don't we apply the same lessons to diseases of poverty and other emerging infectious diseases? De Cock and colleagues note, "It is difficult to explain why investment in separating human drinking water from human feces, the basis of the nineteenth century public health revolution in Europe and North America, has not been a higher political or development priority in resource-poor settings" (*5*, p. 1195). If we acknowledge our interconnectedness through social, economic, and political dimensions, that there exist severe shortcomings in health equity both within and between countries, and that these challenges require cooperation beyond the traditional donor-recipient model, then perhaps we will come to terms with why deaths from malaria, TB, or diarrheal diseases implicate all of us, and matter (*5*). Engagement in global health is not just a humanitarian endeavor; it is a priority for our collective well-being.

As the Ebola crisis wanes and media attention shifts away from West Africa, the underlying determinants of health and disease are still in place. The reemergence of Ebola virus infection attests to this, as do the senseless deaths of those who die from easily preventable diseases. We must hold ourselves to a higher standard, one beyond a 42-day Ebola-free countdown. With self-reflection and in a spirit of solidarity, we must continue to articulate that standard and where our responsibility lies in meeting it.

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Mycobacterium sherrisii Pulmonary Disease, Burkina Faso

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To the Editor: Pulmonary disease caused by nontuberculous mycobacteria (NTM) is increasing worldwide. The most commonly recognized species, with minor geographic differences, are *Mycobacterium avium* complex (MAC), *M. kansasii*, *M. abscessus*, and *M. xenopi* (*1*). Little is known about the role of NTM in pulmonary disease in countries with a high prevalence of tuberculosis (TB). In such settings, smear microscopy (to identify acid-fast bacilli) is the primary, and often the only, diagnostic tool used to diagnose presumptive TB. Therefore, pulmonary disease caused by NTM is frequently misdiagnosed as TB and, because of the patient's lack of response to standard anti-TB treatment, as multidrug-resistant TB (*2*).

In 2012, to detect possible drug resistance, we selected patients with pulmonary TB at the pulmonology division of Ouagadougou University Hospital (Ouagadougou, Burkina Faso), whose cases were classified as failing category II treatment (for patients with history of previous TB treatment). Each patient provided 1 sputum sample for culture and first- and second-line drug susceptibility testing (DST). Culture and DST were performed at the Supranational Laboratory of Milan (Milan, Italy), which provides technical assistance to the National Reference Laboratory in Burkina Faso. Of 314 samples NTM grew in culture for 36 (11%). Most NTM were identified as MAC (20 isolates). In culture of samples from 4 of the remaining patients, *M. sherrisii* grew. We describe the epidemiologic and clinical characteristics of these 4 patients.

Three patients were male. All were born and lived in Burkina Faso and were HIV-negative; their ages ranged from 33 to 57 years. All had a history of having received 2 courses (categories I and II) of treatment for pulmonary TB. All were symptomatic, and their sputum samples were highly positive for acid-fast bacilli (1–10 cells/field; Ziehl-Neelsen stain). One patient did not return for further evaluation after this early assessment; the 3 others underwent a chest radiograph that showed, for each, pulmonary lesions compatible with TB (Table). The clinical specimens investigated in Milan were negative for *M. tuberculosis* complex by specific PCR (GenoType MTBDR*plus*, Hain Lifesciences, Nehren, Germany) and grew NTM in culture. The strains were identified as *M. simiae* with GenoType Mycobacterium CM/AS line probe assay (Hain Lifesciences), but because of the known cross-reactivity of the *M. simiae*–specific probe in this kit (*3*), the 16S rRNA gene was sequenced. All strains showed 100% identity to *M. sherrisii* strain NLA000800640 (GenBank accession no. EU883389), a strain previously isolated from a patient in Tanzania (*4*).

On the basis of these findings, a treatment regimen that included clarithromycin was begun for the 3 patients, in addition to the anti-TB regimen with isoniazid, rifampin, and ethambutol. One patient was lost to follow-up during the first 2 months of treatment and the 2 others died. Further information was available for only 1 of those who died: he died of heart failure after 9 months of treatment. The pulmonary disease may well have been the cause; no autopsy was performed.

The presence of clear signs and symptoms compatible with pulmonary TB and the contemporary exclusion of *M. tuberculosis*, supported by the unresponsiveness to specific treatments and by the negative PCR results of strongly smear-positive sputum samples clearly fulfill the clinical criteria of the America Thoracic Society for NTM pulmonary disease (*5*). Meeting the objective of a second

Table. Clinical features of and microbiological findings from 4 patients with *Mycobacterium sherrisii* infection, Burkina Faso, 2012*

Patient no.	Age, y/sex	HIV status	Smear results	TB treatments	NTM treatment begun	Radiology	Outcome
1	40/F	Negative	3+	2011, 2012	October 2012	Cavitation in the right upper lobe. Bilateral apical bronchopneumonia with small pleural effusion in the right lung and basal emphysema.	Lost to follow-up
2	33/M	Negative	2+	2011, 2012	October 2012	Bilateral apical bronchopneumonia with consolidation in the middle lobe.	Died
3	57/M	Negative	2+	2012	NA	NA	Lost to follow-up
4	36/M	Negative	3+	2011, 2012	December 2012	Massive bilateral pneumonia	Died

*TB, tuberculosis; NTM, nontuberculous mycobacteria; NA, not applicable.

isolation, as required by microbiological criteria, was not possible because a second sputum sample was unavailable.

M. sherrisii is a relatively new species (*6*), closely related to *M. simiae*. Although most of the rare *M. sherrisii* infections reported since 2004 (online Technical Appendix Table 1, http://wwwnc.cdc.gov/EID/article/21/11/14-1809-Techapp1.pdf) were diagnosed in Europe or the United States, about half of the strains were isolated from patients in Africa. Because *M. sherrisii* infection probably is further underestimated by being misidentified as *M. simiae* infection by the commercially available line probe assays, the hypothesis that *M. sherrisii* infection is not so infrequent in the African setting seems therefore reasonable. In addition, the strategy recommended by World Health Organization and based on use of immunochromatographic tests (*7*), does not enable NTM identification. A leitmotiv of most *M. sherrisii* infections reported to date is HIV co-infection, which leads to dissemination of the mycobacterial disease.

This report, although it adds to the record of patients in Africa, does not support the association with HIV infection. Our findings are consistent with the view that the pathogenic potential of *M. sherrisii* is comparable to that of other well-known NTM species (e.g., MAC) responsible for disease both in HIV-positive and HIV-negative patients. The retrospective determination of the MICs of antimicrobial agents potentially active against slowly growing mycobacteria (online Technical Appendix Table 2) confirmed, for the 4 strains of *M. sherrisii*, the well-known multidrug resistance of the species (*8*). The therapeutic failure was thus not surprising because clarithromycin was the only drug among those administered during the treatment that had been shown to be active in vitro. This report provides evidence that conducting appropriate microbiological investigations is essential before initiating a treatment with second-line TB drugs (*9*).

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Histoplasmosis in HIV-Infected Persons, Yaoundé, Cameroon

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To the Editor: In HIV-infected persons in Cameroon (Central Africa), histoplasmosis is still misdiagnosed as tuberculosis because of clinical similarities (*1*,*2*). These patients are automatically given presumptive antituberculous therapy, although tuberculosis is not confirmed. The patients subsequently die of probable disseminated histoplasmosis (DH), and the fungal infection might finally be detected in postmortem tissue samples (*3*). In this context, 3 cases of DH were detected in HIV-infected patients within a 1-year period (2007–2008) in Yaoundé, Cameroon. We initiated this study to investigate the occurrence of histoplasmosis in HIV-infected patients in 4 medical centers for AIDS treatment in Yaoundé from December 2008 through December 2011.

We recruited patients with known HIV status who agreed to participate in the study. Inclusion criteria were CD4 cells <200/mm^3, fever and cough of ≥2 weeks'

duration, weight loss, asthenia, and histoplasmosis-like skin manifestations (i.e., ulcerative lesions and/or umbilicated papules or nodules and/or pustules). Patients under effective antituberculous therapy or antimicrobial drugs for any skin or pulmonary infectious disease were excluded from the study. CD4 cell counts were performed in all patients. Histoplasmosis was diagnosed in sputum, bronchoalveolar fluid (BALF), and bronchial and skin biopsies by direct staining with Gomori's methenamine silver and periodic acid Schiff stains and by culture of sputum and BALF samples on Sabouraud medium. Tuberculosis and bacterial infections were detected in sputum and BALF by using Ziehl-Neelsen and Gram staining and culture on Lowenstein-Jensen and *Streptococcus pyogenes* media. All laboratory examinations were performed at the Centre Pasteur du Cameroun in Yaoundé. Data were collected on an anonymous questionnaire. Means (and SDs) were calculated for quantitative variables, and frequencies were calculated for qualitative variables. The National Ethics Committee, the Ministry of Health of Cameroon, and the medical centers where the study took place approved the study. Patients approved and signed the informed consent form at the time of recruitment.

Our study comprised 56 patients. *Histoplasma capsulatum* was detected in 7 (13%) patients on 6 of 7 skin biopsies and 1 of 3 bronchial biopsies. The median CD4 cell count of *H. capsulatum*–positive patients was 40 cells/mm^3. Similarly, some authors have reported diagnosis of severe DH by using direct staining of skin samples (*4*); in low-income countries, skin involvement is the main presentation of DH because of limited laboratory facilities and/or late diagnosis. In Cameroon until recently, all DH cases in HIV-infected persons were diagnosed by skin biopsy or by chance on peripheral blood smear, thus revealing AIDS at the terminal stage (*3*,*5*). We did not detect *H. capsulatum* infection in sputum or BALF. These results are congruent with findings in Abidjan, Côte d'Ivoire, in 1999 (*6*). African histoplasmosis was not detected in any sample; although this type is endemic to areas with high rates of HIV infection, it is infrequently associated with AIDS patients (*7*).

We detected *Mycobacterium tuberculosis* in 18 (32%) patients and *Candida albicans* in 14 (25%) patients; 3 (0.5%) patients were co-infected with *M. tuberculosis* and *C. albicans*. *M. tuberculosis* was detected in sputum of 9 (21%) of 42 patients and in BALF of 9 (53%) of 17 patients; we detected *C. albicans* in sputum of 13 (31%) patients. Our detection of *M. tuberculosis* in 32% of patients confirms tuberculosis as the main AIDS-defining illness in Cameroon. We did not find tuberculosis and histoplasmosis co-infection, even though it occurs frequently in low-income countries (*1*,*8*).

The limitation in our study was the unavailability of validated sensitive and specific tools for diagnosing histoplasmosis in Cameroon (e.g., detection of the *H. capsulatum* circulating antigen in body fluid using an enzyme immunoassay) (*9*). Thus, using direct staining methods and culture of biopsies and body fluid samples could possibly lead to false-negative results.

Our detection of *H. capsulatum* in 13% of the HIV-infected patients in this study suggests that histoplasmosis is an unknown public health problem in Cameroon that is misdiagnosed as tuberculosis. Accounting for the endemicity of tuberculosis, which is the main HIV-defining illness in Cameroon, and the fatal outcome of DH in HIV-infected patients, practitioners need a high index of awareness to differentiate between tuberculosis and histoplasmosis. A recent report showed major clinical and biologic factors discriminating between these infections (*10*). Knowing these factors may lead practitioners to early diagnosis and treatment of histoplasmosis and in turn reduce the death rate among HIV-infected patients.

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Divergent Gemycircularvirus in HIV-Positive Blood, France

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To the Editor: Gemycircularviruses are a group of recently discovered single-stranded DNA viruses, found initially in fungi in 2010 (*1*). These "myco-like" viruses have a genome ranging from 2.1 to 2.3 kb, containing 2 opposite open reading frames that probably code for a capsid protein (CP) and a spliced replication-associated protein (Rep). Related viruses have been subsequently identified in animal blood and fecal matter, raw and treated sewage, and insects and plant material, suggesting that gemycircularviruses may represent a large group of viruses exhibiting considerable genetic diversity (*2–8*). The presence of these viruses was recently extended to humans after gemycircularvirus sequences were identified in human blood and brain tissue (multiple sclerosis patient), cerebrospinal fluid, and fecal matter (*8*,*9*).

While investigating the virome content of an HIV-positive blood donation, we identified several gemycircularvirus-related sequences. The initial metagenomic approach involved an HIV-1–positive plasma sample (B genotype, ≈530 copies/mL) obtained from the French blood agency national plasma bank in Tours, France. A 4-mL aliquot was prepared for metagenomic analysis after filtration, concentration, and nucleases treatment. Next, particle-protected nucleic acids were recovered and used for the preparation of a next-generation sequencing library and its subsequent analysis (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/21/11/15-0486-Techapp1.pdf). Gemycircularvirus sequences identified among reads (1,680 vs. 82,560 reads total; ≈2%) were assembled into a resulting full-length sequence (GemyC1c) by using CodonCode Aligner version 5.1 (CodonCode Corporation, Centerville, MA, USA). This sequence was verified by using back-to-back specific primers, and the amplicon was cloned and sequenced according to the Sanger method.

The analysis of the GemyC1c sequence (2,109 nt, GenBank accession no. KP987887) revealed a genome divergent from those already available in databases, despite a similar genomic organization (Figure, panel A) and assignment to gemycircularviruses after BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) analysis of putative CP and Rep proteins. This divergence was demonstrated by the phylogenetic analysis of the deduced CP (Figure, panel B), which exhibited ≈72% and ≈44% aa pairwise identity with the 2 closest gemycircularvirus CP sequences available in GenBank (gemycircularvirus c from mongoose feces [Conceicao-Neto N., unpub. data] and HCBI8.215 from cattle blood, respectively). Moreover, GemyC1c CP exhibited ≈30% pairwise identity with viral sequences identified previously in humans (BZ1 from feces, SL1 from cerebrospinal fluid, MSSI2.225 from blood). The deduced spliced Rep (major Rep1 and minor Rep2), seen in such viruses, contained putative rolling circle motifs I (LFTYS), II (HLHAFVD), and III (YATKD) retrieved from gemycircularviruses (*4*).

We subsequently investigated the presence of GemyC1c DNA in 128 HIV-positive plasma samples (French blood agency national plasma bank) along with 256 HIV-negative plasma samples (healthy blood donors, southeastern France; mean donor age 38 years; 136 men; 1 man:1.13 women). Plasma samples were prepared as described previously (*10*), and extracted nucleic acids were tested for GemyC1c DNA by using a specific PCR that included negative, positive, and extraction controls (online Technical Appendix).

Application of the above GemyC1c DNA detection system did not generate any positive signal in the 384 plasma samples in this study, suggesting that the presence

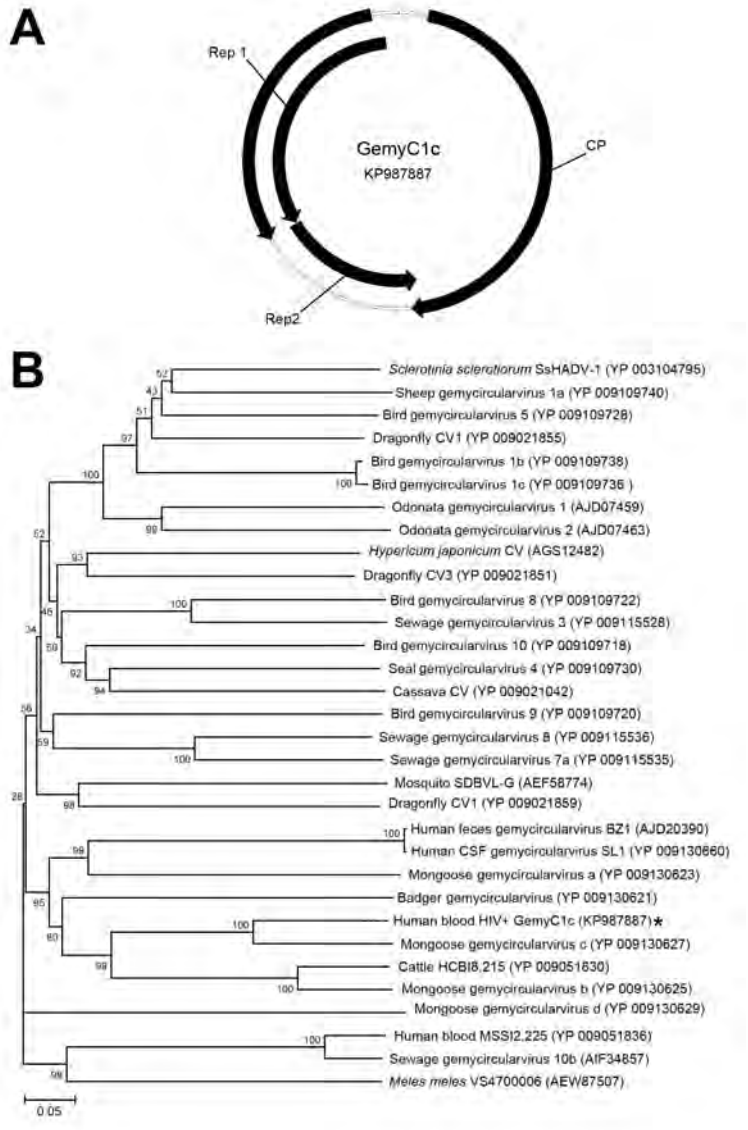

Figure. A) Predicted genomic structure of divergent gemycircularvirus (GemyC1c) isolated from HIV-positive blood, France. Arrows represent major open reading frames. Deduced capsid protein (CP) and spliced replication-associated proteins (Rep1/Rep2) are composed of 318 and 226/126 aa, respectively. B) Neighbor-joining phylogenetic tree constructed by using CP amino acid sequences of GemyC1c (asterisk) and genetically related gemycircularviruses. Bootstrap values were based on 1,000 replicates. Scale bar indicates amino acid substitutions per position.

of this virus in the blood of the populations tested was a rare occurrence. However, it is possible that other, divergent, GemyC1c-related sequences could be present in human blood but remain undetectable by the molecular assay used; the development of universal gemycircularvirus PCR systems is now expected.

Gemycircularviruses are potentially very stable in the environment. Because an unknown part of this group is able to infect fungi, possible contamination from the laboratory environment or nucleic acid extraction methods must be considered. The fact that the same genome was never identified in other libraries generated in our laboratory supports the absence of local contamination; of note, we were also able to detect GemyC1c DNA with PCR by using another plasma aliquot extracted by an alternative method (NucliSENS magnetic extraction; bioMérieux, Marcy l'Étoile, France) In addition, the systematic elimination of the first 35 mL of each blood donation, associated with filtration procedures and control of the temperature of stored plasma (–25°C), contributes to the reduction of bacterial/fungal contamination during blood collection. However, it is not possible to state that the GemyC1c sequence would belong to a human-tropic virus because an association of the virus with an unknown fungus is plausible. Thus, the presence of fungi in the gut, with fungi/virions having traversed the gut lining, or circulating in blood should be considered. Such aspects should prompt future investigations of the effective replication of gemycircularviruses in human or other mammalian cells.

Our discovery of the GemyC1c by a sequence-independent molecular approach was informative for several reasons: 1) this viral sequence would have been undetectable by PCR according to the high genetic divergence existing between GemyC1c and other gemycircularviruses

identified; 2) this finding adds clues to the identification of potential new co-infections occurring in HIV-infected persons; and 3) this finding underlines the need to investigate the virome content of blood samples in a research context of new microbes as potential threats for transfusion. Further studies aimed at exploring genetic diversity and natural history of gemycircularviruses in human hosts are needed.

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Co-infection with Drug-Susceptible and Reactivated Latent Multidrug-Resistant *Mycobacterium tuberculosis*

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To the Editor: Genotyping of *Mycobacterium tuberculosis* (MTB) has identified mixed infections involving >1 MTB strain (*1–4*), which are clinically relevant when different susceptibility patterns are involved (*4–7*). We describe a tuberculosis (TB) case-patient with mixed infection in an area of moderate incidence. In a low-resistance setting (monoresistance 4.6%; multiresistance 1.7%), 1 of the strains was drug susceptible and the other was multidrug-resistant (MDR). Molecular fingerprinting and epidemiologic research revealed that the infection corresponded to a recent infection by a susceptible strain and reactivation of an MDR TB strain. The patient was an HIV-negative woman, 47 years of age, who had immigrated to Spain from Romania and had been living in Almería for >3 years. TB was diagnosed in May 2014; she had experienced symptoms for 2 months. Her diagnosis was confirmed 3 years after being studied in Almería as a close contact of her husband, also from Romania, who had tested positive for MDR TB (resistant to rifampin and isoniazid). When she was observed in the contact trace, she tested positive for purified protein derivative, had been vaccinated against the *M. bovis* bacillus Calmette-Guérin strain, and had no radiologic findings or clinical symptoms. Based on the susceptibility profile of her husband, prophylaxis was not prescribed. Her husband adhered to anti-TB treatment for 20 months; all microbiological control test results had been negative since 2 months after starting therapy.

Because her infection was thought to originate from previous contact with an MDR TB case-patient, we assessed her sputum samples for resistance using GenoType-MTBDR-plus (Hain-Lifescience, Nehren, Rhineland-Paltinate, Germany). The test showed hybridization with the same mutant probes (*rpo*B-MUT3; *kat*G-MUT1) as those of her husband, the assumed index MDR TB case-patient (Figure, panel A). The pattern was indeterminate because the hybridization for the mutant probes was faint, and intense hybridization was observed for all the *wt* probes (Figure, panel A). Results suggested the simultaneous presence of an MDR strain and a susceptible strain in a respiratory specimen. The presence of the MDR TB strain was confirmed by the phenotypic antibiogram in a BBL MGIT mycobacteria growth indicator tube (Becton Dickinson, Franklin Lakes, NJ, USA) after the isolate had been cultured. GeneXpert (Cepheid, Sunnyvale, CA, USA) was used to analyze 2 respiratory specimens. Results indicated susceptibility to rifampin, revealing the limitations of this test: the use of probes targeting the *wt* sequences failed to detect resistant strains that coexist with a susceptible strain (*7*).

To ascertain the likelihood of 2 co-infecting strains, we analyzed the specimen and the cultured isolate by mycobacterial interspersed repetitive unit–variable number tandem repeat (MIRU-VNTR), which is highly sensitive for detecting complex infections (*8*,*9*). Double alleles were found at 12 loci (Figure, panel B), confirming co-infection.

Peaks in the electropherograms suggested that 1 of the 2 strains was under-represented and its proportion was lower in the cultured isolates (Figure, panel C), indicating that culturing diminished its representation of the minority strain. This finding was consistent with the inability of the GenoType test to detect the MDR strain when applied to the cultured isolate (Figure, panel A). We also detected lower fitness for the MDR strain compared to the susceptible strain ($p<0.01$) (online Technical Appendix Table, http://wwwnc.cdc.gov/EID/article/21/11/15-0683 Techapp1.pdf).

Proportions of the resistant and susceptible strains were determined by plating on Middlebrook 7H11 +/- isoniazid (0.4 μL/mL) and counting single colonies. Because 2% of the colonies were of the resistant strain, we separated the strains and concluded: 1) the strain cultured in the presence of an antimicrobial drug coincided in the population exclusively with the MDR strain from the husband (as shown by MIRU-VNTR); and 2) the co-infecting MDR strain was a minority strain that was under-represented in the cultured isolate.

To analyze the origin of the susceptible strain, we investigated its MIRU-VNTR type in the population-based

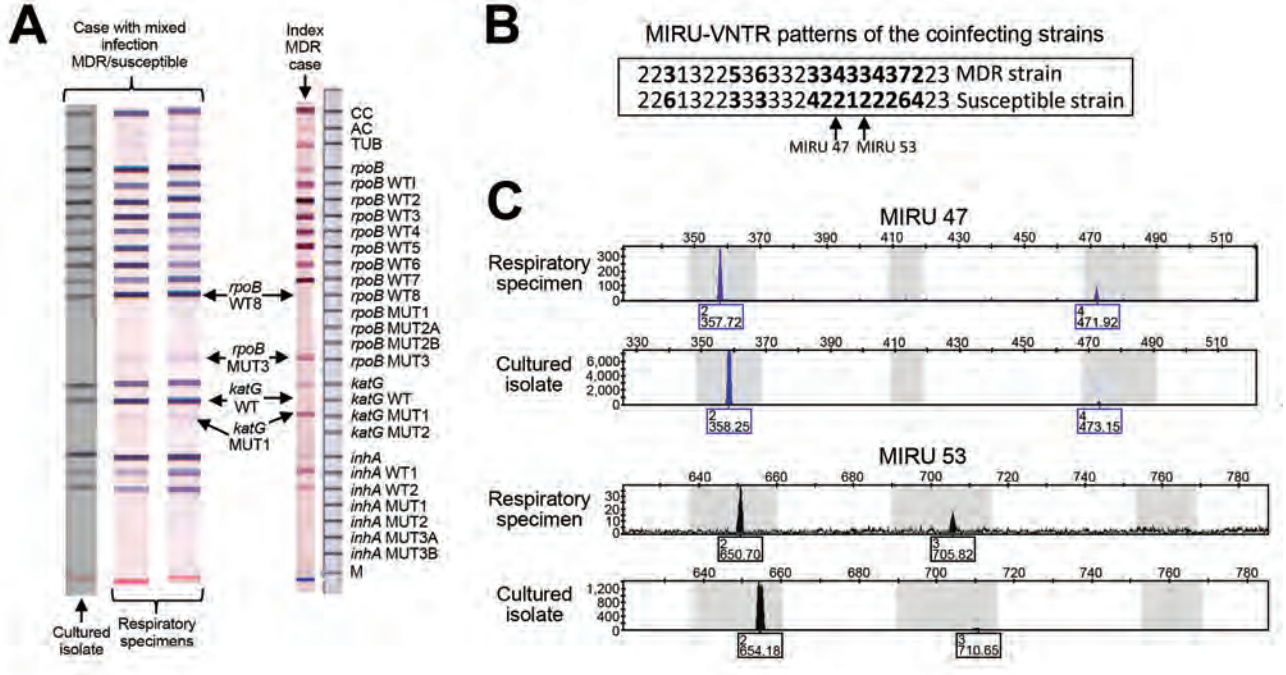

Figure. Identification of co-infection with drug-susceptible and reactivated latent multidrug-resistant Mycobacterium tuberculosis (MDR TB). A) Genotype of multidrug-resistant tuberculosis (MDR TB) and results for the MDR TB index patient and for 2 respiratory specimens and 1 cultured isolate from the case-patient who had a mixed MDR/susceptible infection. The same indeterminate intense-wt/faint mutant pattern test was repeated by using another 2 specimens. B) Mycobacterial interspersed repetitive units–variable number tandem repeat (MIRU-VNTR) types for the 2 strains involved in the co-infection. The results from the loci in which the analysis yielded double alleles are in bold text. Values for MIRU47 and 53 are indicated. C) Selection of 2 electropherograms representative of 2 (MIRU47 and 53) of the 12 loci with double alleles caused by mixed infection. Data that were obtained from direct analysis of a respiratory specimen or from a cultured isolate are shown.

molecular epidemiology survey (*10*) and found another 4 cases (from 2008, 2011 [2 cases], and 2014). Three case-patients had emigrated from Romania, and all 5 case-patients lived in the same area of Almeria. These data indicated that the susceptible strain was circulating in the geographic/epidemiological context of the current case-patient before and when she tested positive for that strain; therefore, she likely acquired the susceptible strain through recent transmission.

The presence of susceptible and resistant strains in a patient should be considered even in moderate incidence settings and where resistance rates are not high. Underdetection of these cases could lead to misinterpretation when MDR became apparent after treatment of susceptible strains. Diagnostic laboratories could easily screen for mixed infections by applying MIRU-VNTR. However, only by integrating clonal analysis, refined molecular typing, and epidemiologic data from universal genotyping programs can we clarify the reasons underlying complex MTB infections. For this case-patient, a recent infection with a susceptible strain coincided with or could have triggered reactivation of a latent infection involving an MDR strain acquired through close contact years previously. We emphasize the alteration of the true clonal complexity of an infection induced by culturing specimens and that some commercial tests do not identify complex MTB infections. These findings are particularly relevant when the infection involves resistant strains such as those found in this case-patient.

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Sensitivity to Polymyxin B in El Tor *Vibrio cholerae* O1 Strain, Kolkata, India

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To the Editor: The epidemiology of cholera, especially in Africa and Asia, has periodically changed in subtle ways (*1*). The recent cholera epidemic in Haiti, a Caribbean country with no cholera cases in decades, affected >500,000 persons, caused ≈8,000 deaths, and brought this illness to the forefront of Haitian public health concerns

(*2*,*3*). This life-threatening disease is caused by *Vibrio cholerae*, a waterborne bacterium with >200 serogroups, 2 of which, O1 and O139, cause epidemic or pandemic cholera. *V. cholerae* O1 is categorized as classical and El Tor biotypes, which differ biochemically and have different levels of virulence. Classical strains typically cause more severe illness than El Tor strains, which result in mild or moderate and sometimes asymptomatic cases. However, El Tor strains have replaced classical strains as the cause of cholera; the classical biotype is believed to be extinct, and El Tor strains currently prevail. However, the genetic traits specific to classical strains are still present in environmental and clinical *V. cholerae* isolates. Currently, all clinical strains of *V. cholerae* in Kolkata produce classical cholera toxin. Such phenotypic and genetic changes in *V. cholerae* are being monitored worldwide.

Several phenotypic and genetic laboratory tests are used to determine whether isolates are classified as classical or El Tor biotypes. Among phenotypic traits distinguishing the 2 biotypes, sensitivity to polymyxin B (50 U) is considered a reliable indicator and stable phenotype for biotyping. Research has shown that the genome of *V. cholerae* strains is undergoing cryptic changes that influence the strains' virulence, rapid transmission, and spread (*4*). Our previous findings showed El Tor strains with few biotype traits of classical strains (*5*).

Since the seventh cholera pandemic, which occurred during the 1960s and 1970s and was caused by El Tor strains, the El Tor biotype had been resistant to polymyxin B, a cationic antimicrobial peptide. However, when cholera strains first appeared in patients in Kolkata, India, in June 2012, *V. cholerae* O1 was found to be sensitive to polymyxin B

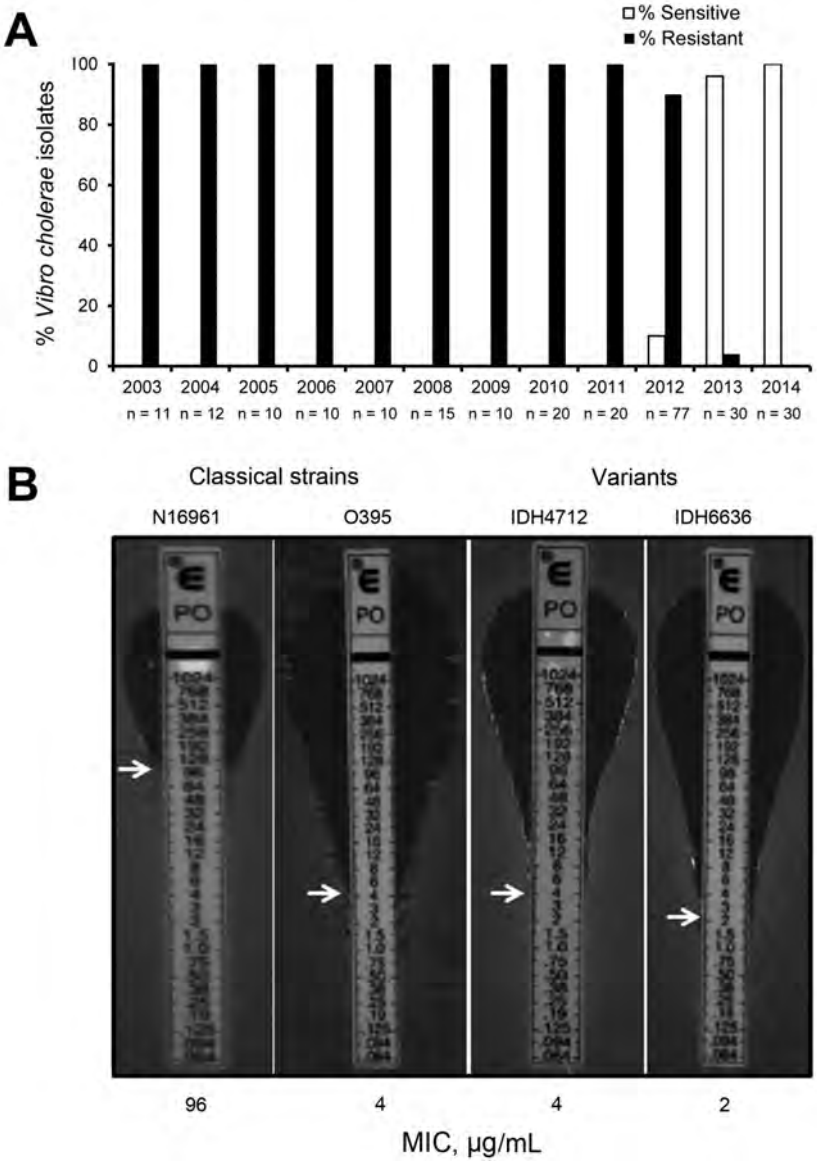

Figure. Isolation profile of polymyxin B–sensitive *Vibrio cholerae* strains in Kolkata, India, 2003–2014. A) Yearly occurrence of polymyxin B sensitivity and resistance in *V. cholerae* O1 El Tor variant strains isolated from Kolkata patients. During the study period, 255 strains were tested; n values indicate the number of strains tested each year. Polymyxin B–sensitive strains first appeared in Kolkata in June 2012. The first isolate in January 2013 was resistant, but, thereafter, all strains isolated during 2013–2014 were sensitive to polymyxin B, a biotyping marker for classical strains. B) MIC of polymyxin B in El Tor variant strains (classical and El Tor). MICs are indicated by white arrows. Polymyxin B sensitivity, a characteristic of classical strains, was displayed by El Tor variant strains. Data represent 3 biologic repetitions.

(*6*). To determine whether this phenomenon occurred earlier, we tested 255 clinical strains isolated from patients in Kolkata during 2003–2014 and found that, from March 2013, polymyxin B–sensitive El Tor strains had replaced resistant strains (Figure, panel A). The MIC of polymyxin B, determined by Etest (bioMérieux, Marcy l'Etoile, France), confirmed that the El Tor strains were susceptible to this antimicrobial drug (Figure, panel B). In this assay, the El Tor strain (N16961) was highly resistant to polymyxin B (MIC 96 μg/mL), whereas the variant strains in Kolkata showed a drastic reduction in resistance (*6,7*).

To confirm additional changes in biotype attributes in the variant Kolkata isolates during 2003–2014, we used the Voges-Proskauer test to determine production of acetylmethyl carbinol and found that the tested strains produced acetoin and were positive for chicken erythrocytes agglutination. The *rtsC* gene encoding the activator protein, which is absent from classical biotype strains but present in El Tor strains, was found in all the tested strains of the El Tor biotype. Biotype-specific CTX prophage repressor *rstR* was amplified with the El Tor–specific primers, indicating presence of El Tor *rstR*. The *tcpA* gene has distinct alleles specific to classical and El Tor biotypes of O1. Our study showed that all strains yielded amplicons with the El Tor-*tcpA*–specific primers but not with the classical-*tcpA*–specific primers. However, these strains had a single-base substitution at the 266-nt position of *tcpA*, also present in variant strains from Haiti. Furthermore, *Vibrio* seventh pandemic (VSP) gene clusters VSP I and VSP II are unique to El Tor strains of the seventh pandemic. We found presence of VSP I and II encoding genes in all our tested strains, indicating that the strains are El Tor, but with specific classical traits.

We also checked the strains' sensitivity to many antimicrobial drugs: tetracycline, trimethoprim/sulfamethoxazole, streptomycin, erythromycin, gentamicin, ciprofloxacin, and azithromycin, and all strains were sensitive to all drugs except trimethoprim/sulfamethoxazole and streptomycin. All strains isolated during 2013–2014 were fully resistant to trimethoprim/sulfamethoxazole and streptomycin, but 55% of strains isolated before 2012 were sensitive to these drugs.

Genes encoding lipid IVA acyltrasferase (*msbB*), biofilm formation, antimicrobial peptide resistance (*carR*), and 3 aminoacyl lipid modification (*almEFG*) have been shown to contribute to polymyxin resistance in *V. cholerae* (*6–8*). Analysis of these genes from the newly emerged polymyxin-B–sensitive strains may provide additional useful information. We found that these strains contained Haitian variant *ctxB* (*ctxB7*) similar to the classical cholera toxin. Our earlier studies identified many new attributes of Haitian *V. cholerae* variant strains in Kolkata since 2003 (*9,10*).

We report the emergence of El Tor strains producing classical cholera toxin. These strains have lost an El Tor biotype marker and acquired a vital classical biotype characteristic, a change that has probably altered the regulatory mechanisms of lipid A modification machinery in *V. cholerae* (*6–8*). This change is a major event in the history of cholera after 1961, when El Tor strains first appeared. The recent changes in *V. cholerae* O1 strains should be carefully monitored to determine their clinical and epidemiologic implications.

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G2P[4]-RotaTeq Reassortant Rotavirus in Vaccinated Child, United States

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To the Editor: Group A rotaviruses (RVAs) are a leading cause of acute gastroenteritis-associated deaths among children <5 years of age in developing countries (*1*). The genome of RVA consists of 11 double-stranded RNA segments that code for 11 or 12 viral proteins (VP1–VP4, VP6, VP7, nonstructural protein 1 [NSP1]–NSP5/6) (*2*). In 2008, the Rotavirus Classification Working Group established a system of extended classification that was based on the sequences of all 11 gene segments and used the notations Gx-P[x]-Ix-Rx-Cx-Mx-Ax-Nx-Tx-Ex-Hx for the genes VP7, VP4, VP6, VP1–VP3, NSP1–NSP5, respectively (*3*). Similar to other RNA viruses, RVAs show high genomic diversity, which is generated primarily through point mutations, reassortment, rearrangement, and recombination events.

In 2006 and 2008, two live-attenuated vaccines, RotaTeq (Merck, Whitehouse Station, NJ, USA) and Rotarix (GlaxoSmithKline, Rixensart, Belgium), respectively, were introduced in the United States (*4*). RotaTeq is a pentavalent human bovine reassortant vaccine that contains 4 G types (G1, G2, G3, and G4; VP7 gene) plus the P[8] VP4 type on a bovine WC3 (G6P[5]) backbone (*5*). In 2012, Bucardo et al. reported finding a vaccine-derived nonstructural protein 2 (NSP2) gene in 2 wild-type RVA strains with a G1P[8] genogroup 1 backbone (*6*). Each of these strains had been found during routine surveillance in Nicaragua, where RotaTeq was introduced in 2006, suggesting reassortment of the vaccine strain with circulating wild-type strains. The authors also examined alignments of the NSP2 gene and found no differences at functional domains between the vaccine-derived NSP2 and the circulating wild-type NSP2 (*6*). This finding could explain why a vaccine-derived NSP2 reassortant was viable.

During the 2010–11 surveillance season, the New Vaccine Surveillance Network identified an RVA strain, RVA/human-wt/USA/2011729115/2011/G2P[4] (2011729115), which also contained a vaccine-derived NSP2 gene. This specimen was obtained from a 4-year-old child through routine active surveillance in the emergency department at Texas Children's Hospital (Houston, TX, USA). RVA double-stranded RNA was extracted from a fecal sample from the child by using Trizol reagent (Life Technologies, Grand Island, NY, USA). The sequencing templates were prepared by using sequence-independent whole-genome reverse transcription PCR amplification (*7*) with slight modifications. PCR amplicons were sequenced by the Illumina Miseq 150 paired-end method at the Genomics Laboratory, Hudson Alpha Institute for Biotechnology (Huntsville, AL, USA). Illumina sequence reads were analyzed by using CLC Genomics Workbench 6.0. (http://www.clcbio.com/products/clc-genomics-workbench/). A combination of de novo assembly followed by mapping to a G2P[4] reference strain was used to obtain the full-length genome of strain 2011729115. The sequences were submitted to GenBank under accession nos. KR701624–KR701634. Genotype assignment for each gene was performed by using a combination of BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and RotaC 2.0 (http://rotac.regatools.be/). For each gene, multiple alignments were made by using the MUSCLE algorithm implemented in MEGA 5.1 (*8*). Maximum-likelihood trees were constructed for each gene in PhyML 3.0 (http://www.atgc-montpellier.fr/phyml/) by using the optimal model for each alignment as identified by jModeltest 2 (TrN+I) and approximate-likelihood ratio test (aLRT) statistics computed for branch support (*9*,*10*).

The full genotype constellation for strain 2011729115 is G2-P[4]-I2-R2-C2-M2-A2-N2-T2-E2-H2, which is a DS-1 like constellation. The G2P[4] strains from the 2010–11 season shared 97.6%–100% nt identity for the NSP2 gene, except for strain 2011729115, which exhibited only 86.8%–87.3% nt identity with the other circulating G2P[4] strains. BLAST analysis of sequences deposited in GenBank indicated that the 2011729115 NSP2 gene was 100% identical to published RotaTeq NSP2 gene sequences and clustered with the 5 RotaTeq NSP2 sequences in phylogenetic analysis (Figure). The NSP2 protein functions as a single-stranded RNA binding protein, nucleic acid helix destabilizer, nucleoside triphosphatase (NTP), nucleoside diphosphate kinase (NDP), and RNA triphosphatase (RTPase) (*2*). No amino acid differences in the RNA binding and NDP/NTP/RTPase domains were found between the NSP2 gene from strain 2011729115 and the G2P[4] strains (data not shown), suggesting that this vaccine-derived NSP2 protein can function like the wild-type version.

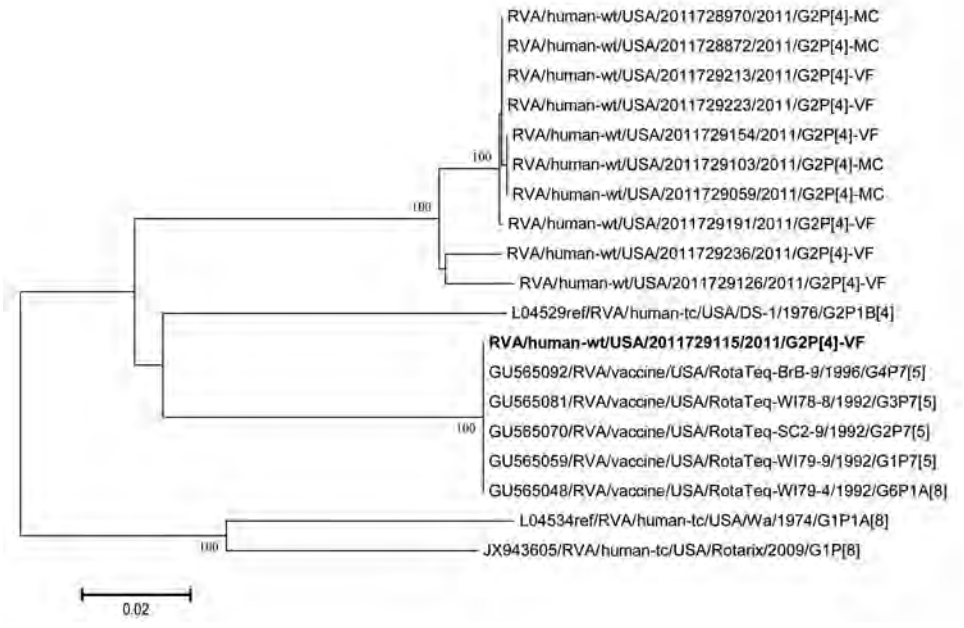

Figure. Maximum-likelihood tree for the rotavirus (RVA) nonstructural protein 2 (NSP2) gene showing phylogenetic clustering with wild-type G2P[4] strains identified during the 2010–11 season, United States, and RotaTeq (Merck, Whitehouse Station, NJ, USA) vaccine strains. The tree was created by using MEGA 5.1 (*8*). Approximate-likelihood ratio test values >70% are shown next to supported nodes. Boldface indicates strain 2011729115. Scale bar indicates number of nucleotide substitutions per site.

In conclusion, we identified an NSP2 gene RotaTeq reassortant in an RVA with a DS-1 like genotype. Although the child from whom the NSP2 reassortment specimen was obtained had been vaccinated with a complete regimen of 3 doses of RotaTeq in 2007, no other RotaTeq genes could be detected in the fecal sample. This finding could suggest a reassortment event and infection independent of the vaccination in 2007, although precisely where this reassortment event took place is difficult to ascertain. The existence of a viable NSP2 wild-type reassortant was also proposed by Bucardo et al. (*6*). Whether the RotaTeq NSP2 gene provides any fitness advantage to the virus during infection of vaccinated children also is unclear; during the 2010–11 season, other cases of G2P[4]-associated gastroenteritis in vaccinated children, in which the RotaTeq NSP2 gene was lacking, were reported. Our finding of strain RVA/human-wt/USA/2011729115/2011/G2P[4], a wild-type RVA with a vaccine-derived NSP2 gene, in a child with acute gastroenteritis in the United States highlights the need for continued rotavirus surveillance, domestically and internationally, after vaccine introduction.

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Multidrug-Resistant Tuberculosis in Child Successfully Treated with 9-Month Drug Regimen

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To the Editor: Approximately 480,000 persons acquired multidrug-resistant tuberculosis (MDR TB) in 2013 (*1*). Of the 32,000 children who acquire MDR TB annually, few are identified and administered appropriate treatment (*2*). World Health Organization (WHO)–recommended treatment for MDR TB lasts 20–24 months, including 8 months of daily drug injections (*3*). Because many children have paucibacillary TB, a shorter protocol may be sufficient, especially for early or nonsevere disease.

In Bangladesh, 87% of MDR TB patients who received a 9-month regimen had a favorable outcome; compared with patients who received the WHO regimen, fewer had adverse events or were lost to follow-up (*4*,*5*). A randomized controlled trial of this regimen is ongoing (*6*); however, the trial excludes children, and no detailed data are available for use of this regimen in children. The regimen is being implemented in several countries under operational research conditions (*7*).

Along with the Ministry of Health of Uzbekistan, and in accordance with WHO advice (*8*), Médecins Sans Frontières investigated the efficacy, tolerability, and safety of the shortened regimen in Karakalpakstan, Uzbekistan (*9*), where rates of second-line drug resistance are high (*1*) and *katG*-mediated isoniazid resistance predominates. Unlike the Bangladesh study (*4*), the Karakalpakstan study used moxifloxacin instead of gatifloxacin and included scheduled electrocardiograms (ECGs) and graded assessments of side effects to monitor for safety, including cardiac toxicity. All drugs in the regimen were previously used safely in children (*10*). For children, limited data are available regarding use of 2 new TB drugs, bedaquiline and delamanid; thus, the shortened regimen could represent the best opportunity to improve their outcomes and access to treatment. We report the successful treatment of MDR TB in a child who received the 9-month drug regimen. This retrospective research fulfilled Médecins Sans Frontières Ethics Review Board criteria for analysis of existing program data. Written informed consent was provided by the child and his parents.

In November 2013, a 14-year-old boy in Karakalpakstan received a diagnosis of pulmonary MDR TB after seeking medical care for a sore throat without cough, fever, weight loss, or major concurrent conditions. His mother (a close contact) had experienced symptoms of pulmonary TB since 2011 and, after a period of self-treatment, received a diagnosis of MDR TB with confirmed absence of preextensively or extensively drug-resistant TB; she completed appropriate treatment in September 2013. In accordance with national guidelines, the boy did not receive treatment for latent TB.

Clinical examination of the boy (weight 43 kg, body mass index 17.2 kg/m^2) was unremarkable and showed no signs of extrapulmonary disease. A chest radiograph showed a left midzone interstitial infiltrate. Sputum sample testing (Xpert MTB/RIF; Cepheid, Sunnyvale, CA, USA) confirmed rifampin-resistant *Mycobacterium tuberculosis*. Sputum smear microscopy and liquid-based culture (BACTEC MGIT 960; Becton Dickinson, Franklin Lakes, NJ, USA) were negative. Baseline biochemical, hematologic, and ECG results were within normal limits. Serologic test results were negative for HIV and hepatitis B and C viruses.

Together, a history consistent with TB disease, radiographic evidence, and molecular testing results were considered sufficient indication for treatment of MDR TB. After psychosocial counseling and health education sessions, the boy, with his family's agreement, consented to daily outpatient treatment with isoniazid (400 mg), ethambutol (800 mg), pyrazinamide (1,600 mg), prothionamide (500 mg), moxifloxacin (400 mg), capreomycin (750 mg), and clofazimine (100 mg) beginning in December 2013.

Treatment initiation was complicated by drug-associated nausea and vomiting, headache, tinnitus, and abdominal pain. Despite early aggressive management in line with study protocols, occasional vomiting continued. Intensive counseling ensured good adherence; only 3 days were missed. Corrected QT prolongation was excluded by use of ECG monitoring during treatment initiation.

After 4 months of treatment, the boy's sputum smear microscopy and culture results remained negative, so the continuation phase of treatment was initiated. The daily regimen consisted of ethambutol (800 mg), pyrazinamide (1,600 mg), prothionamide (500 mg), moxifloxacin (400 mg), and clofazimine (100 mg).

In May 2014, after 6 months of treatment, the boy returned to school while still receiving treatment. In August 2014, the regimen was completed without incident, and at a 6-month follow-up, the boy had not experienced a relapse.

According to study protocol, he will be followed for 1 year posttreatment to monitor for relapse.

The shortened treatment regimen has several potential benefits for children. The shorter treatment period enables an earlier return to school and social activities, the shorter duration of anti-TB injectable drug use may lessen ototoxicity, and fewer adverse effects and shorter duration could improve treatment adherence.

The reluctance to include children in TB research studies may result from difficulties in confirming a diagnosis (due to paucibacillary disease and difficulty in obtaining specimens); such confirmation is often a prerequisite for treatment. Other barriers include lack of second-line TB drug formulations and pharmacokinetic data for children, ethics review issues, and informed and parental consent issues. Clinicians and TB program managers could consider the 9-month treatment regimen for children. We advocate inclusion of children of all ages in research investigating the efficacy and safety of a 9-month regimen and emphasize the importance of separately reporting data for children.

Acknowledgment

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Human Infection with *Sporolactobacillus laevolacticus*, Marseille, France

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To the Editor: *Sporolactobacillus laevolacticus*, formerly known as *Bacillus laevolacticus*, is a gram-positive, acid-tolerant, catalase-positive, facultatively anaerobic and mesophilic bacteria initially isolated from the rhizosphere of wild plants (*1*,*2*). However, there have been no reports of its isolation from humans. We report *S. laevolacticus* associated with a wound infection and cellulitis in a patient hospitalized in Marseille, France.

In March 2015, a 47-year-old man with no underlying disease was admitted to the emergency unit of the North Hospital in Marseille, France. He had an infected wound on his right foot that occurred after he jogged barefoot during a vacation in Comoros, but the patient did not know how he obtained the wound and had not taken any antiinflammatory drugs. The foot became swollen, red, hot, and painful. He visited a doctor during his vacation and was prescribed antiinflammatory drugs and antimicrobial drugs, including a second-generation cephalosporin and ofloxacin.

The patient returned to Marseille, but the infection persisted. At admission, the patient was apyretic but had high levels of C-reactive protein (85.7 mg/L [reference range 1–3 mg/L]) and fibrinogen (8.35 g/L), which indicated inflammation. His leukocyte count was normal (9.29 $\times 10^9$ cells/L) but his procalcitonin level (0.19 µg/L) was increased, which suggested that the infection had not been

[1]These authors contributed equally to this article.

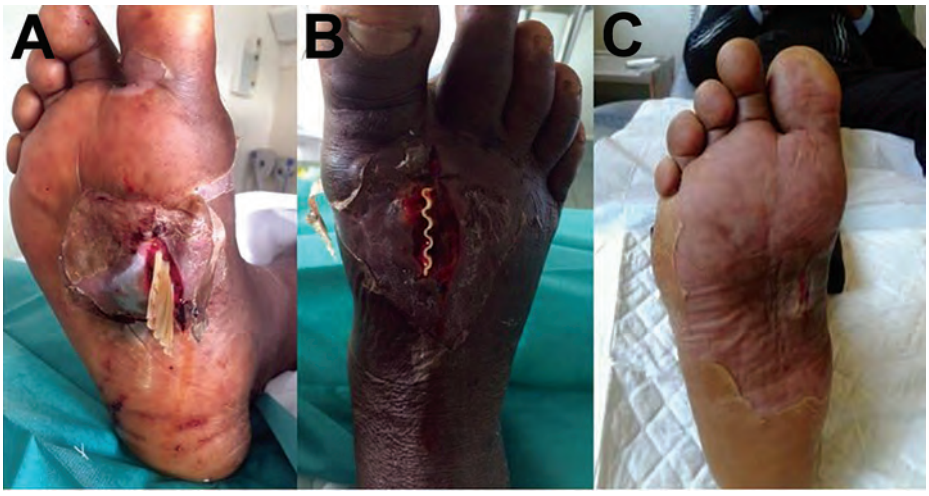

Figure. A) Foot of a 47-year-old man showing wound infected with *Sporolactobacillus laevolaticus*, Marseille, France. B) Drainage of a cellulitis abscess. C) Extent to which the wound on the arch of the foot had healed 6 weeks after surgery and antimicrobial drug therapy. A color version of this figure is available online (http://wwwnc.cdc.gov/EID/article/21/11/15-1197-F1.htm).

cured. A cellulitis abscess was suspected, and surgical cleaning and drainage was performed on March 10 (Figure, panels A, B).

Samples were collected during surgery and probabilistic antimicrobial drug therapy, including tazocillin, clindamycin, and vancomycin, was initiated. Abscess puncture liquid collected during surgery was sterile when incubated directly on Columbia and Polyvitex agar plates (bioMérieux, Craponne, France). However, a surgical sample inoculated into a blood culture bottle grew gram-positive bacilli after 4 days.

Subculture colonies were identified by using matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry (Bruker, Leipzig, Germany) as *S. laevolaticus* (score 1.88). Identification was confirmed by PCR amplification of the 16S RNA gene (*3*). A 944-bp sequence showed 99.5% similarity with that of a known *S. laevolaticus* strain (GenBank accession no. AB362648) by BLAST analysis (http://www.ncbi.nlm.nih.gov).

The *S. laevolaticus* strain was susceptible to amoxicillin, amoxicillin/clavulanate, imipenem, metronidazole, clindamycin, and vancomycin. The antimicrobial drug regimen was then changed to clindamycin and trimethoprim/sulfamethoxazole, and the patient showed an excellent clinical outcome. The patient was considered clinically cured 7 weeks later (Figure, panel C).

S. laevolacticus has been studied for its capacity to survive extreme conditions and for its fermentation system (*4*–*8*). The fact that the bacterium has not been previously isolated from humans might be because it was isolated only from plant rhizospheres (*2*), so human studies have not been conducted. In addition, conventional identification methods, such as the VITEK 2 system (bioMérieux) or the API system (bioMérieux), cannot identify *S. laevolacticus*. Since September 2009, we have used MALDI-TOF mass spectrometry in North Hospital for routine identification of bacterial species isolated from clinical samples (*9*). This strategy increases our capacity to detect rare bacterial species, including emerging pathogens (*10*).

The bacterial species was accurately identified by using MALDI-TOF and then confirmed by using a 16S RNA PCR. Because the bacterium was originally isolated from a plant rhizosphere and the patient was hospitalized with an open wound in the foot and bacteremia, we speculate that the infection was the direct result of close extended contact between the wound and soil infected with the bacteria. This case confirms that *S. laevolaticus* can be responsible for human infections and suggests that this bacterial species could be an emerging opportunistic pathogen responsible for human infections.

Acknowledgment

We thank TradOnline (http://www.tradonline.fr/en/) for providing English corrections.

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The Fantastic Laboratory of Dr. Weigl: How Two Brave Scientists Battled Typhus and Sabotaged the Nazis

By **Arthur Allen,** W.W. Norton & Company, New York, New York, USA, 2014; ISBN: 978-0-393-08101-5; Pages 384; Price US $26.95

Allen's narrative is set in eastern Europe just before and during the Second World War. The subject is typhus and 2 microbiologists who were engaged frenetically in producing vaccines against it.

The lives of the microbiologists—Rudolf Weigl, a German zoologist, and Ludwig Fleck, a Jewish physician—intersected in Lwow, eastern Poland. When the First World War broke, Weigl, stationed nearby to lead a laboratory to control typhus among Russian prisoners of war, recruited Fleck, then a student.

Rickettsia prowazekii had just been identified as the agent of typhus. Its propagation required that lice feed on infected humans. Reckoning that the rectal lining of the louse is chitinous and so can withstand mechanical trauma, Weigl inoculated lice anally with these rickettsiae. The experiment worked. When he inadvertently jabbed his hand with contaminated glassware and fever later arose, he had his wife place lice on him to feed. After he recovered, Weigl inoculated, successfully, rickettsiae from the lice that had fed on him to other lice. Breakthrough: propagating *R. prowazekii* without using humans was now possible.

After the Bolshevik revolution, Weigl returned to Lwow to produce vaccine by attenuating *R. prowazekii* through passage in lice and animals. To scale up vaccine production, Weigl resumed using humans to feed lice. With as many as 40 cages strapped to the leg, a person can feed 25,000 lice a month.

Fleck, too, returned to Lwow. When German troops took over this area, thousands of Jews were dispossessed and killed. Fleck's discovery of rickettsial antigens in urine—which could be put to diagnostic use—saved his life. Retained to direct the sanitation laboratory, he contemplated using the antigens as vaccine, but that proved to be a nonstarter: to inoculate Aryans with an excretal product of diseased non-Aryans was unconscionable to his Nazi controllers.

The typhus menace continued to beset German-occupied Europe. Weigl, imposed upon to scale up his vaccine for the troops, expanded the number of louse feeders. Being a feeder became a much sought-after occupation, and louse feeding became a cover for underground operations.

Fleck was transferred to Buchenwald, where the Gestapo was meting out the now infamous atrocities to its inmates, and where vaccine production from *R. prowazeckii* harvested from rabbit lungs was to begin. His laboratory staff were an incompetent lot, unable to isolate, let alone identify, rickettsiae. Nonetheless, Fleck considered that production of a fake vaccine might be an effective way to undermine the Nazi war effort. The scam operation went ahead, and useless vials of rabbit-lung extracts were delivered to the front. The ruse was never discovered.

After liberation, the Soviets occupied Poland. Weigl, duly taking up an academic position, soon faced a contretemps: an apparatchik of mediocre accomplishments denounced him as a Nazi collaborator. Fleck's ending was more salubrious. Assuming a microbiology professorship, then testifying in the Nuremberg trials, he finally emigrated to Israel.

Allen's narrative is well documented, written rivetingly for general readers. They will assimilate, become reminded of, and appreciate how acts of human depravity can generate devastations of catastrophic proportions. Of comfort is that other human actions —the noble ones— can go some way to contain, mitigate, and repair the damages inflicted.

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ABOUT THE COVER

Unknown (contemporary). Cotton canvas, pastel, acrylic, ink. 39 × 59 in/99.06 × 149.86 cm.
Parque dos Continuadores, Maputo, Mozambique. Personal Collection, Philip Lederer, USA. Photograph by David Swerdlow.

Celebrating the Fabric of Commonplace Society

Byron Breedlove and Nkuchia M. M'ikanatha

Batik is an ancient creative art that uses wax and dye to decorate cloth. Although evidence of this art has been documented in many parts of the world, probably its origin is in the Island of Java, Indonesia. The word batik is derived from the Indonesian word *ambatik*, which means a cloth with little dots. Batik involves brushing or drawing molten wax onto designated areas of a cloth so that those areas can then resist coloration. Next, the cloth is dyed and the waxed sections retain their original color. After drying the cloth, typically by sunlight, the artist repeats this process by waxing, dyeing, and drying to create more intricate and colorful designs. In the last step, the artist applies the final dye and removes the wax, yielding a beautifully decorated cloth (batik) that may be displayed, used, or worn.

Although contemporary batik draws from this traditional process, it has changed in several ways. Today, batik artists use other forms of dyeing, different tools, and new recipes for the wax; they also use an expanded range of materials, including silk, cotton, wool, leather, paper, wood, and ceramics. Moreover, cultural influences on batik patterns and motifs are noticeable in many parts of world, including communities in Asia and Africa. In 2009, UNESCO designated handcrafted batik an intangible

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cultural value passed down for generations. That designation symbolizes not only our shared creativity but also our diversity—representing what connects humanity to the past, present, and future. The untitled contemporary batik displayed on this month's cover came from a street market in Maputo, Mozambique, in April 2012. In Maputo and other cities in Africa, including Freetown in Sierra Leone, batiks are sold on street corners; often the artist, including the creator of this month's cover, is unknown.

Mozambican batiks frequently express daily scenes with striking colors—that in this example draw you to elongated figures, dazzling coloration, flattened perspective, and a celebratory occasion, in time of tranquility. Festooned in an array of bright color, the villagers conveying various foods converge toward a pair of figures, one hovering over a large blue mortar, the other holding a long pole. The shorter figures to the right may be kneeling in reverence; the standing figures all appear to be on their tiptoes—perhaps it has recently rained. There is no infusion of allegory, certainly no irony, though there may be intended subtleties that convey cultural messages in the choice of colors and patterns.

Because of their universal recognition, batiks can represent commonplace life, culture, and society, yet they also highlight the many bright moments that stand out from the daily rigors, chores, and routines. These striking clothes literally embody the fabric of everyday life, which at times succumbs to natural and anthropogenic forces. Thus, batiks serve as metaphors for what is lost in a population overpowered by natural or other emerging threats—this undoubtedly happened during the Ebola epidemic in West Africa. Joanne Liu, International President of Médecins Sans Frontières, stated that when she first arrived in West Africa in 2014, Ebola "was destroying families and ripping apart the very fabric of society, while national authorities and a handful of aid organisations desperately struggled against this unrelenting, invisible foe."

The World Health Organization has documented more than 28,000 reported cases and more than 11,000 reported deaths attributed to Ebola. According to the United Nations Development Group, "West Africa as a whole may lose an average of at least US$3.6 billion per year between 2014 and 2017, due to a decrease in trade, closing of borders, flight cancellations and reduced Foreign Direct Investment and tourism activity, fueled by stigma." The close connection among African nations means that the Ebola crisis also affects those countries in which disease incidence was low or zero. Countries beyond Africa monitored international travelers in addition to other measures implemented in response to the Ebola epidemic, demonstrating that infectious diseases are not contained by borders or geography.

Through a concerted effort, the fabric of society in West Africa is in the process of being repaired, bringing back stability and normalcy. The possibility of a vaccine for Ebola tantalizes the world while attention to global health security has been reignited. The colorful batik on this month's cover reminds us to keep celebrating the quotidian.

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EMERGING INFECTIOUS DISEASES®

Upcoming Issue

- Status of Stored Rinderpest Virus, 2013–2015
- Opportunistic Pulmonary *Bordetella hinzii* Infection after Avian Exposure
- Zoonotic Leprosy in the Southeastern United States
- Infection Risk for Persons Exposed to Highly Pathogenic Avian Influenza A H5 Virus–Infected Birds, United States, December 2014–March 2015
- Influenza A(H6N1) Virus in Dogs, Taiwan
- Asymptomatic MERS-CoV Infection in Humans Possibly Linked to Infected Camels Imported from Oman to United Arab Emirates, May 2015
- Hendra Virus Infection in Dog, Australia, 2013
- Replication Capacity of H9N2 Influenza Virus in Pet Birds and Mammals, Bangladesh
- Severe Leptospirosis in Martinique, 2010–2013
- Vectorborne Transmission of *Leishmania infantum* from Hounds, United States
- Kinetics of Serologic Responses to MERS Coronavirus Infection in Humans, South Korea
- Pyrethroid- and DDT-Resistant, Organophosphate-Susceptible *Anopheles* Mosquito Species, Western Kenya
- Association between Human Q Fever and Animals, Taiwan, 2004–2012
- Methicillin-Resistant *Staphylococcus aureus* Prevalence among Captive Chimpanzees, Texas, USA, 2012
- Oropharyngeal Tularemia Outbreak Associated with Drinking Contaminated Tap Water, Turkey, July–September 2013
- Novel *Waddlia cocoyoc* Intracellular Bacterium from *Artibeus intermedius* Fruit Bats, Mexico
- Epidemic Diarrheal Virus among Farmed Pigs, Ukraine
- Tembusu-Related Flavivirus in Ducks, Thailand
- Life-Threatening Sochi Virus Infections, Russia
- Increased Number of Human Cases of Influenza Virus A(H5N1) Infection, Egypt, 2014–15

Complete list of articles in the December issue at http://www.cdc.gov/eid/upcoming.htm

Upcoming Infectious Disease Activities

October 31–November 4, 2015
APHA
American Public Health Association 143rd Annual Meeting and Expo
Chicago, IL, USA
http://apha.org/events-and-meetings/annual

December 6–9, 2015
2015 National HIV Prevention Conference
Atlanta, GA, USA
http://www.cdc.gov/nhpc/index.html

February 8–10, 2016
ASM Biodefense and Emerging Diseases Research Meeting
Arlington, VA, USA
biodefense@asmusa.org
http://www.asmbiodefense.org/

February 22–25, 2016
CROI
Conference on Retroviruses and Opportunistic Infections
Boston, MA, USA
http://www.croiconference.org/

March 2–5, 2016
ISID
17th International Congress on Infectious Diseases
Hyderabad, India
http://www.isid.org/icid/

November 4–7, 2016
International Meeting on Emerging Diseases and Surveillance
Vienna, Austria
http://imed.isid.org/

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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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 75% passing score) and earn continuing medical education (CME) credit, please go to http://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 "Register" link on the right 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/about-ama/awards/ama-physicians-recognition-award.page. 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

Neurologic Disorders in Immunocompetent Patients with Autochthonous Acute Hepatitis E

CME Questions

1. Your patient is a 52-year-old man with an acute onset of severe shoulder pain and weakness and moderate elevation in transaminase levels. According to the retrospective case series by Blasco-Perrin and colleagues, which of the following statements about the overall aspects of neurologic disorders occurring in immunocompetent patients infected with hepatitis E virus (HEV) is correct?

A. The 4 syndromes reported were mononeuritis multiplex, Parsonage-Turner syndrome (PTS), ischemic stroke, and hemorrhagic stroke
B. Ten of the 15 patients had overt hepatitis with jaundice
C. All of the patients had complete, spontaneous resolution of their neurologic symptoms without HEV treatment
D. Patients with acute neurologic manifestations and abnormalities in transaminase levels should be screened for HEV

2. According to the retrospective case series by Blasco-Perrin and colleagues, which of the following statements about mononeuritis multiplex occurring in immunocompetent patients infected with HEV is correct?

A. Two of the 15 patients had mononeuritis multiplex
B. This condition is characterized by asymmetric, asynchronous, and painful segmental-nerve involvement
C. Nerve biopsy result proved that this was a vasculitic process
D. All of the patients had neurologic sequelae at last follow-up

3. According to the retrospective case series by Blasco-Perrin and colleagues, which of the following statements about PTS occurring in immunocompetent patients infected with HEV is correct?

A. PTS, also called brachial neuritis or neuralgic amyotrophy, occurred in 4 of the 15 patients
B. PTS is a common condition associated with sudden, acute, severe elbow pain followed by severe amyotrophy
C. All of the patients with PTS had neurologic resolution by the last follow-up
D. This condition is known to be caused only by direct HEV infection of the affected nerve(s)

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

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 75% passing score) and earn continuing medical education (CME) credit, please go to http://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 "Register" link on the right 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/about-ama/awards/ama-physicians-recognition-award.page. 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

Uncommon *Candida* Species Fungemia among Cancer Patients, Houston, Texas, USA

CME Questions

1. You are seeing a neutropenic 65-year-old woman admitted to the hospital for fever while receiving chemotherapy for acute lymphocytic leukemia. Her initial blood culture results demonstrate growth of a *Candida* species. Which of the following *Candida* species was most prevalent among uncommon *Candida* species causing bloodstream infections in the current study?

A. *C. famata*
B. *C. dubliniensis*
C. *C. lusitaniae*
D. *C. guilliermondii*

2. Which of the following trends was noted among cases of uncommon *Candida* bloodstream infections in the current study?

A. A minority of patients had hematologic malignant diseases
B. The proportion of cases of candidemia resulting from uncommon *Candida* species increased from 1998 to 2013
C. The incidence of infection with *C. guilliermondii* increased more than any other species from 2006 to 2013
D. There was no relationship between infections with uncommon *Candida* species and the use of echinocandins

3. The patient had been treated with an echinocandin as prophylaxis. Which of the following statements regarding breakthrough fungemia in the current study is most accurate?

A. Fungemia was very rare among patients treated with antifungal prophylaxis
B. Most cases of breakthrough fungemia were among patients taking an azole
C. The most common species causing breakthrough fungemia was *C. lusitaniae*
D. Breakthrough fungemia was associated with higher rates of admission to the intensive care unit and higher mortality rates

4. The patient experiences severe complications associated with this infection. Which of the following variables is most significant as a risk factor for 28-day mortality in the current study?

A. Persistent neutropenia
B. Age older than 60 years
C. Infection with *C. guilliermondii* in particular
D. Failure to remove a central venous catheter

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.

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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.

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.

Commentaries. Thoughtful discussions (500–1,000 words) of current topics. Commentaries may contain references (not to exceed 15) but no abstract, figures, or tables. Include biographical sketch.

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 (500–1,000 words) are published online only. They should be submitted no later than 6 months after the conference and focus on content rather than process. 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.

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.

Etymologia. Etymologia (100 words, 5 references). We welcome thoroughly researched derivations of emerging disease terms. Historical and other context could be included.

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.

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