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Tuberculosis

March 2016

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La muerte de Girardot en Bárbula
(*The death of Girardot in Bárbula*), 1883.

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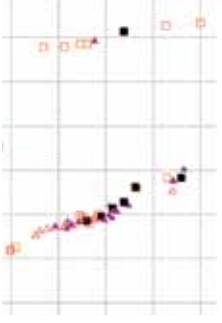
About the Cover p. 573

Perspective



p. 384

Synopses

p. 390

Research

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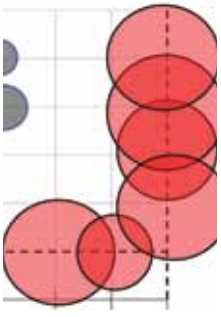
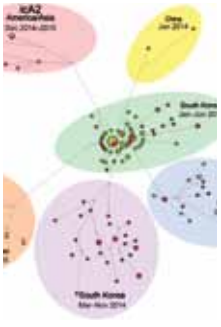


p. 505

p. 509



Dispatches

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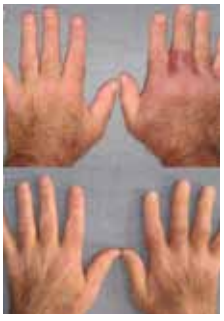

p. 560

p. 568

Letters

About the Cover

Online Reports

Global Introduction of New MDR TB Drugs—Balancing Regulation with Urgent Patient Needs
http://dx.doi.org/10.3201/eid2203.151228

Global Progress and Challenges in Implementing New Medications for Treating Multidrug-Resistant Tuberculosis
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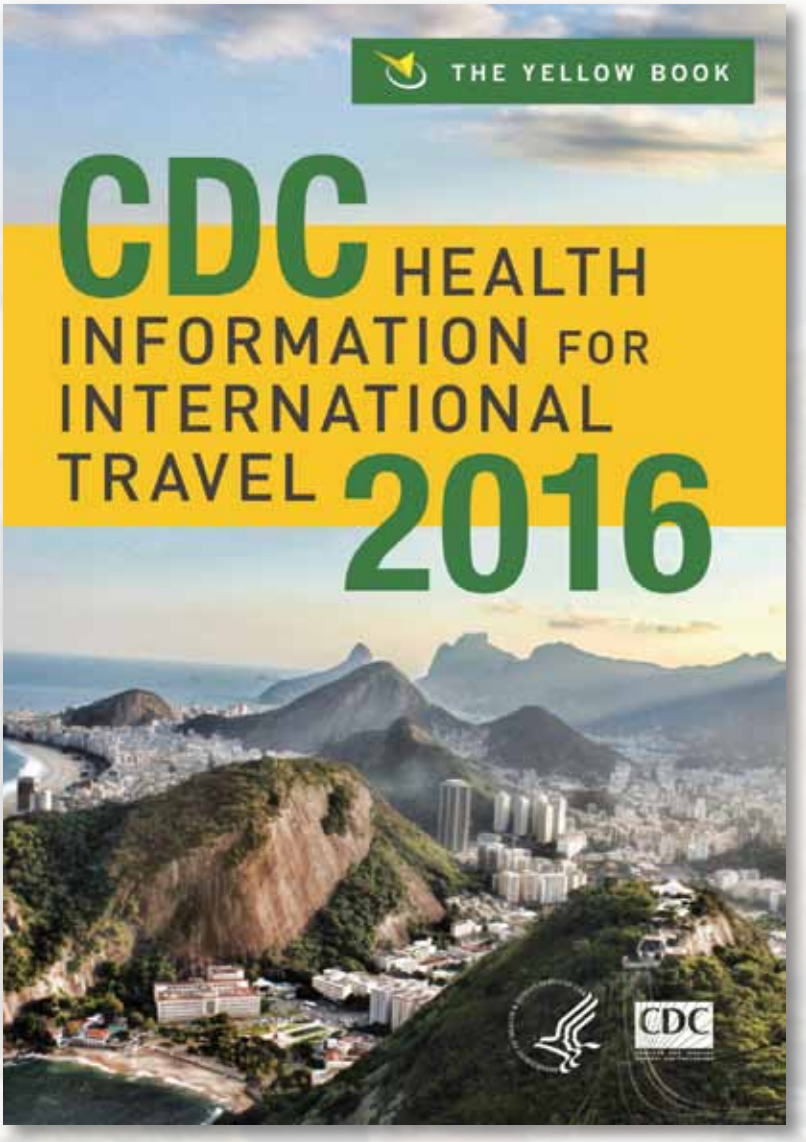

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Leveraging Advances in Tuberculosis Diagnosis and Treatment to Address Nontuberculous Mycobacterial Disease

Ravikiran M. Raju,[1] Sagar M. Raju,[1] Yanlin Zhao, Eric J. Rubin

The nontuberculous mycobacteria (NTM), defined as any mycobacterial pathogen other than *Mycobacterium tuberculosis* or *Mycobacterium leprae*, are a diverse group of pathogens that collectively cause a substantive but often unappreciated worldwide burden of illness. Although NTMs may cause illness similar to *M. tuberculosis*, these pathogens generally do not respond to classic tuberculosis (TB) drug regimens, resulting in misdiagnosis and poor treatment, particularly in resource-poor settings. Although a few high-quality epidemiologic surveys have been made on the topic, existing evidence suggests that NTM-associated disease is much more common than previously thought: more common than TB in the industrialized world and likely increasing in prevalence globally. Despite this evidence, these organisms remain markedly understudied, and few international grants support basic science and clinical research. Here we suggest that the considerable efforts in developing new treatments and diagnostics for TB can be harnessed in the fight against NTM-associated illnesses.

In recent years, major investments in basic research related to *Mycobaterium tuberculosis* have culminated in the large-scale rollout of the GeneXpert (Cepheid, Sunnyvale, CA, USA) diagnostic platform, the approval of bedaquiline for treatment of patients with drug-resistant tuberculosis (TB), and a deeper fundamental understanding of how the bacteria causes disease. These advancements stand in stark contrast to the poor understanding of the nontuberculous mycobacteria (NTMs). The NTMs are a group of organisms within the genus *Mycobacterium* (excluding *M. tuberculosis* and *M. leprae*) that cause a spectrum of diseases that include TB-like lung disease; localized infections of the lymphatic system, skin, soft tissue, or bone; and systemic disease (*1*). Previous studies have helped uncover NTM prevalence in industrialized countries in which differentiating between TB and NTM infections is much less challenging because of the availability of molecular techniques for detecting and identifying microorganisms. However, recent studies that have been done to examine the NTM burden of illness in industrialized settings have consistently uncovered an unexpectedly large prevalence (Figure).

Major obstacles to adequately addressing NTM disease include the challenges of diagnosis and treatment as well as the lack of active research to understand the pathogenesis of these organisms. In each of these arenas, it is critical that we address gaps in the knowledge and capacity to deal with NTM-associated illness. By increasing funding to programs that seek to expand basic knowledge of NTMs and leveraging advancements in TB diagnostics and therapeutics, we can begin to form a deeper understanding of these pathogens and develop appropriate measures to address them. Here, we outline some of the challenges surrounding the diagnosis and treatment of NTMs and research of these organisms and propose avenues for how the road paved by the fight against TB can serve as a scaffold for advancing our understanding of these related, neglected pathogens.

The Challenges of Diagnosis

NTMs share many characteristics with *M. tuberculosis* that make the bacteria difficult to differentiate in resource-poor settings. The standard method for diagnosing TB is through microscopic examination of sputum smears, but when this approach is used, NTMs appear identical to *M. tuberculosis*. Without molecular methods, which are unavailable in much of the developing world, these organisms are difficult to distinguish. Furthermore, in resource-limited settings, patients are often assumed to have *M. tuberculosis* infections because the clinical manifestations of many NTMs can mimic those of TB. In a study in Nigeria, Pokam et al.

Author affiliations: Harvard School of Public Health, Boston, Massachusetts, USA (R.M. Raju, S.M. Raju, E.J. Rubin); Chinese Center for Disease Control and Prevention, Beijing, China (Y. Zhao)

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[1]These authors contributed equally to this article.

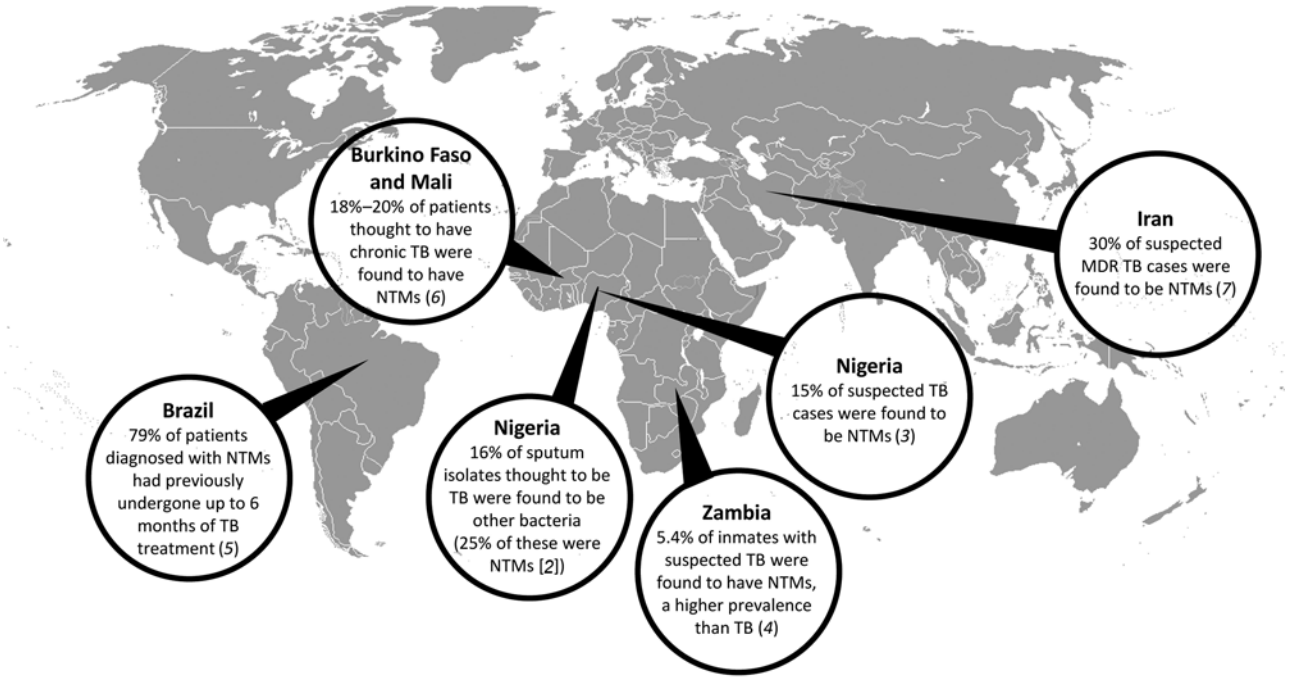

Figure. Summary of key studies of the epidemiology of nontuberculous mycobacteria (NTM) disease in countries populated by low- and middle-income residents. TB, tuberculosis; MDR TB, multidrug-resistant tuberculosis.

found that 16.5% of culture and sputum isolates thought to be *M. tuberculosis* were bacteria other than *M. tuberculosis* upon molecular typing; 25% of these misdiagnosed cases (4% overall) were found to be caused by NTMs (*2*). In another study in Nigeria, Aliyu et al. found that of 1,603 suspected TB cases, 15% were found to be NTM infections (*3*). Recent evidence has suggested that the rate of confusion between *M. tuberculosis* and NTMs may be even larger. Turnbull et al. discovered that inmates in a prison in Zambia who had symptoms of cough and an abnormal chest radiograph image showed an NTM prevalence of 5.4% compared with a 3.8% rate for *M. tuberculosis* (*4*).

These findings have substantial implications for global health approaches to TB. Given that traditional treatments for *M. tuberculosis* infection are ineffective against most NTMs, the unexpectedly high rate of NTMs is likely a contributing factor to perceived TB treatment failure. In Brazil, a national mycobacterial referral center found that of 174 patients with pulmonary NTM, 79% had undergone TB treatment for up to 6 months before NTM infection was diagnosed (*5*). Studies conducted in Burkino Faso and Mali found that 18%–20% of patients suspected of having chronic TB were found to have NTM in their sputum (*6*). Similarly, a study in Iran showed that as many as 30% of suspected cases of multidrug-resistant TB were in fact NTMs, further suggesting the generalization of this phenomenon (*7*). Understanding the true prevalence of NTMs in the developing world is especially valuable considering that evidence suggests that NTM infection may interfere with the Bacille Calmette-Guérin vaccine, a widely used tool in preventing TB infections in the developing world (*8*).

These studies must be taken with some caution, as it is often difficult to distinguish whether the NTMs are a true source of infection or a contaminant in biological specimens or laboratory equipment. To account for this, the American Thoracic Society and the Infectious Disease Society of America guidelines for NTM diagnosis require isolation and growth of the pathogen on ≥2 separate occasions from the same patient to diagnose a pulmonary NTM infection (*9*). Because clinicians in most countries in the developing world often make diagnosis of TB on the basis of clinical symptoms, these guidelines may place a tremendous burden on laboratories in resource-poor settings. Any new diagnostic platform for the NTMs must account for the issues behind species differentiation and contamination and do so in a way that is feasible for application globally.

The Challenges of Treatment

The difficulty of diagnosing NTMs and the frequent confusion of these pathogens with TB is compounded by the fact that standard TB treatments are often ineffective against NTM infections. Anti-TB medications produce a disappointing ≈50% response rate (*10*) in NTM-associated disease. As a result, misdiagnosis and mistreatment have huge implications on patient outcomes. Even within the NTM

class, there is a substantial difference between the various species, which defies a one-size-fits-all treatment approach. This group encompasses pathogens with huge varieties of growth rates, host preferences, and inherent resistance to antibacterial drugs.

The introduction of macrolides, such as clarithromycin, for treatment for NTMs did improve cure rates for certain species, but in a retrospective study by Huang et al., many patients treated with these drugs for at least 12 months continued to have symptoms, and chronic illness was documented among patients who were successfully treated (*11*). Moreover, macrolide resistance is now well documented (*12*). The recommendation of multidrug regimens to counter such resistance is a logical next step, but often these regimens are minimally studied, and few if any have been investigated in a rigorous clinical trial. Therefore, while many clinicians rely on multidrug regimens for the treatment for NTM disease, the ideal combination of agents, duration of therapy, and true efficacy remain unvalidated and unknown.

The Challenges of Current Research Paradigms

Fundamentally, poor understanding of the NTMs arises from a lack of investment. There have been few clinical studies of treatment for NTM-associated disease; most date from a time when advanced HIV infection was common in industrialized countries and opportunistic NTM infections were seen at an alarming frequency among HIV/AIDS patients. We conducted a search using the RePORTER tool (http://projectreporter.nih.gov/reporter.cfm) to find currently active grants from the US National Institutes of Health for this topic and found 228 grants related specifically to research on mycobacterial pathogens. Of these, only 5 (2.2%) were awarded to study specific aspects of the NTMs. These 5 grants cover a wide range of unmet needs, from understanding NTM susceptibility to novel drug discovery. However, this level of attention is clearly insufficient to address the many gaps that exist.

As prevalent as they are, many basic facts about the NTMs remain unknown. For example, it was largely thought that environmental exposure was the sole method of infection. However, a recent report that described whole genome sequencing as a molecular epidemiologic tool suggested that, in the context of cystic fibrosis patients, which is a population exceptionally susceptible to these pathogens, there may be a possibility of person-to-person transmission of *M. abscessus* (*13*). If true, control measures similar to those used for TB transmission might be effective, at least for highly susceptible persons. Although this study suggests that alternate modes of transmission may exist, it is still widely believed that environmental transmission is the major source of NTM infection, and numerous reservoirs such as household water sources have been identified (*14*). However, it can often be difficult to trace infections to a specific environmental source, which is a problem that is compounded by delayed and often incorrect diagnoses.

Additionally, it remains unclear why anti-TB medications are not effective treatment options. NTMs have complex cell walls, systems that modify both antibacterial drugs and their targets, and an extensive array of drug efflux pumps, but all these mechanisms also exist in *M. tuberculosis*. It may be that in NTMs, differing synergy of these and other mechanisms might conspire to produce a poor response to therapy. However, these differences must be studied to determine their significance. Such questions highlight the numerous areas in which our ability to address these pathogens would benefit from a better fundamental understanding.

Addressing the Challenges: Leveraging Advancements in the TB Field

Although there are no simple solutions to the challenges of effectively addressing the NTMs, recent advancements in the TB field have potential for synergistic effects. One of the major breakthroughs in TB diagnostics over the past decade was the move toward using molecular methods such as the GeneXpert system. Although GeneXpert testing distinguishes fairly well between NTMs and *M. tuberculosis*, it does not distinguish within the broad category of NTMs, which is a necessary prerequisite to effective treatment. However, this advancement provides an opportunity to reconfigure molecular diagnostic platforms to include at least common NTM pathogens, providing a rapid and specific detection method. Even so, this method would not be a panacea. Because NTMs can colonize humans without causing disease and can contaminate biologic samples and laboratory equipment, simply finding the organism does not provide a definitive diagnosis. However, even raising the possibility can alert a clinician to consider the diagnosis of NTM-associated illness, limiting misdiagnosis and, as a result, incorrect treatment. These systems would also provide researchers with a tool to uncover the true burden of illness from NTMs.

Substantial efforts have been invested in anti-TB drug development. These have yielded 2 new approved antibiotics, bedaquiline and delamanid; several others are in clinical development. These drug discovery platforms could easily be transferred to screening for NTM-active compounds, and some of the new agents have already been shown to have activity against NTMs. For example, bedaquiline is more effective than currently existing antimycobacterial agents in treating *M. ulcerans* in a mouse model of infection and has shown promise as a salvage therapy for *M. avium* and *M. abscessus* (*15*,*16*). Moreover, new oxazolidinones, a class of drug effective against many NTMs, are currently being developed for TB and might

prove clinically useful (*17*). Although many antitubercular compounds have poor activity against NTMs, new agents could serve as starting points that could be optimized; for example, bedaquiline derivatives have been found to have broad-spectrum activity (*18*).

While building on discoveries in the TB field, developing new interventions to diagnose, treat and prevent NTM-associated illness will likely require a better basic understanding of these organisms. Mycobacteria are extremely diverse; >150 species have been identified to date and vary in pathogenicity, virulence, mode of transmission, and antibiotic susceptibility. Here, too, the path paved by TB biologists could afford some insight into NTM physiology. The sequencing of the *M. tuberculosis* genome and the development of genetic tools to probe the bacterium's physiology afforded novel insights into how this pathogen causes disease. Similarly, the sequencing of many NTM species, now being completed, could lead to novel discoveries that highlight why these organisms have been so difficult to treat in the past and why exactly they diverge from *M. tuberculosis* (*19*).

None of these measures will be taken without increased funding. The prevalence of NTMs not only in developing countries but also in the United States and many parts of Asia suggests that many public agencies should be willing to support NTM research. However, public funding is not the only avenue for increasing investment. Considering the prevalence and chronicity of these organisms in the industrialized world, a potentially substantial market of affected entities may exist. Resources from private interests and pharmaceutical companies could be leveraged to develop novel diagnostics and therapeutics if encouraged by advancements in the academic setting in our basic understanding of these pathogens.

The evidence that has been uncovered to date points to the NTMs as a source of substantial and growing burden of illness. NTM infections not only effect thousands of people, requiring lengthy and taxing treatments that are often not available in resource-poor settings; they also muddy the waters in the global fight against TB by draining resources resulting from misdiagnosis and mistreatment. Only through a concerted effort by researchers, clinicians, industry, and global health policy stakeholders to understand and address these issues can we respond to this large and neglected threat.

Dr. Raju is a physician and scientist and is currently a pediatric resident at Children's Hospital Boston, Boston, Massachusetts, in the Urban Health and Advocacy Track. His professional and research interests are in the biology of essential physiologic processes in *M. tuberculosis* and nontuberculous mycobacteria, with the overall goal of developing novel therapeutics for these neglected diseases.

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Address for correspondence: Ravikiran Raju, Harvard Institutes of Medicine, Rm 1007, 4 Blackfan Circle, Boston, MA 02115, USA; email: Ravikiran.raju@childrens.harvard.edu

SYNOPSIS

Epidemiology of Histoplasmosis Outbreaks, United States, 1938–2013

Kaitlin Benedict, Rajal K. Mody

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Learning Objectives

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

- Identify epidemiologic features of reported US histoplasmosis outbreaks during 1938–2013, based on a literature review
- Determine risk factors associated with reported US histoplasmosis outbreaks during 1938–2013
- Describe clinical features and outcomes in reported US histoplasmosis outbreaks during 1938–2013

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Disclosures: ***Kaitlin Benedict, MPH,*** *and* ***Rajal K. Mody, MD, MPH,*** *have disclosed no relevant financial relationships.*

Histoplasmosis has been described as the most common endemic mycosis in the United States. However, histoplasmosis is not nationally notifiable. Its presumed geographic distribution is largely derived from skin test surveys performed during the 1940s, and information about its local features comes primarily from outbreak investigations. We conducted a literature review to assess epidemiologic features of histoplasmosis outbreaks in the United States. During 1938–2013, a total of 105 outbreaks involving 2,850 cases were reported in 26 states and the territory of Puerto Rico. Common exposure settings were chicken coops and buildings or other structures undergoing renovation or demolition. Birds, bats, or their droppings were reported to be present in 77% of outbreak settings, and workplace exposures were reported in 41% of outbreaks. The continued occurrence of histoplasmosis outbreaks, particularly work-related ones involving known disturbance of bird or bat droppings, highlights the need to increase awareness of the disease.

Author affiliation: Centers for Disease Control and Prevention, Atlanta, Georgia, USA

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Histoplasmosis is caused by inhalation of the microconidia of *Histoplasma* spp. fungi, which are thermally dimorphic (i.e., environmental mold which converts to a yeast at 37°C). Infection can range from asymptomatic to life-threatening disease, depending on host status, inoculum size, and other factors (*1*). In the United States, histoplasmosis-endemic areas were established during the 1940s–1950s by using nationwide skin testing to evaluate histoplasmin sensitivity among young adults (*2*). The highest percentages of positive reactions (60%–90%) were noted in areas surrounding the Ohio and Mississippi River valleys; percentages of positive reactions decreased with increasing distance from these valleys (*2*). *Histoplasma* spp. grow particularly well in organic matter enriched with bird or bat droppings and likely exist in microfoci within and outside the broadly defined endemic regions (*3*–*5*). Environmental disruption of *Histoplasma* habitats is often a key factor associated with histoplasmosis outbreaks (*3*–*5*).

Although most infections are acquired sporadically, reports describing epidemiologic features of histoplasmosis in the United States usually involve investigations of localized outbreaks (*3*), which have not been comprehensively reviewed since the 1970s (*6*). We provide an update on the epidemiologic features of documented histoplasmosis outbreaks and identify potential opportunities to prevent them.

Methods

During February 2015, we searched Medline, Embase, Scopus, CINAHL (Cumulative Index to Nursing and Allied Health Literature), ProQuest, and CAB (Centre for Agriculture and Biosciences) Abstracts without date or language restrictions and used combinations of the terms "histoplasmosis," "*Histoplasma*," "outbreak," "cluster," "epidemic," and "United States." Using the digital archive of scientific literature produced by the Centers for Disease Control and Prevention (CDC Stacks, http://stacks.cdc.gov/), we searched for reports published in Morbidity and Mortality Weekly Report before 1981. We reviewed references pertaining to outbreak investigations in all relevant articles. In addition, we searched abstracts from major infectious disease and epidemiology conferences and searched records from CDC's Mycotic Diseases Branch for information about unpublished outbreaks and about details of investigations published as conference abstracts. We abstracted clinical and epidemiologic data of interest from reports of outbreaks that met inclusion criteria (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/22/3/15-1117-Techapp1.pdf). The Cochran-Armitage test for trend was used to assess changes by decade.

An outbreak was defined as ≥2 cases of histoplasmosis associated with a common environmental source. Outbreaks were included if ≥1 case had laboratory evidence of histoplasmosis or if *Histoplasma* spp. were recovered from the common source. A case was defined as an illness clinically compatible with acute histoplasmosis, as determined by the authors of the original reports. Laboratory evidence of infection was defined as any of the following: positive culture or histopathology, presence of H or M immunoprecipitin bands on immunodiffusion, complement fixation titer ≥1:8, a positive *Histoplasma* antigen enzyme immunoassay result in urine or serum, or a positive serologic test, as stated in the report. We did not include clusters of histoplasmosis cases transmitted through organ transplantation or those involving infections acquired abroad. Prior outbreaks described anecdotally in published reports were not included unless the exact number of cases was stated.

Radiographic evidence of infection included pulmonary infiltrates, lesions, nodules, or cavitation; hilar or mediastinal lymphadenopathy; or unspecified findings indicating acute pulmonary histoplasmosis, as noted by the original authors. Outbreak onset was determined by the date on which the first patient became ill. We assessed reports for statements regarding average duration between suspected exposure and symptom onset and calculated the median. For outbreaks in which the incubation period was expressed as only a range, we calculated medians for the minimum and maximum number of days. Outbreak duration was defined as the interval between symptom onset of the first case and onset of the last case.

For reports not stating an exact number of patients hospitalized but mentioning a general proportion (e.g., "most patients were hospitalized"), we used a conservative estimate (i.e., 1 + half the number of patients). For reports not including number of deaths, we assumed that no deaths resulted if no patients were hospitalized or if all hospitalized patients recovered. We assumed that all cases occurred among adults ≥18 years of age if an outbreak occurred entirely among workers.

Setting was defined as the location where exposures occurred, such as a chicken coop, cave, or building. Farm settings not specifically associated with a chicken coop were classified as "farm." We used "residential area" to classify an outdoor residential area that was not a farm and that had no exposures associated with a specific building or structure. Outbreaks in which persons were suspected to have been exposed throughout a city because of windborne dispersal of infectious material from a common source were classified as "citywide windborne." When outdoor exposures resulted from a common activity, but no specific setting was described, we categorized the setting as "unspecified outdoor area."

Specific activities suspected to have initiated the outbreak were not mutually exclusive and included soil disruption (e.g., digging or excavation); disruption of plant matter (e.g., trees, wood, leaves, or vegetable matter); demolition, construction, or renovation activities; caving; and known disturbance of large accumulations of bird or bat droppings

(e.g., scraping droppings from a bridge or shoveling accumulations of droppings from a building's roof). We also assessed each report for statements about the mere presence of birds, bats, or their droppings because some outbreaks were not related to obvious disturbance of droppings, but birds, bats, or droppings were described as present in the areas of suspected exposure. Outbreaks were categorized as work-related if at least 1 case occurred in a worker as a direct result of his or her occupational activities and if those activities were believed to have initiated the outbreak. Outbreaks were classified as having workplace exposures if some patients were exposed in their workplace but were not directly involved in the outbreak-initiating activities.

Results

This review includes 105 reported histoplasmosis outbreaks comprising 2,850 cases during 1938–2013 (Figures 1, 2). The range of outbreak size was 2–383 cases (mean 27; median 6). Seventeen (16%) outbreaks had 2 cases; 29 (28%) had 3–5; 18 (17%) had 6–9; 12 (11%) had 10–19; 10 (10%) had 20–29; and 19 (18%) had ≥30. All but 2 outbreaks had ≥1 case with laboratory evidence of histoplasmosis. Laboratory evidence was reported for 1,884 (66%) cases; 873 (31%) had no laboratory evidence; and 93 (3%) cases in 5 outbreaks had no information about percentage of cases with laboratory evidence.

Radiographic evidence of histoplasmosis was reported for 500 (81%) cases from 68 outbreaks. For 70 outbreaks that included 1,630 cases with complete data on patient age, 51% of cases occurred among children <18 years old. The preponderance of cases among children was driven by 2 large school-related outbreaks (*7,8*). If these 2 outbreaks are excluded, 82% of cases occurred among adults. For 53 outbreaks that included 1,318 cases with complete data about patient sex, 60% of cases occurred among males. Outbreaks were reported from 26 states and Puerto Rico (Figure 3). The following states had the most reported cases: Indiana, 790 (28%); Ohio, 415 (15%); Iowa, 213 (8%); Michigan, 182 (6%); Illinois, 155 (5%); Nebraska, 144 (5%); and Arkansas, 143 (5%). Onsets of most (72%) outbreaks occurred during May–November (Figure 4).

For 32 outbreaks with reported overall attack rates, the median attack rate was 63% (range 9%–100%). A median incubation period (median 10 days) was included in 5 reports; a mean (median 13 days) was included in 9 reports; and 25 reports stated a range. Median minimum and maximum incubation periods were 7 and 15 days, respectively. Median outbreak duration was 13 days (n = 51 outbreaks; range 1 d to >5 y).

Treatment, Hospitalizations, and Outcomes

Information on antifungal therapy was reported for 34 outbreaks; 120 (6.7%) of 1,804 patients received antifungal treatment (Table 1). For reports of 62 outbreaks that included precise numbers of hospitalized patients, 265 (14.7%) of 1,801 patients were hospitalized. Inclusion of 2 large outbreaks with reported approximate numbers of hospitalized patients increased the minimum number of patients hospitalized to 610 (26.9%) of 2,269 (data not shown). The percentage of patients hospitalized generally decreased over the decades ($p<0.0001$), except for the 1970s, when a low number of patients were reported as hospitalized. For 72 outbreaks with death data, 25 (1.1%) of 2,232 patients died; as with hospitalizations, the percentage of patients who died decreased over time ($p<0.0001$) (Table 1).

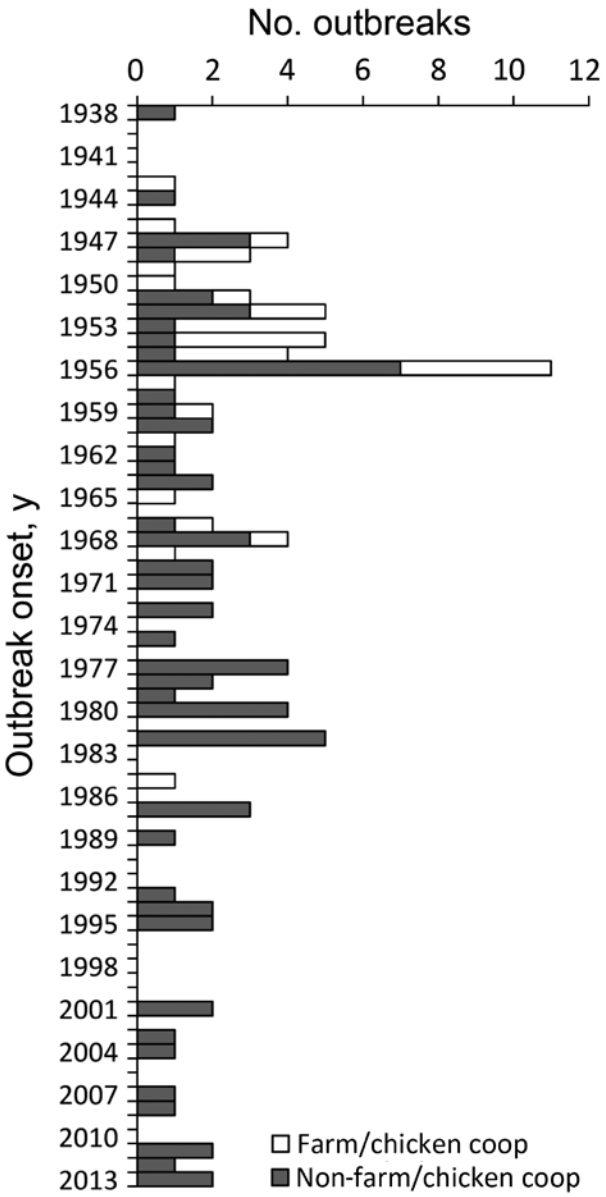

Figure 1. Number of histoplasmosis outbreaks by year of onset and setting, United States, 1938–2013 (N = 105).

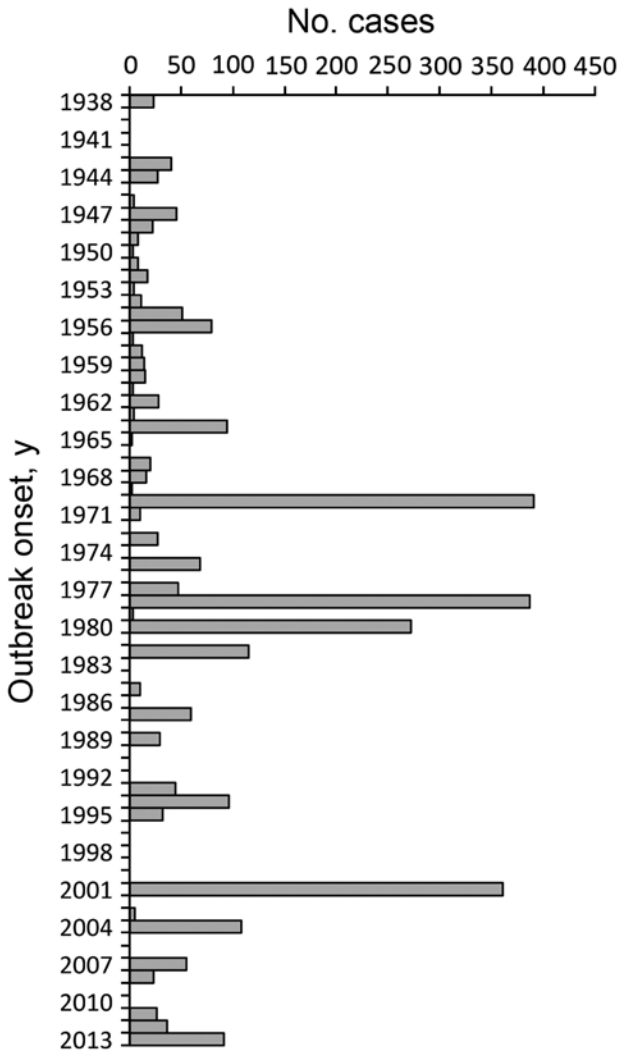

Figure 2. Number of outbreak-related cases of histoplasmosis by onset year, United States, 1938–2013 (N = 2,850).

Settings and Activities

The most frequent settings for outbreaks were buildings (19 outbreaks), chicken coops (17 outbreaks), farms (12 outbreaks), and unspecified outdoor areas (11 outbreaks) (Table 2). Despite the frequency of outbreaks in these settings, outbreaks in these locations generally involved fewer cases than outbreaks in other settings. Citywide windborne outbreaks had the highest median number of cases (65, range 28–381); these cases were believed to have originated from environmental disturbances at a stream bank (2 outbreaks), a golf course (1 outbreak), and either an abandoned amusement park or a tennis complex (1 outbreak). Most (28/29) outbreaks associated with farms or chicken coops occurred during 1943–1969; the last occurred in 1985. More recent outbreaks (i.e., occurring during 1987–2013) were associated with various settings. The 9 cave-associated outbreaks represented some of the southernmost outbreak locations (Florida, Texas, and Puerto Rico); 4 of 5 outbreaks in Puerto Rico were associated with caves.

Disturbance of bird or bat dropping accumulations was described in 42 (40%) outbreaks; soil disruption in 34 (32%); plant matter disruption in 21 (20%); and demolition- or construction-related activities in 26 (25%). Details were insufficient to determine specific activities that may have precipitated 9 outbreaks. In 1 report, none of these activities was explicitly mentioned. Presence of bats (or bat droppings) was described in 24 (23%) outbreaks; presence of birds (or bird droppings) in 59 (56%); presence of either bird or bats in 81 (77%); and presence of birds and bats in 2 (2%). Reported birds included chickens (24 [41%] of 59 bird-related outbreaks); blackbirds, including starlings, grackles, and unspecified blackbirds (19 [32%]); pigeons (9 [15%]); and gulls (1 [2%]). Type of bird was not described in 8 (14%) bird-related outbreaks.

Work-Related Outbreaks and Workplace Exposures

Thirty-five (33%) outbreaks were work-related; 26 occurred among workers only, and 9 affected workers and nonworkers. Fifteen (43%) work-related outbreaks took place at a building, and 6 (17%) occurred at outdoor structures: bridges (4 outbreaks) and water towers (2 outbreaks). Occupations involved in work-related outbreaks were primarily construction, demolition, or maintenance. Presence of birds, bats, or droppings were reported in 30 (86%) work-related outbreaks. For 8 outbreaks not classified as work related, some patients were exposed in their workplace but were not directly involved in outbreak-initiating activities. Altogether, cases acquired in the workplace were described in reports of 43 (41%) outbreaks. In 4 of those outbreaks, at least 1 public health investigator or laboratory worker became ill with histoplasmosis.

Other Features

Animals, primarily dogs, were infected in 5 outbreaks. In 6 outbreaks, ≥1 person was infected in a state other than the state of residence or other than the state where illness began. Of 72 outbreak investigations that used the histoplasmin skin test, 71 occurred before 1990. Environmental sampling was performed in 83 outbreak investigations; results were positive in 57 (69%). Environmental sampling was more commonly performed in outbreak investigations before 1990 than after (77/89 [87%] vs. 6/16 [38%]). Decontamination with formalin was described for 6 outbreaks (*7,9–13*).

Discussion

During 1938–2013, a total of 2,850 cases of histoplasmosis resulted from 105 reported outbreaks in various settings in 26 US states and Puerto Rico. Outbreak locations were

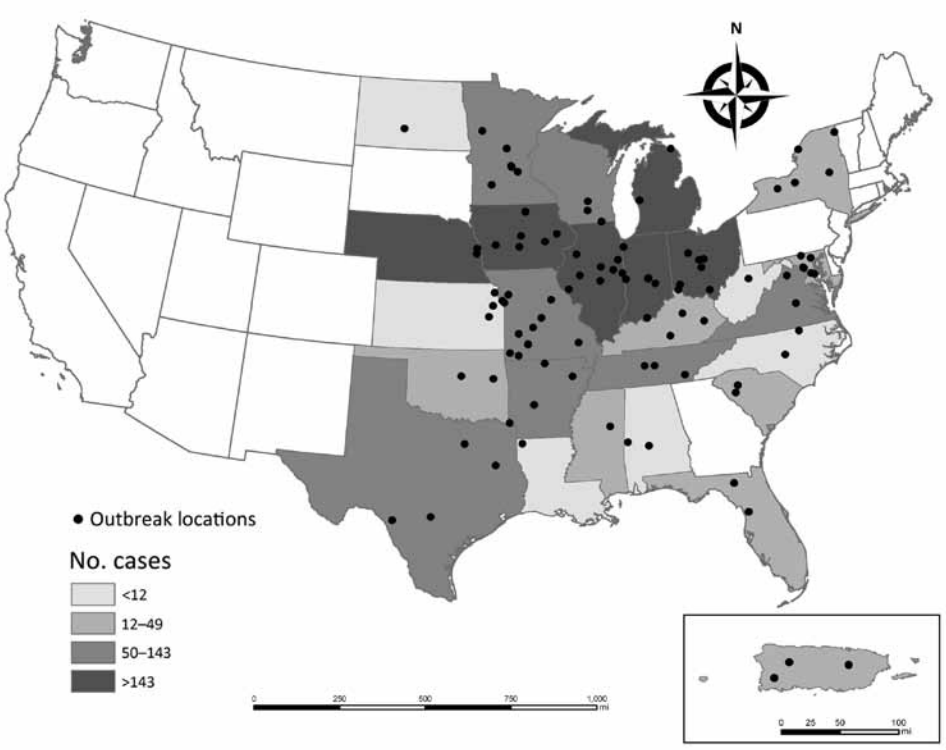

Figure 3. Locations of histoplasmosis outbreaks and number of outbreak-related cases, by state or territory (Puerto Rico, inset), United States, 1938–2013. City or county was not available for 1 outbreak in Ohio; 1 in Iowa; 2 in Tennessee; 1 in Missouri; and 1 in North Carolina. Points were placed in the center of the state for these outbreaks. Three of the 5 outbreaks in Puerto Rico occurred in the same cave system and appear as a single point on the map. Histoplasmosis is a reportable disease in Arkansas, Delaware, Illinois, Indiana, Kentucky, Michigan, Minnesota, Nebraska, Pennsylvania, and Wisconsin.

generally consistent with the known distribution of histoplasmosis; only a few outbreaks occurred in states believed to have a low level of endemicity (i.e., Florida, Minnesota, New York, North Dakota, and South Carolina [*2*]). The apparent decrease in the number of outbreaks over time may be largely because of the decline in reported farm- or chicken coop–associated outbreaks. However, the continued occurrence of histoplasmosis outbreaks highlights the need for increased awareness about ways to reduce exposures, particularly in the workplace and other settings where bird or bat droppings are present and environmental disruption occurs.

The association between histoplasmosis outbreaks and environmental disturbance, particularly in the presence of bird or bat droppings, is well recognized. We found 77% of outbreaks were reported to have evidence of bird or bat droppings; the actual percentage is likely higher, as our analysis was limited to data provided in published reports. The magnitude of environmental disturbance can range from minor, such as walking on contaminated ground or setting up tents (*14,15*), to large-scale, such as excavation or clearing foliage in a bird-roosting site (*13,16,17*). Among reports of outbreak investigations with sufficient information, only 1 outbreak was not described as associated with disturbance of bird or bat droppings, soil or plant matter disruption, or demolition or construction. This outbreak was suspected of being related to a load of coal that was dumped outside the windows of an Arkansas classroom, thus dispersing potentially contaminated coal dust; although chicken manure had been dumped on the school property during the previous year, the manure was last disturbed ≈6 months before the outbreak (*18*).

Many histoplasmosis outbreaks show the potential for cases to occur among persons who did not participate directly in the outbreak-initiating activities. Such cases often occurred in a workplace (*7,8,11,12,17,19,20*). More than 30% of outbreaks were work-related (i.e., with workers involved in outbreak-initiating activities), and ≈40% of outbreaks affected persons in their workplace (i.e., workers may or may not have been involved in the outbreak-initiating activities). For workers who disrupt contaminated soil or accumulations of bird or bat droppings, the National Institute for Occupational Safety and Health has developed guidance for workers and employers about ways to reduce exposures to *H. capsulatum*: excluding birds or bats from buildings; posting warnings and communicating health risks to workers; controlling dust during activities such as construction, demolition, and excavation in known endemic areas; properly disposing of potentially contaminated waste; and selecting and wearing appropriate personal protective equipment (PPE) (*21*). Several outbreak reports described cases among workers despite use of PPE; these cases confirm that PPE must be used correctly and consistently to be effective (*22–25*).

Our findings almost certainly underestimate the number of histoplasmosis outbreaks because fungal disease

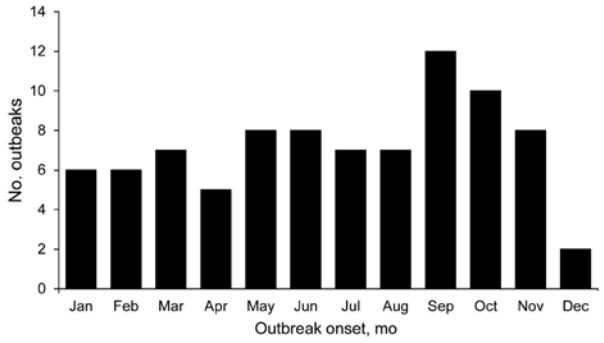

Figure 4. Number of histoplasmosis outbreaks by onset month (reported for 86 of 105 outbreaks), United States, 1938–2013.

outbreaks are not nationally notifiable and many outbreaks likely go unpublished or unrecognized. Although diagnostic tests for histoplasmosis have improved during the past few decades, the infection can be challenging to diagnose, and outbreaks can be difficult to recognize. Even among outbreaks occurring since 1995, some patients had delayed diagnoses (*26*) or received unnecessary treatment for suspected bacterial infections (*8,26,27*). During a recent outbreak at a prison in Illinois, 42 inmates became ill within a 48-hour period (*28*); fever and headache were predominant symptoms, rather than respiratory symptoms, and a viral infection was initially suspected when 10 of 18 nasopharyngeal swabs tested positive for adenovirus but were negative with repeat testing (M.A. Arwady, unpub. data). This example highlights the nonspecific symptoms of acute pulmonary histoplasmosis (e.g., fever, cough, headache, fatigue, and chest pain), which can persist for weeks or months (*1,29,30*). Histoplasmosis can also be acquired outside the United States, so this illness should be considered for persons who have these symptoms and have recently traveled, especially to Central or South America. Outbreaks of internationally acquired histoplasmosis among travelers are known to occur, but those reports were outside the scope of this article.

In our analysis, the percentage (66%) of symptomatic patients with positive laboratory results likely underrepresents the true percentage of patients who would test positive because only a subset of ill persons were selected for or received laboratory testing during some outbreak investigations (*7,22*). Although we were unable to evaluate the type of diagnostic tests on an individual level, serologic tests are the most commonly used diagnostic method for acute pulmonary histoplasmosis. Complement fixation and immunodiffusion tests for histoplasmosis are each ≈80% sensitive, but antibodies can take up to 6 weeks to develop (*1*). A small number of reports in this analysis included cases with low complement fixation titers (≥1:8). Although titers in this range are weak diagnostic evidence on their own, compatible signs, symptoms, and shared exposures strengthen the suspicion for histoplasmosis. *Histoplasma* antigen detection tests were first developed in the mid-1980s and are also useful for detecting acute disease, particularly among persons with immunocompromising conditions, disseminated histoplasmosis, or intense exposures (*1,31,32*).

Several examples show the potential for large histoplasmosis outbreaks. The outbreak with the most reported symptomatic cases (n = 383) occurred in 1970, when a group of junior high students in Ohio raked and swept a central courtyard where birds had roosted (*7*). A similarly large (but unusually prolonged and severe) citywide windborne outbreak of 381 symptomatic cases occurred during September 1978–August 1979 in Indianapolis, Indiana, where 2 activities and settings were suspected outbreak sources: demolition of an amusement park and construction of a tennis stadium (*30*). A serosurvey performed as part of that investigation revealed a large number of presumably asymptomatic persons who had laboratory evidence of acute histoplasmosis; the authors of the report extrapolated that >100,000 persons were infected (*30*).

Generally, an estimated <1% of persons infected with *Histoplasma* spp. develop symptoms, and infection likely results in at least partial protection from future infection; however, reactivation histoplasmosis can occur in immunosuppressed persons (*1*). Thus, the true public health effects of a histoplasmosis outbreak are challenging to quantify, in part because some exposed persons may develop serious disease years after the exposure when they later develop immunocompromising conditions.

Most mild-to-moderate histoplasmosis cases are self limited, but patients with more severe cases require antifungal treatment (*29*). The percentage of histoplasmosis outbreak patients treated in each decade likely reflects the development of antifungal drugs commonly used to treat histoplasmosis: amphotericin B (approved by the Food and Drug Administration in 1958) and itraconazole (approved in 1992). Accordingly, the observed declines in outbreak-associated hospitalizations and deaths (*16,30,33–37*) over time may partially result from the improvement and availability of histoplasmosis-related treatment and diagnostic

Table 1. Antifungal drug treatments, hospitalizations, and deaths resulting from histoplasmosis outbreaks, by decade, United States, 1938–2013*

Decade	No. (%) patients		
	Treated	Hospitalized	Died
1938–1949	0	57 (64.8)	1 (1.7)
1950–1959	7 (21.9)	28 (37.3)	4 (3.3)
1960–1969	7 (6.5)	24 (48.0)	5 (2.9)
1970–1979	50 (5.5)	17 (3.7)	15 (1.8)
1980–1989	10 (4.5)	72 (27.5)	0 (0.0)
1990–1999	14 (20.6)	38 (22.1)	0 (0.0)
2000–2013	32 (6.8)	29 (4.1)	0 (0.0)
Total	120 (6.7)	265 (14.7)	25 (1.1)

*Percentages are of reported cases. Number and percent of cases from outbreaks were included when information was available.

Table 2. Outbreaks and cases of histoplasmosis, by outbreak setting, United States, 1938–2013

Setting	No. outbreaks				No. cases*			
	Total	1938–1963	1964–1989	1989–2013	Total	Mean	Median	Range
Building	19	7	7	5	437	23	12	2–138
Chicken coop	17	14	3	0	105	6	4	2–40
Farm†	12	10	2	0	52	4	4	2–10
Outdoors, not specified	11	6	5	0	65	6	2	2–30
Cave‡	9	2	6	1	96	11	6	3–24
Residential area	7	4	2	1	74	11	8	3–26
Outdoor structure§	7	3	3	1	142	20	7	2–101
School or university	6	2	2	2	866	144	44	12–383
Other¶	5	2	0	3	195	39	27	6–108
Citywide windborne	4	1	3	0	538	135	65	28–381
Bamboo field	3	0	2	1	27	9	6	3–18
Prison	3	0	1	2	163	54	72	6–85
Campsite	2	0	1	1	90	45	45	36–54
All outbreaks	105	51	37	17	2,850	27	6	2–383

*Means and medians have been rounded to nearest whole number.
†Not specifically a chicken coop.
‡Four of 9 cave-associated outbreaks occurred in Puerto Rico.
§Bridge (4 outbreaks), chapel (1 outbreak), and water tower (2 outbreaks). These outbreaks were work related except for the chapel-related outbreak.
¶Agricultural processing plant, storm cellar, park, landfill, and paper factory.

testing. We estimate that 15%–27% of patients in outbreaks require hospitalization but that only ≈1% of acute cases are fatal; however, this percentage of fatalities may be underestimated because we assumed that no deaths occurred during outbreaks in which no patients were hospitalized.

Alternatively, decreased numbers of hospitalizations and deaths could be associated with changes in exposure types experienced at different outbreak settings. The apparent absence of chicken coop–associated outbreaks after the 1960s may reflect a true outbreak reduction related to an application of knowledge obtained during outbreak investigations but could also indicate a bias toward reduced reporting of these smaller outbreaks over time. With increased popularity of backyard chicken flocks over the past decade (*38*), public health officials and healthcare providers should continue to be aware of the potential for histoplasmosis outbreaks and sporadic cases related to keeping chickens.

Although the small peak in reported outbreaks and outbreak-related cases during the late 1970s and early 1980s coincided with the start of the HIV epidemic, the temporal association does not appear as strong as that described between sporadic cases and the HIV epidemic. Similarly, unlike sporadic cases, numbers of outbreaks or outbreak-related cases did not decrease appreciably after introduction of antiretroviral therapy (*39*), suggesting that histoplasmosis outbreaks are influenced more by environmental factors than by host factors.

Historically, skin testing, environmental testing, and decontamination of environmental material with formalin often played central roles in histoplasmosis outbreak investigation and control but are currently of limited or no relevance. The histoplasmin skin test was frequently used as an epidemiologic tool in outbreak investigations to test patients (either during the outbreak or as part of clinical follow-up) or other exposed persons or to establish skin-test positivity rates within a specific geographic area or population. Nevertheless, the skin test was not a useful diagnostic test because of concern about cross-reactivity with other endemic mycoses, and the reagents have not been commercially available in the United States since 2000 (*1,21*). Similarly, environmental recovery of *H. capsulatum* from outbreak settings contributed substantially to knowledge of this species' natural habitat and provided essential epidemiologic linkage between cases and exposure settings. However, the current utility of environmental testing for *H. capsulatum* in outbreak situations is less clear because traditional culture-based detection methods are resource intensive, and detection of the organism in the environment likely would not change public health recommendations for outbreak control. Molecular methods to detect *H. capsulatum* in environmental samples appear promising but are not yet widely used. As these technologies advance, they may serve as faster, less expensive methods to analyze environmental samples than culture-based methods (*21*). Use of formalin to decontaminate contaminated soil or other environmental material is no longer recommended (*21*) because this substance is a health hazard and use is impractical for large areas or settings such as caves (*40*).

Overall, outbreak-associated cases likely represent a small proportion of histoplasmosis cases (*39*). Histoplasmosis is reportable to public health authorities in 10 states, but passive surveillance almost certainly underestimates the true number of diagnosed cases in these areas. Histoplasmosis is often described as the most common endemic mycosis in the United States. This description is perhaps accurate on the basis of the potentially large number of asymptomatic infections suggested by the nationwide skin test surveys of the 1940s–1950s (*2*); however, current estimates of symptomatic cases and of the economic and

public health effects caused by histoplasmosis are unavailable. Future work is needed to better define the true burden of both outbreak-associated and sporadic cases of histoplasmosis. Increased awareness among healthcare providers, public health and occupational safety professionals, and the public is also needed so that appropriate methods can be used to reduce exposures in known endemic areas during disruption of bird or bat droppings or other contaminated material.

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Ms. Benedict is an epidemiologist in the Mycotic Diseases Branch, Division of Foodborne, Waterborne, and Environmental Diseases, National Center for Emerging and Zoonotic Infectious Diseases, CDC. Her research interests include the epidemiology of fungal infections and health communication.

Dr. Mody is a medical officer in the Commissioned Corps of the US Public Health Service and leads the branch epidemiology team in the Mycotic Diseases Branch at CDC. His research interests include the prevention and epidemiology of fungal infections.

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Address for correspondence: Kaitlin Benedict, Centers for Disease Control and Prevention, 1600 Clifton Road NE, Mailstop C09, Atlanta, Georgia 30329-4027, USA; email: jsy8@cdc.gov

Avian Influenza A(H5N1) Virus in Egypt

Ghazi Kayali, Ahmed Kandeil, Rabeh El-Shesheny, Ahmed S. Kayed, Asmaa M. Maatouq, Zhipeng Cai, Pamela P. McKenzie, Richard J. Webby, Samir El Refaey, Amr Kandeel, Mohamed A. Ali

In Egypt, avian influenza A subtype H5N1 and H9N2 viruses are enzootic in poultry. The control plan devised by veterinary authorities in Egypt to prevent infections in poultry focused mainly on vaccination and ultimately failed. Recently, widespread H5N1 infections in poultry and a substantial increase in the number of human cases of H5N1 infection were observed. We summarize surveillance data from 2009 through 2014 and show that avian influenza viruses are established in poultry in Egypt and are continuously evolving genetically and antigenically. We also discuss the epidemiology of human infection with avian influenza in Egypt and describe how the true burden of disease is underestimated. We discuss the failures of relying on vaccinating poultry as the sole intervention tool. We conclude by highlighting the key components that need to be included in a new strategy to control avian influenza infections in poultry and humans in Egypt.

An unprecedented increase in the number of human infections with the highly pathogenic avian influenza A(H5N1) virus was observed in Egypt during the 2014–15 winter season. The World Health Organization reported that 31 cases were confirmed in 2014, of which 27 were in persons infected as of September (*1*). The Ministry of Health and Population in Egypt confirmed 31 cases in 2014 and 88 in January and February 2015. Thus, the official number of cases during September 2014–February 2015 was 114, including 36 deaths. Furthermore, in February 2015, the first human case of subtype H9N2 virus infection in Egypt was reported. These events compelled national and international authorities to examine the reasons behind the increase in human infections and implement control measures.

In Egypt, highly pathogenic avian influenza subtype H5N1 virus was first reported in poultry in 2006 and was declared to be enzootic in 2008 (*2*,*3*). As an initial response, the government of Egypt devised a comprehensive response plan that included increasing awareness, culling infected poultry, zoning and movement restrictions, and emergency vaccination of parent flocks (*3*,*4*). However, the virus continued to circulate, and infections were reported in more governorates. The authorities then decided to increase vaccination to cover all commercial flocks and backyard poultry (*3*). Eventually, vaccination became the only tool used to control H5N1 virus in Egypt, as other aspects of the control plan became neglected. This strategy failed to control the spread of H5N1 virus, given that outbreaks in poultry continued to occur.

The inadequate control measures enabled H5N1 viruses to mutate. Genetic drift in the hemagglutinin (HA) gene was observed each year and was more profound after 2008, when the virus was declared enzootic (*4*–*6*). Two subclades of H5N1 viruses, 2.2.1 and 2.2.1.1, co-circulated in poultry from late 2009 through 2011 (*5*–*7*). Subclade 2.2.1.1 viruses are thought to have emerged as escape mutants because of vaccine pressure (*8*). As of 2012, subclade 2.2.1.1 viruses were rarely detected, but subclade 2.2.1 viruses continued to evolve to form a new phylogenetic cluster (*5*). Subclade 2.2.1 and 2.2.1.1 viruses were also antigenically distinct (*9*–*11*).

Most human cases of H5N1 infection in Egypt were caused by infection with subclade 2.2.1 H5N1 viruses, which are abundant in backyard poultry (*12*). Epidemiologic analysis of human H5N1 cases reported during 2006–2010 showed that the case-fatality rate was 34% and differed significantly by sex (higher among female patients), age (increased with age), and time to hospitalization (decreased with faster hospitalization) (*4*,*13*). By 2015, most of the reported H5N1 human cases worldwide were in Egypt (37%, 292/784) (*1*).

This increase in human infections and the continuous circulation of H5N1 and H9N2 viruses in poultry in Egypt have raised concerns for public health and animal health. Here we analyze the current situation of H5N1 viruses in Egypt. We discuss the evolution of the viruses in poultry, describe the epidemiology of human infection, analyze the effect of poultry vaccination, and provide insight on how to move forward for controlling H5N1 virus circulation in poultry.

Avian Influenza in Poultry in Egypt

Surveillance

In Egypt, a systematic surveillance program for avian influenza viruses has been in place since 2009. The program, a

Author affiliations: St. Jude Children's Research Hospital, Memphis, Tennessee, USA (G. Kayali, P.P. McKenzie, R.J. Webby); National Research Centre, Giza, Egypt (A. Kandeil, R. El-Shesheny, A.S. Kayed, A.M. Maatouq, M.A. Ali); Georgia State University, Atlanta, Georgia, USA (Z. Cai); Ministry of Health and Population, Cairo, Egypt (S. El Refaey, A. Kandeel)

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collaborative effort between the Center of Scientific Excellence for Influenza Viruses in Egypt and the St. Jude Center for Excellence for Influenza Research and Surveillance in the United States, provided an important tool for understanding the ecology of avian influenza viruses in poultry in Egypt. Commercial and semicommercial farms, abattoirs, backyard flocks, and live bird markets located in different governorates are sampled on a monthly basis regardless of the presence of disease symptoms in poultry. In each governorate, the same locations are continuously sampled by a poultry veterinarian, who collects 1 swab sample per bird; depending on the size of the poultry population, as many as 5 birds are sampled per flock. Birds are not randomly selected, and samples also are collected from sick or dead birds found onsite. Samples are mostly from chickens, but ducks, geese, turkeys, pigeons, and quails also are sampled. This program enabled detection of genetic and antigenic changes in H5N1 viruses in Egypt. It provided important epizootiologic data and described the poultry sectors acting as reservoirs of H5N1 viruses.

The rate of avian influenza infection during August 2009–July 2010 was 5%, was exclusively attributable to H5N1 infection, and was more concentrated in the commercial production sector (*14*). From August 2010 through January 2013, the positivity rate increased to 10%, and the infection was detected in all poultry production sectors (*6*). In 2011, H9N2 viruses emerged and were detected by this program and other surveillance activities in Egypt (*6*,*15*,*16*). The monthly positivity rate of avian influenza infection from August 2009 through December 2014 (Figure 1) showed that avian influenza infection in poultry follows a seasonal pattern, with sharp increases during the colder months (November through March). After the detection of H9N2 virus in 2011, the positivity rate during colder months was higher than that in the period when H5N1 was the only virus infecting poultry, exceeding 20% in some months. Furthermore, co-infection with H5N1 and H9N2 viruses was frequently detected (*6*).

We further analyzed data collected through the surveillance program during February 2013–December 2014, when 4,858 cloacal and 3,049 oropharyngeal samples were collected (range 120–700 samples monthly). The positivity rate for any avian influenza infection was 4.7%. A higher rate of infection was observed in oropharyngeal swab samples. Detection rates differed significantly by governorate, species, and poultry production sector. No significant differences by the birds' health status or age were observed (Table 1). The same seasonal pattern observed during August 2009–July 2010 (Figure 1) was observed during this period (Figure 2). H9N2 virus was more frequently detected as a single virus causing infection or as co-infecting the same bird with H5N1 virus (Figure 3). Both H5N1 and H9N2 viruses were circulating in the more recent surveillance months, when more human cases were reported.

Genetic Analysis

Phylogenetic analysis of all the HA genes of H5N1 viruses in Egypt available in GenBank shows considerable evolution over time (Figure 4). H5N1 was first detected in 2006 and remained relatively stable until 2008. In 2008, subclade 2.2.1.1 emerged and continued in circulation until early 2011. At the same time, clade 2.2.1 viruses also were in

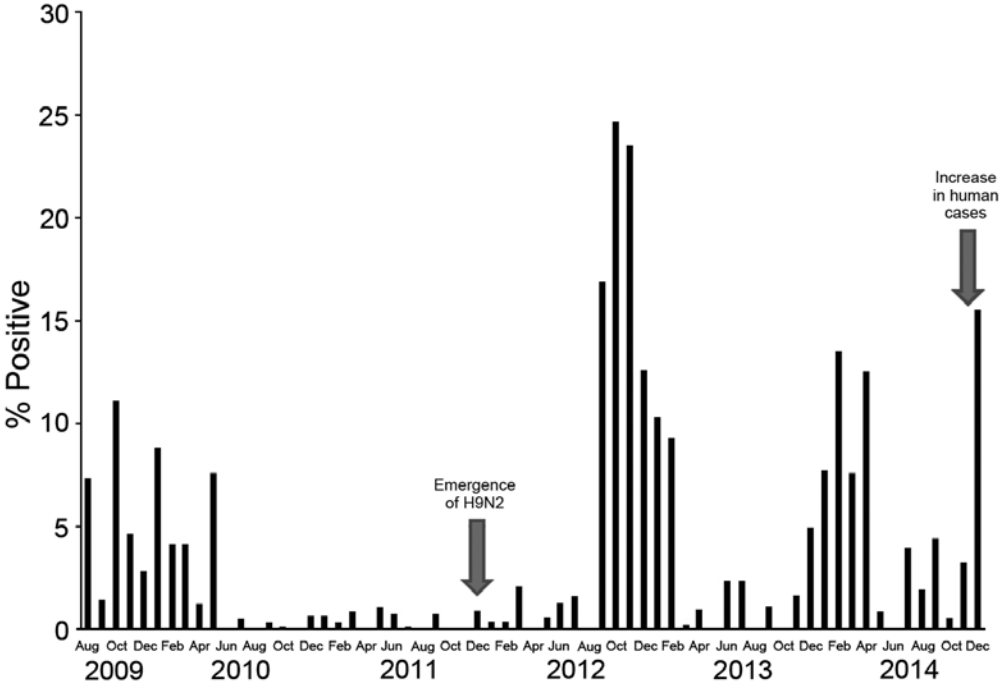

Figure 1. Monthly positivity rate of poultry infection with avian influenza viruses (all types), Egypt, August 2010–December 2014. A seasonal pattern is shown by sharp increases in rates during colder months (November–March). Emergence of H9N2 virus in poultry and an increase in human H5N1 cases are indicated.

Table 1. Epizootiologic data on avian influenza viruses (all types), Egypt, February 2013–December 2014*

Variable	Collected samples, no. (%)†	Influenza A–positive samples, no. (%)‡	p value§
Sample type			
Cloacal	4,858 (61.4)	112 (2.3)	<0.01
Oropharyngeal	3,049 (38.6)	234 (7.7)	
Governorate			
Cairo	1,116 (14.1)	9 (0.8)	<0.01
Daqhaliya	2,031 (25.7)	136 (6.7)	
Qalubiya	809 (10.2)	22 (2.7)	
Menofiya	13 (0.2)	0	
Sharqiya	2,160 (27.3)	123 (5.7)	
Fayyoum	1,642 (20.8)	48 (2.9)	
BeniSuef	30 (0.4)	4 (13.3)	
Asiut	69 (0.9)	4 (5.9)	
El Minya	38 (0.5)	0 (0)	
Species			
Chickens	6,863 (86.8)	322 (4.7)	0.01
Ducks	606 (7.7)	15 (2.5)	
Geese	58 (0.7)	0	
Pigeons	243 (3.1)	2 (0.8)	
Turkey	57 (0.7)	1 (1.8)	
Quail	80 (1.0)	6 (7.5)	
Location			
Abattoir	150 (1.9)	0	0.01
Commercial farm	4,359 (55.1)	200 (4.6)	
Backyard flock	1,678 (21.2)	61 (3.6)	
Live bird market	1,720 (21.8)	85 (4.6)	
Bird health status			
Healthy	5,799 (73.3)	238 (4.1)	NS
Ill	1,629 (20.6)	86 (5.3)	
Dead	479 (6.1)	22 (4.6)	
Age group, y			
0–1	7,819 (98.9)	342 (4.4)	NS
>1	88 (1.1)	4 (4.5)	

*NS, not significant.
†Percentage of total samples collected.
‡Of samples in category.
§By χ^2 test comparing positivity rates across variable categories.

circulation. Within this group, further drift was observed, and as of 2011, viruses grouped together and formed a new cluster characterized by a set of mutations (*5*). Viruses from late 2013 and 2014 branched together within this new cluster. Some avian H5N1 viruses in 2014 possessed mutations R140K in antigenic site A and A86V in antigenic site E of the HA gene, similar to other viruses in the new cluster. The new cluster was classified as clade 2.2.1.2 (*17*).

Phylogenetic analysis of the neuraminidase (NA) gene and the internal genes showed a similar pattern of evolution as that for the HA gene (online Technical Appendix, http://wwwnc.cdc.gov/eid/article/22/3/15-0593-Techapp1.pdf). Currently circulating viruses belong to the new cluster. No major mutations were observed in viruses circulating during 2013–2014.

Genetic analysis of the H9N2 viruses in poultry in Egypt showed that those viruses belonged to the G1 lineage. Also, they possessed several genetic markers of increased transmission to mammalian hosts (*18*).

Antigenic Analysis

The genetic drift of H5N1 viruses in Egypt led to antigenic variability. When tested against a panel of H5N1 virus monoclonal antibodies, subclades 2.2.1 and 2.2.1.1 are antigenically distinct (*6,10,11*). A more recent analysis revealed that several strains from 2013 and 2014, especially those with the R140K mutation in the HA gene, had a distinct antigenic composition compared with other viruses of the new cluster (Figure 5).

Human Infection with H5N1 Viruses

Human Cases

During 2006–2015, the estimated number of confirmed human cases of H5N1 infection in Egypt was 292, with a 34% case-fatality rate. The number of reported cases by year for 2006–2015 (Figure 6) shows that, before the 2014–15 winter season, the annual number of cases never exceeded 40. However, in just 2 months, January and February 2015, a total of 88 cases were reported. The most recent cases occurred in persons who reported exposure to backyard poultry (70%), bred domestic birds (26%), slaughtered poultry (14%), or were exposed to dead birds (4%). The main clinical signs and symptoms were fever (98%), sore throat (94%), and cough (83%). Overall, the case-fatality rate was lower than the 67% calculated for human cases

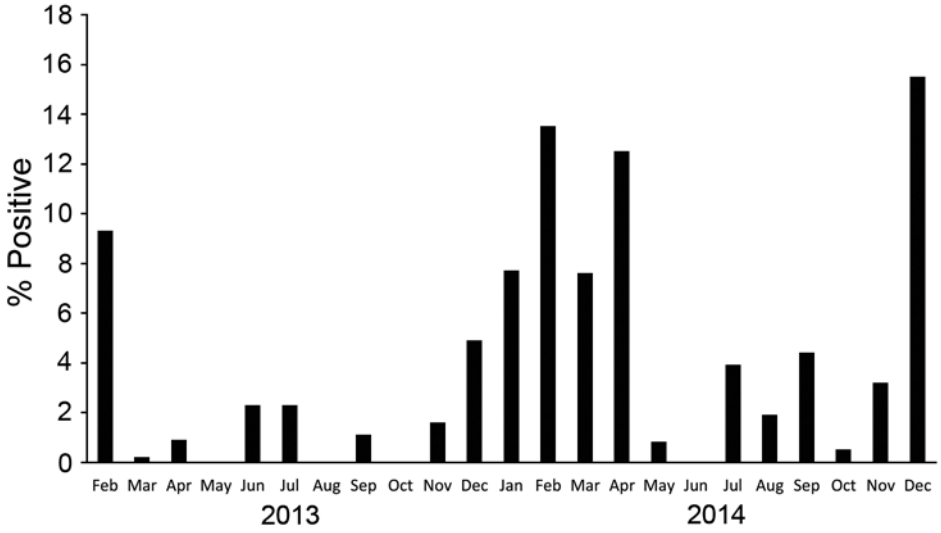

Figure 2. Monthly positivity rate of infection with avian influenza viruses (all types), Egypt, February 2013–December 2014. As in Figure 1, a seasonal pattern is shown by sharp increases in rates during colder months (November–March).

globally, excluding those from Egypt. Within Egypt, the case-fatality rate annually ranged from 10% to 75%.

Characterization of Recent Human Viruses

We sequenced the full genomes of 2 H5N1 viruses isolated from infected humans in Egypt during November 2014 (A/Egypt/MOH-NRC-7271/2014 and A/Egypt/MOH-NRC-7305/2014; GenBank accession nos. KP7022162–KP7022177). We also sequenced the HA segment of a third virus, A/Egypt/MOH-NRC-8434/2014 (GenBank accession no. KR063683.1), isolated from a human in December 2014. In addition, we sequenced 2 poultry viruses (A/duck/Egypt/A10353A/2014 and A/chicken/Egypt/A10351A/2014) obtained from the same places around the same time the human cases were detected (Figure 4). The poultry and human viruses branched together. The strains of human influenza viruses from Egypt carried several mutations that were novel or rare in previously circulating strains and have not been characterized before. Two viruses had mutations M66I, I529V, and E249K in the polymerase basic 2 gene. These mutations were present in 1 virus isolated from a chicken in November 2014. Mutations M66I and I529V were previously seen in chicken viruses from January 2014. Mutation I529V was previously seen in a single chicken isolate from 2013.

Sequencing results for the 2 human viruses isolated in November 2014 for which the full genome sequences were obtained showed the presence of mutation G22E in the polymerase basic 1–frameshift 2 (PB1-F2) gene; the mutation was not found in other viruses. Sequencing results also showed 3 unique mutations in the polymerase protein of the 2 viruses: L342M, E351D, and F708L.

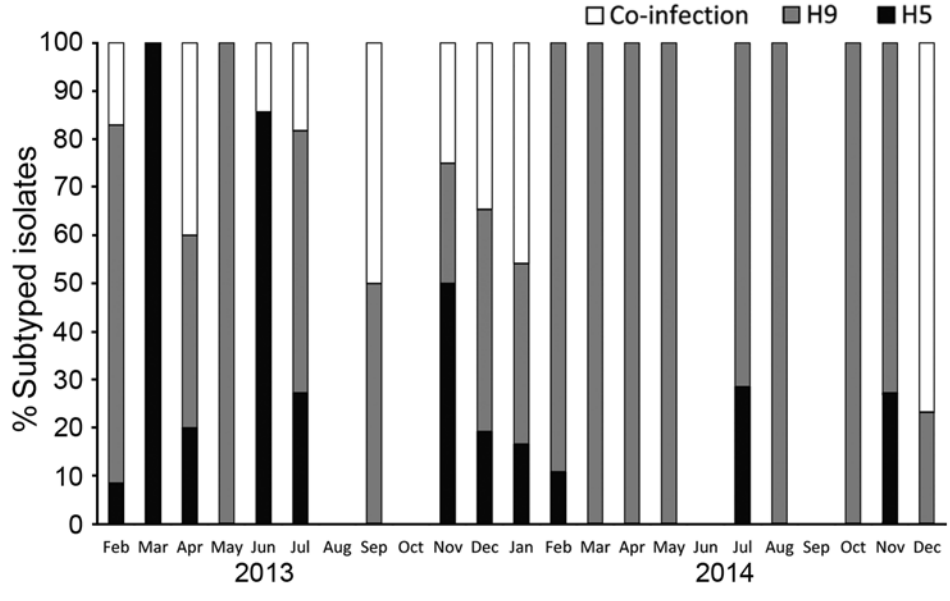

Figure 3. Subtypes of influenza A viruses detected in poultry by using reverse transcription PCR, by month, Egypt, February 2013–December 2014.

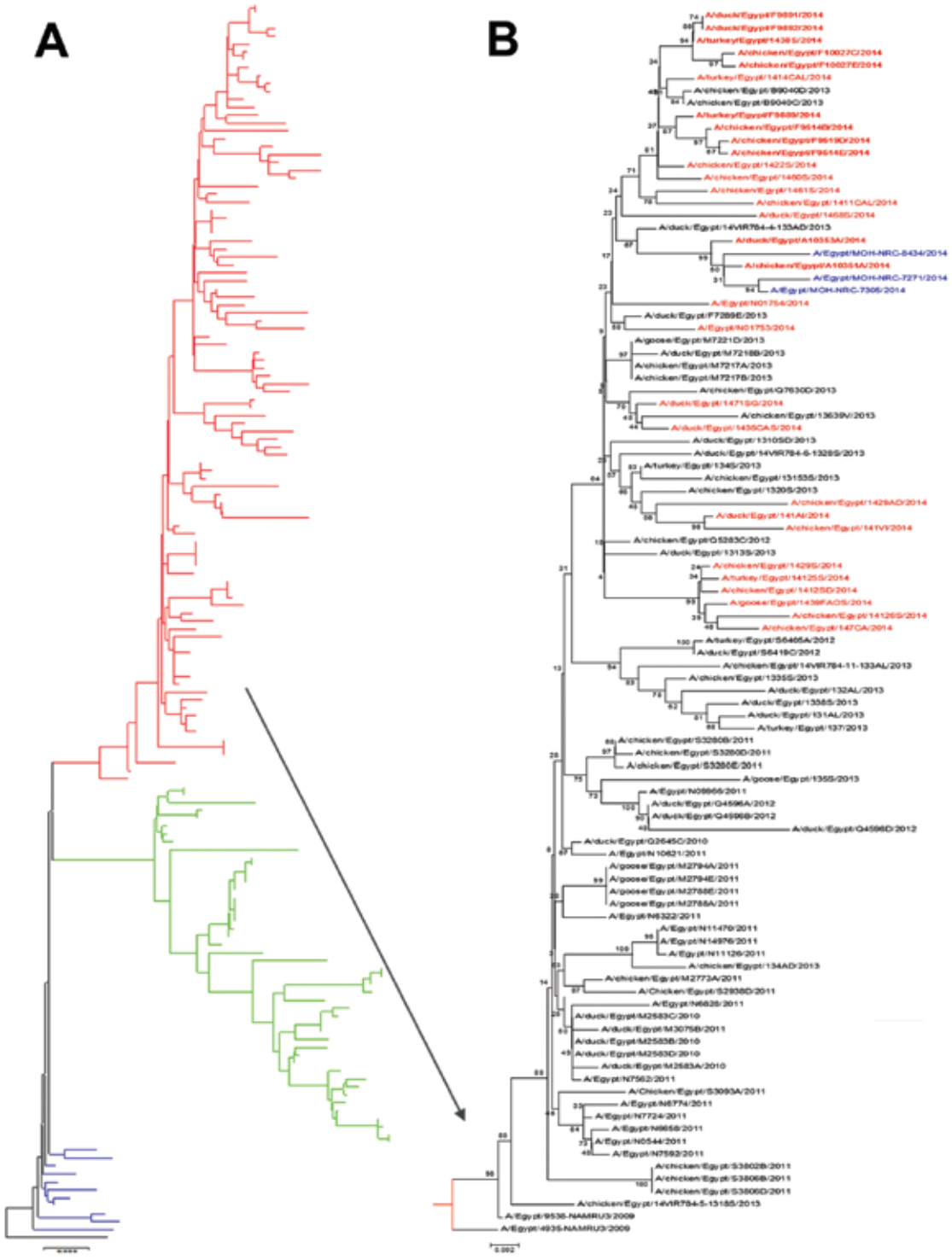

Figure 4. Phylogenetic tree of the hemagglutinin genes of avian influenza subtype H5N1 viruses isolated in Egypt during 2006–2014 and reference isolates from GenBank. Phylogenetic analysis was conducted by using the neighbor-joining algorithm with the Kimura 2-parameter model. Strain A/bar-headed goose/Qinghai/3/2005 was used as the root for the tree, and the reliability of phylogenetic inference at each branch node was estimated by the bootstrap method with 1,000 replications. Evolutionary analysis was conducted by using MEGA6 (http://www.megasoftware.net). A) Clade 2.2 viruses from 2006–2008 are shown in blue, subclade 2.2.1.1 viruses are shown in green, and clade 2.2.1 viruses are shown in red. B) Human viruses sequenced for this study are shown in blue. Boldface red font indicates avian viruses isolated in 2014 and sequenced for this study; lightface red font indicates other viruses from GenBank. *Indicates that 2014 viruses were grouped in 1 lineage. Scale bars indicate nucleotide substitutions per site.

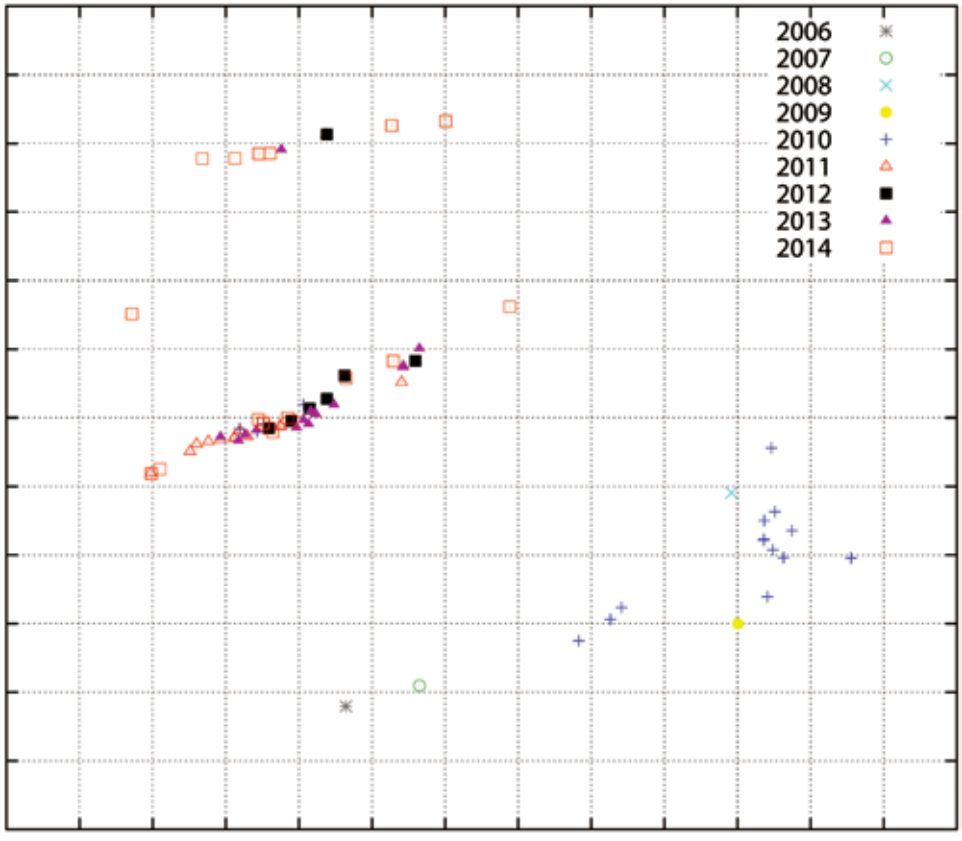

Figure 5. Antigenic cartography of reactivity of highly pathogenic avian influenza A(H5N1) virus isolates from Egypt, 2006–2014. The map was produced by using hemagglutination inhibition assay data generated with a panel of monoclonal antibodies and by using AntigenMap (http://sysbio.cvm.msstate.edu/AntigenMap). One unit (grid) represents a 2-fold change in the assay results. Each mark on the map represents results for 1 isolate.

These mutations were also seen in a chicken virus obtained during the same month that the humans became ill, and mutations L342M and E351D were previously seen in chicken viruses isolated in January 2014. Mutation K373R was common in the HA protein of all 3 human viruses and was previously observed in a chicken virus from the same period and in 2 human viruses isolated in 2009. The NA protein of the 2 fully sequenced human viruses had novel mutations V43I, I94V, V264I, and V304I, which were also present in the chicken isolate of the same period. One novel mutation, R452K, was observed in the nucleoprotein gene of the 2 fully sequenced viruses; this mutation was also seen in the chicken isolate of the same period, 2 chicken isolates from January 2014, and 1 chicken isolate from 2013.

The results of our genetic analysis indicate that the viruses infecting humans in November and December 2014 had a genetic composition almost exactly the same as that of the avian viruses circulating at that time. These human and avian viruses had a set of mutations throughout the genome that places the viruses on the same phylogenetic branch (online Technical Appendix). The role of these mutations, whether individually or in combination, is not known. Determining whether the mutations we found

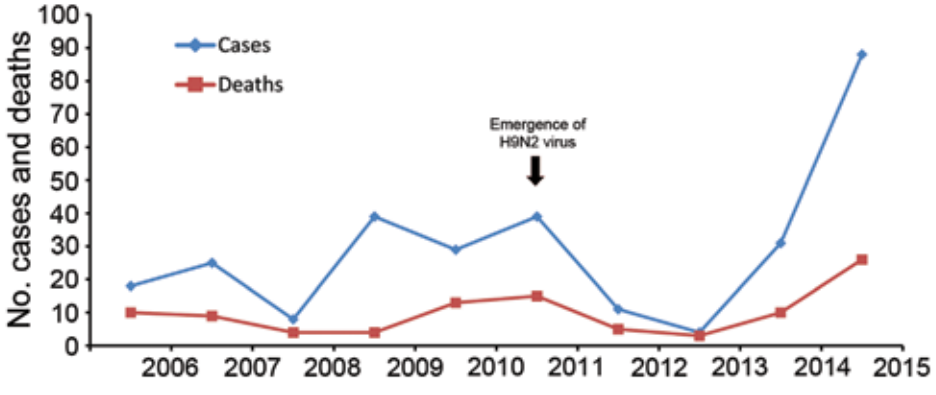

Figure 6. Human cases of avian influenza A(H5N1) virus infection and associated deaths, Egypt, 2006–2015. Data for 2015 include cases confirmed in January and February only. For reference, the emergence of H9N2 virus in poultry is shown (arrow).

contributed to the recent increase in human H5N1 infections in Egypt would require basic science research that might fall under the "gain of function" category, in which viruses are genetically manipulated under laboratory conditions to study their effects on mammalian hosts.

Extent of Avian Influenza Infection in Egypt

In Egypt, the number of reported human cases of avian influenza infection appears to be underestimated. An underestimation might result in an overestimation of the case-fatality rate, but it would certainly underestimate the extent

Table 2. H5 commercial inactivated oil-emulsion vaccines used for immunization of poultry against avian influenza A(H5N1) virus, Egypt, 2006

Vaccine trade name	Virus used	Lineage	Sequence similarity, %	Manufacturer, city, country
AI-VAC H5	A/chicken/Italy/22A/1998(H5N9)	Classical	90.7	FATRO, Ozzano dell'Emilia, Italy
CEVAC FLUKEM	A/chicken/Mexico/232/1994(H5N2)	Classical	84	Ceva, Mexico City, Mexico
VOLVAC IV KV	A/chicken/Mexico/232/1994(H5N2)	Classical	84	Boehringer Ingelheim, Ingelheim am Rhein, Germany
AIV Vaccine	A/turkey/Engl and /N28/1973(H5N2)	Classical	91.4	Yebio, Qingdao, China
AIV Vaccine	A/turkey/Minnesota/3689–1551/1981(H5N2)	Classical	89.8	Lohmann, Waterville, Maine, United States
Nobilis Influenza H5N2	A/duck/Potsdam/1402/1986(H5N2)	Classical	91.6	Merck, Kenilworth, New Jersey, United States
Optimune AIV	A/turkey/Wisconsin/1968(H5N9)	Classical	88.3	Ceva Biomune, Mexico City, Mexico
Avian Influenza H5	A/chicken/Mexico/232/1994(H5N2)	Classical	84	Avimex Animal Health Mexico City, Mexico
VOLVAC IV +ND KV	A/chicken/Mexico/232/94(H5N2) and Lasot and V	Classical	84	Boehringer Ingelheim, Ingelheim am Rhein, Germany
CEVAC NEW FLU-KEM	A/chicken/Mexico/232/94(H5N2) and Lasot and V	Classical	84	Ceva, Mexico City, Mexico
ITA FLU	A/chicken/Mexico/232/1994(H5N2)	Classical	84	Laprovet, Notre-Dame-d'Oé, France
Reassortant AIV (subtype H5N1) Vaccine (strain Re-1)	RGA/goose/Guangdong/1996(H5N1)(Re-1)	Clade 0	93.8	Zhaoqing DaHuaNong Biology Medicine, Sihui, China
Reassortant AIV (Subtype H5N1) Vaccine (srain Re-1)	RGA/goose/Guangdong/1996(H5N1)(Re-1)	Clade 0	93.8	Yebio, Qingdao, China
PoulvacFluFend H5N3 RG	RGA/chicken/VN/C58/2004(H5N3)	Clade 1	95.1	Fort Dodge Animal Health, Fort Dodge, Iowa, USA
MEFLUVAC	RGA/chicken/Egypt/Q1995D/2010(H5N1) and RGA/chicken/Egypt/M2583D/2010(H5N1)	Clade 2.2	93.8 and 99.6	ME-VAC, Cairo, Egypt
SER-VACC FLU	RGA/chicken/Egypt/M2583D/2010(H5N1)	Clade 2.2	99.6	Veterinary Serum and Vaccine Institute, Cairo, Egypt
ME FLUVAC H5+H9	RGA/chicken/Egypt/Q1995D/2010(H5N1) and A/chicken/Egypt/114940v/NLQP/2011(H9N2)	Clade 2.2	93.8	ME-VAC, Cairo, Egypt
ME FLUVAC One	RGA/duck/Egypt/M2583D/2010(H5N1)	Clade 2.2	99.6	ME-VAC, Cairo, Egypt
ME FLUVACH5+ ND	RGA/chicken/Egypt/Q1995D/2010(H5N1) and NDV/chicken/Egypt/11478AF/2011(ND)	Clade 2.2	93.8	ME-VAC, Cairo, Egypt
ME FLUVAC Super H5 +H9+ ND	RGA/chicken/Egypt/Q1995D/2010(H5N1), RGA/duck/Egypt/M2583D/2010(H5N1), A/chicken/Egypt/114940v/NLQP/2011(H9N2), and NDV/chicken/Egypt/11478AF/2011(ND)	Clade 2.2	93.8 and 99.6	ME-VAC, Cairo, Egypt
Egy FLU	RGA/chicken/Egypt/18-H/2009(H5N1)	Clade 2.2	94.9	Harbin Veterinary Research Institute, Harbin, China
Inactivated Reassortant Avian Influenza Virus Vaccine (H5N1 Subtype, Re-6 Strain)	RGA/duck/Guangdong/S1322/2006(H5N1)(Re-6)	Clade 2.3.2	Not performed	Yebio, Qingdao, China
Reassortant AIV (Subtype H5N1) Vaccine (Strain Re-5)	RGA/duck/Anhui/1/2006(H5N1)(Re-5)	Clade 2.3.4	94.9	Merial, Duluth, Georgia, United States
Reassortant AIV (Subtype H5N1) Vaccine (Strain Re-5)	RGA/duck/Anhui/1/2006(H5N1)(Re-5)	Clade 2.3.4	94.9	QYH, Beijing, China

of human infection with avian influenza viruses. Results from a controlled, serologic cohort study of persons in Egypt exposed and not exposed to poultry estimated the seroprevalence of antibodies against H5N1 (titers >80) at 2% (*19*). If this seroprevalence were to be extrapolated to the entire poultry-exposed population in Egypt, the true number of infections would amount to several hundred thousand. These figures are even more striking when it comes to human infection with H9N2 viruses. The seroprevalence of H9N2 antibodies detected in the same cohort study (*19*) ranged from 5.6% to 7.5%, whereas just 1 case of H9N2 infection was reported.

H5N1 viruses elicit a poor humoral immune response, providing low antibody titers that typically fade over a short period (*20*,*21*). Thus, relying on serologic testing to detect prevalence or incidence of infection can yield underestimated results. This outcome was evident when we used a microneutralization assay to test serum samples from 38 contacts of persons with confirmed H5N1 infection; no antibodies against H5N1 virus were found, but 5 contacts had low levels (<1:20) of antibodies against H9N2 virus. This finding suggests that the extent of avian influenza infection in humans is even higher than what is currently thought. Human genetic predisposition to infection with avian influenza viruses is an important epidemiologic question that is not well studied, although some reports suggest that genetics play a role in susceptibility to infection (*22*,*23*). Hence, estimating the true incidence of human infection with avian influenza viruses and determining the accompanying risk factors need further study.

H5 Influenza Vaccines for Poultry

As of 2006, at least 24 commercial inactivated avian influenza H5 vaccines were licensed for use at poultry farms in Egypt (Table 2). Different viruses were used as vaccine seed strains, including classical H5 lineage viruses and reverse genetics–engineered reassortant viruses containing H5N1 virus HA and NA genes and the remaining genes from A/Puerto Rico/8/1934(H1N1). Farm owners decide which vaccine to use, if any. Amino acid sequence similarities between vaccine strains and the consensus sequence of H5N1 isolates circulating in Egypt during 2013–2014 ranged from 84.0% to 99.6% of A/chicken/Mexico/232/94 (H5N2) and reverse genetics–engineered A/chicken/Egypt/M2583D/2010 (H5N1), respectively. Serum samples obtained from chickens vaccinated with commercial vaccines or an experimental vaccine based on clade 2.2.1 A/chicken/Egypt/M7217B/2013(H5N1) were tested against H5N1 viruses isolated in Egypt during 2006–2014 (Figure 7). Commercial vaccines showed variable reactivity against earlier antigens, but reactivity declined as the virus mutated. The experimental vaccine was highly reactive with all antigens, especially for more recent viruses. The genetic dissimilarity and poor reactivity between commercial vaccines and currently circulating viruses indicate that the vaccines are not efficacious in the field. These vaccines confer partial protection and thus might lead to vaccine-induced escape mutants, thereby complicating, rather than solving, the problem of H5N1 virus circulation in Egypt. Previous reports have indicated that improper antigenic matching between vaccines and circulating viruses might reduce vaccine efficacy (*24*–*27*).

All vaccines used in Egypt are licensed by the Ministry of Agriculture and Land Reclamation on the basis of laboratory evaluation results. For influenza virus vaccines, this evaluation involves vaccinating poultry and challenging them with an H5N1 virus. Until recently, the challenge virus was a 2008 H5N1 virus isolate, A/chicken/Egypt/1709-6/2008(H5N1) (GenBank accession no. EU 717857).

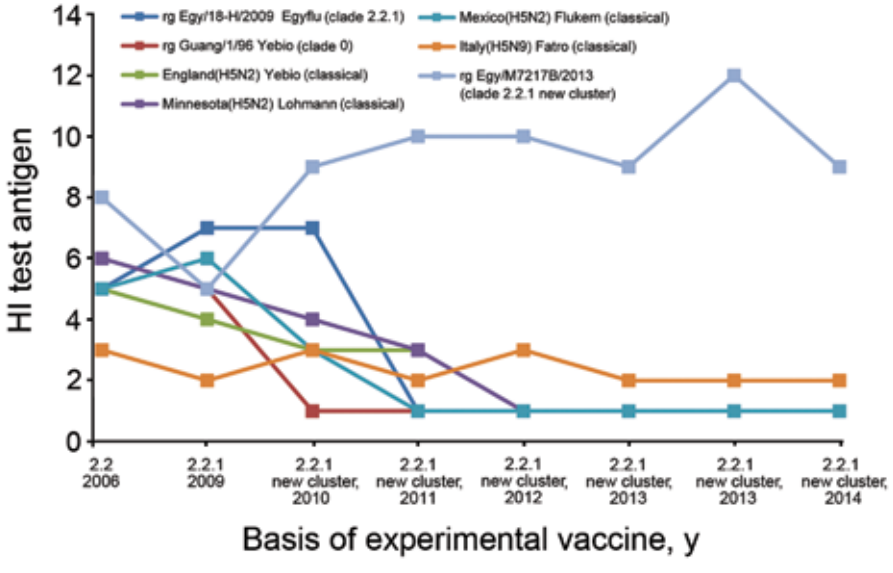

Figure 7. Cross-reactivity of antisera raised against commercial and experimental inactivated H5 vaccines against avian influenza A(H5N1) virus isolates from Egypt, 2006–2014. Antisera from chickens immunized with the H5 vaccines were tested by using a hemagglutination inhibition (HI) assay against virus isolates from Egypt during 2006–2014 (x-axis). Egy, Egypt; Guang, Guangdong; rg, reverse genetics–engineered reassortant.

Against the 2008 isolate, those vaccines were efficacious in the laboratory setting but not in the field because the circulating viruses were not antigenically matched to the vaccine seed strains, highlighting the failure of the only tool used to control avian influenza among poultry in Egypt.

Proposed Avian Influenza Control Plan

The avian influenza situation in Egypt is deteriorating, evident in the fact that H5N1 and H9N2 viruses are enzootic in poultry and that incidence of H5N1 and H9N2 infection is increasing. The problem is also evident in the sharp increase in human H5N1 cases and the detection of the first human H9N2 case. Thus, it is imperative that authorities in Egypt devise and implement an aggressive control plan to curb the spread of disease in human and animal populations. The control plan must include the following elements: 1) mapping of the unlicensed, small-scale poultry farms that have become abundant in rural areas; 2) increasing the biosecurity levels of these small farms by using inexpensive tools; 3) revamping veterinary and public health surveillance and conducting joint human–animal interface surveillance and risk-assessment exercises; 4) encouraging poultry owners to report outbreaks and providing them appropriate compensation; 5) intervening, when poultry outbreaks are reported, by culling infected poultry and setting monitoring zones around each focus point; 6) properly decontaminating infected farms; 7) encouraging the use of disinfectants in backyards where poultry are raised; 8) increasing awareness about the effects of avian influenza; 9) testing patients with suspected influenza forH5N1 and H9N2 virus; and 10) reevaluating the vaccination strategy, including that for H9N2 virus. If vaccination is to remain an important tool in the control plan, then the following aspects should be considered: 1) matching vaccine strains to currently circulating strains; 2) matching challenge strains to currently circulating strains; 3) maintaining high vaccination coverage; 4) ensuring vaccine efficacy not only in a laboratory setting but also in the field; and 5) evaluating vaccine efficacy on an annual basis.

Conclusions

Egypt is one of the few countries where H5N1 virus has become enzootic and is the only country with a high number of H5N1 outbreaks among poultry and cases among human. During the 2014–15 winter season, a sudden and substantial increase in human infection with H5N1 viruses was observed. There is no obvious or confirmed reason for this increase, but data indicate the following: 1) H9N2 virus is co-circulating and co-infecting with H5N1 viruses, 2) H5N1 viruses causing the infections possess some mutations that were rarely seen in the past, and 3) the poultry vaccination program is failing. However, our perspective was limited to the data available through our surveillance program, which might not be representative of the epizootiology of avian influenza virus in Egypt. Regardless of the causes of the recent increase in human H5N1 cases, this situation evolved because of the ineffective control strategy that was implemented. Controlling the situation requires a One Health approach, but certainly the greater share of responsibility now lies with the veterinary side.

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Dr. Kayali is a staff scientist at the St. Jude Children's Research Hospital, Memphis, TN. His research interests are the epidemiology of influenza and viral zoonotic diseases.

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Addresses for correspondence: Ghazi Kayali, Division of Virology, Department of Infectious Diseases, St. Jude Children's Research Hospital, 262 Danny Thomas Pl, Memphis, TN 38105, USA; email: ghazi.kayali@stjude.org

Patient Report and Review of Rapidly Growing Mycobacterial Infection after Cardiac Device Implantation

Varun K. Phadke, David S. Hirsh, Neela D. Goswami

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Learning Objectives

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

- Determine the clinical and etiologic considerations regarding mycobacterial cardiac implantable electronic device infections, based on a case report and review
- Identify antibiotic resistance of mycobacterial cardiac implantable electronic device infections
- Evaluate management of mycobacterial cardiac implantable electronic device infections

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Mycobacterial infections resulting from cardiac implantable electronic devices are rare, but as more devices are implanted, these organisms are increasingly emerging as causes of early-onset infections. We report a patient with an implantable cardioverter-defibrillator pocket and associated bloodstream infection caused by an organism of the *Mycobacterium fortuitum* group, and we review the literature regarding mycobacterial infections resulting from cardiac device implantations. Thirty-two such infections have been previously described; most (70%) were caused by rapidly growing species, of which *M. fortuitum* group species were predominant. When managing such infections, clinicians should consider the potential need for extended incubation of routine cultures or dedicated mycobacterial cultures for accurate diagnosis; combination antimicrobial drug therapy, even for isolates that appear to be macrolide susceptible, because of the potential for inducible resistance to this drug class; and the arrhythmogenicity of the antimicrobial drugs traditionally recommended for infections caused by these organisms.

Author affiliation: Emory University, Atlanta, Georgia, USA

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Infection is an uncommon but potentially devastating complication of cardiac implantable electronic device (CIED) implantation (*1*). Staphylococcal species cause most of these infections, followed by other pyogenic bacteria (*1*). CIED infections caused by mycobacteria have been reported infrequently; most of these infections result from the rapidly growing nontuberculous mycobacteria. We report a patient with an infection caused by a rapidly growing mycobacterium (RGM) in the *Mycobacterium fortuitum* group that developed following placement of an implantable cardioverter-defibrillator (ICD). We also review the published literature of cardiac device–associated infections caused by mycobacteria, focusing particularly on RGM.

Patient Report

A 60-year-old man with chronic systolic heart failure, emphysema, and hepatitis C infection was admitted to Grady Memorial Hospital (Atlanta, Georgia, USA) with 2 days of purulent drainage from his ICD pocket site. Just before his admission, pain had developed, followed by spontaneous dehiscence (i.e., separation of the surgical incision along the suture line) of the pocket. He reported no fever or constitutional symptoms. Two months earlier, a single-chamber ICD had been inserted uneventfully in his left prepectoral region after he had an episode of ventricular fibrillation that led to cardiac arrest. At the time of device implantation, he had been receiving intravenous vancomycin and piperacillin/tazobactam for concomitant hospital-acquired pneumonia, and he was prescribed oral cephalexin for 5 days after the procedure. During outpatient follow-up visits at weeks 2 and 4 after device insertion, the patient's wound was noted as unremarkable.

At the time of the new admission, the patient was afebrile with unremarkable vital signs. Physical examination showed mild, nontender edema over the ICD pocket; a 1-cm, shallow ulceration of the incision site; scant serous drainage; and minimal surrounding erythema. The patient had no peripheral stigmata of infective endocarditis, and the remainder of the examination was unremarkable. Laboratory studies showed a normal leukocyte count (5,500 cells/μL) with a slight (70%) neutrophil predominance. Blood cultures were collected, and he was started empirically on intravenous vancomycin for a suspected pocket infection. Transthoracic echocardiography was performed and showed no valvular vegetations. A transesophageal echocardiogram was deferred because of considerable laryngeal stenosis caused by traumatic endotracheal intubation at the time of his recent cardiac arrest.

On hospital day 2, the entire device, including the ICD generator and leads, was removed and sent for culture. Purulent fluid and necrotic tissue were noted in the pocket during explantation, and a drain was left in place at the time of wound closure (Figure, panel A). Gram stain of the pocket exudate showed beaded gram-positive rods. Within 5 days, the generator pocket tissue culture and 1 of 2 sets of blood cultures were growing an aerobic, gram-positive rod that also appeared beaded on Gram stain. Culture of the lead tip remained sterile. Because of the Gram-stain appearance of the blood culture isolate, an acid-fast stain was performed in the microbiology laboratory, and results showed the organism to be acid fast. At the request of the inpatient infectious diseases consultation service, the isolate was subcultured to mycobacterial growth media and was identified as an RGM. An HIV test result was negative.

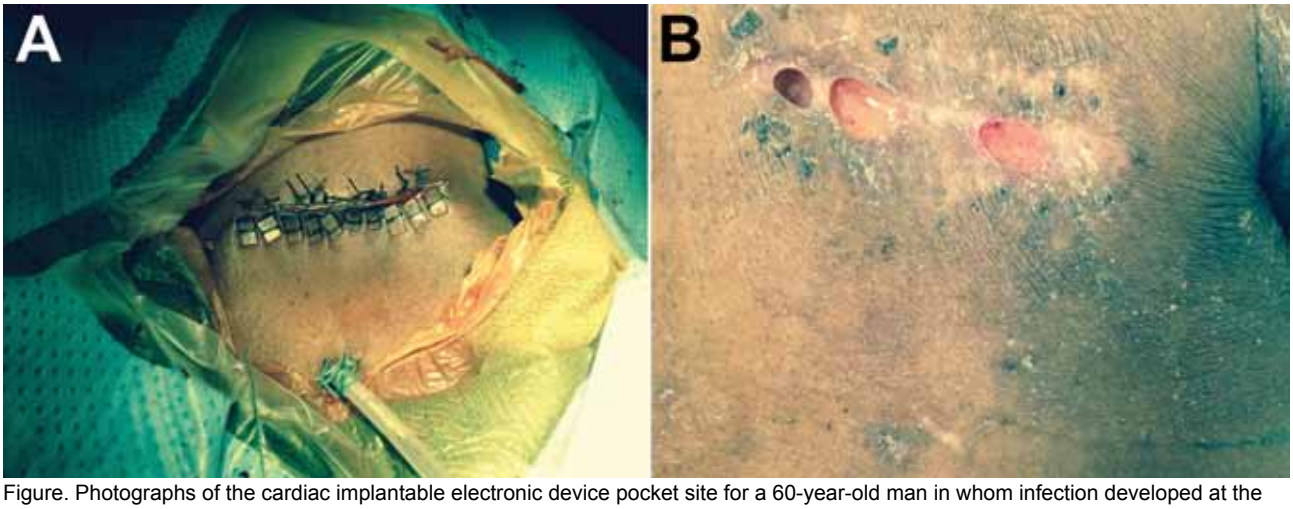

Figure. Photographs of the cardiac implantable electronic device pocket site for a 60-year-old man in whom infection developed at the implantation site of a cardiac implantable electronic device, Atlanta, Georgia, USA. A) Device pocket site after explantation. The wound was closed with pledged Ethibond sutures (Ethicon, Somerville, NJ, USA), and a Jackson-Pratt drain (closed-suction drainage system consisting of an internal drain connected by plastic tubing to a flexible bulb) was tunneled into the inferior aspect of the pocket. The drain was removed 24 hours postoperatively, and a small incision was left open to heal by secondary intention. B) Device pocket site 6 weeks after suture removal. Most of the incision healed well, with evidence of localized dehiscence (i.e., spontaneous partial separation of the surgical incision along the suture lines).

The patient's antimicrobial drug regimen was empirically changed to intravenous cefoxitin, oral ciprofloxacin, and oral clarithromycin, a combination selected because of a clinical suspicion of *M. fortuitum* infection, surmised from the limited literature on RGM-associated CIED. Because of uncertainty about the capacity for outpatient therapeutic drug monitoring and because the patient had undergone surgical debridement and was thought to be clinically improved at the time of discharge, intravenous aminoglycoside therapy was deferred pending species identification and test results regarding antimicrobial drug susceptibility. A baseline electrocardiogram obtained before initiation of antimicrobial drugs showed a corrected QT interval (i.e., duration from start of Q wave to end of T wave in the heart's electrical cycle) of 511 ms (reference <430 ms).

One week after device extraction, a computed tomography scan of the patient's chest was performed, and results showed a residual, subclinical, peripherally enhancing, 2.7 × 1.0–cm collection of air and fluid over the left pectoralis muscle. Immediate device reimplantation was deferred. He was discharged on hospital day 14 with a wearable cardioverter-defibrillator to use until his physicians believed that device reimplantation was safe. The therapeutic plan at discharge was for at least 6 months of antimicrobial drug therapy, beginning with the cefoxitin, ciprofloxacin, and clarithromycin regimen and subsequently tailored to the organism and susceptibility profile when these data became available.

Five weeks after device removal, the patient returned for outpatient follow-up and reported full adherence to his prescribed antimicrobial drug regimen. On examination, most of the wound over the previous device pocket had healed, but localized dehiscence and ongoing purulent drainage was evident (Figure, panel B). He was referred to plastic surgery for ongoing management of his wound. Identification and antimicrobial drug susceptibilities of the organism were still pending, so his antimicrobial drug regimen was not changed. Unfortunately, he was lost to follow-up; 2 months later, he discontinued use of his wearable cardioverter-defibrillator and died of out-of-hospital cardiac arrest.

By use of high-performance liquid chromatography, the patient's isolate was identified as a *Mycobacterium fortuitum* group organism. Further genetic testing for species-level identification was not performed. The following susceptibilities were identified: cefoxitin intermediate (MIC 64 μg/mL), ciprofloxacin susceptible (MIC ≤0.12 μg/mL), and clarithromycin resistant (MIC 8 μg/mL after 14 days of incubation) (Table 1).

Table 1. Antimicrobial drug susceptibility profile of patient's *Mycobacterium fortuitum* group isolate*

Antimicrobial drug	MIC, μg/mL	Interpretation*
Amikacin	≤1	Susceptible
Cefoxitin	64	Intermediate
Ciprofloxacin	≤0.12	Susceptible
Clarithromycin	8	Resistant†
Doxycycline	>16	Resistant
Imipenem	8	Intermediate
Linezolid	2	Susceptible
Moxifloxacin	≤0.25	Susceptible
Tigecycline	0.12	‡
Trimethoprim/sulfamethoxazole	1/19	Susceptible
Tobramycin	>16	Resistant

*According to breakpoints defined by the Clinical and Laboratory Standards Institute (*2*).
†Clarithromycin MIC after 14 d of incubation.
‡No accepted breakpoints from the Clinical and Laboratory Standards Institute exist for tigecycline.

Discussion

The RGM species are ubiquitous environmental organisms that have been isolated from soil, food, natural and municipal water, various plants and animals, and hospital surfaces (*3*). These organisms are not believed to be permanent members of the human bacterial flora but often become transient colonizers after frequent exposure. The most commonly encountered RGM species in clinical practice are *M. abscessus*, *M. chelonae*, and *M. fortuitum*, but >100 species have been identified (*3*). These organisms are capable of growth on standard mycobacterial (e.g., Middlebrook 7H11 or Lownstein-Jensen) and routine bacteriologic (e.g., sheep's blood and MacConkey agar) growth media. However, colonies may take ≥5 days to appear on standard media, exceeding the incubation time of routine cultures in many clinical microbiology laboratories. Even when growth is observed, these organisms often appear as beaded gram-positive bacilli on routine Gram stain and may be misidentified as contaminants (*4*). Therefore, a high index of suspicion for a potential RGM infection is needed for an accurate diagnosis.

Despite increasing recognition that these organisms can cause infections associated with prosthetic devices and surgical sites, RGM infections complicating implanted cardiac devices are still uncommon. We searched the available literature using PubMed with no starting date restrictions through March 31, 2015, and identified only 32 previously reported cases of CIED infections caused by any mycobacterial species. Including our patient, 23 (70%) of 33 reported infections were caused by an RGM species (*5–24*) (Table 2). We found 2 reports of CIED infections caused by *M. avium* complex (*26,27*) and 8 reports of infections caused by *M. tuberculosis* complex organisms (*28–33*). Of the 23 RGM infections, 21 (91%) were reported in the past 10 years, a trend likely resulting from improvements in microbiologic techniques and increased recognition of these organisms as causative pathogens. Mean age of case-patients with RGM infections was 65.4 years, consistent with age trends for CIED implantation. Sixteen (70%) case-patients had infections associated with permanent pacemakers. Among 21 case-patients for which time of onset was reported, 5 (24%) infections developed >6 months after the most recent device manipulation.

Although cardiac devices can become secondarily infected because of seeding from incidental bloodstream infections, the RGM species are uncommon causes of bacteremia. Instead, the source of early-onset CIED infections is more likely inoculation of the organism into the pocket at the time of the implantation procedure. This source contrasts with the probable source for the 8 reported CIED infections caused by *M. tuberculosis* complex. Manifesting ≥11 months after device manipulation, these infections more likely resulted from reactivation disease, mycobacteremia, and secondary seeding of the device.

The most commonly isolated organisms have been in the *M. fortuitum* group, which account for ≈50% of mycobacterial CIED infections in patients and nearly two thirds of infections caused by an RGM species. The *M. fortuitum* group has historically included *M. fortuitum* and *M. peregrinum*, although *M. mageritense* and others have also been proposed as members of this group (*3*). The preponderance of *M. fortuitum* infections among patients with cardiac device implantations mirrors trends observed for poststernotomy (*34*) and postaugmentation mammoplasty (*35*) infections caused by RGM. Although most skin and

Table 2. Clinical and demographic information for published cases of cardiac device infections due to rapidly growing mycobacteria*

Year (ref)	Age, y/ sex	Organism	Type	Onset†	Bacteremia/ lead infection‡	IE§	Macrolide resistant	Device removed	Antimicrobial drug therapy	Outcome
Mycobacterium fortuitum group										
1998 (*6*)	74/M	*M. fortuitum* + *M. chelonae*	PPM	13 d	NR/NR	NR	NR	Yes	FQ + AG × 4 wk	Cured
2005 (*9*)	62/F	*M. fortuitum*	PPM	6 mo	Yes/yes	Yes	No	Yes	CLR + CIP × 4 wk, DOX + CIP × 24 wk	Cured
2005 (*10*)	74/M	*M. peregrinum*	ICD	6 wk	Yes/NR	NR	No	Yes	CLR + CIP × 6 wk	Cured
2005 (*8*)	72/M	*M. fortuitum*	PPM	2 wk	No/NR	No	Yes	Yes	CIP + AG × 2 wk, CIP × 6 mo	Cured
	61/M	*M. fortuitum*	ICD	17 mo	No/yes	No	Yes	Yes	LVX × ≥1 y¶	Cured
2006 (*11*)	80/M	*M. fortuitum*	PPM	18 d	Yes/NA	No	No	No	CLR + CIP × 6 wk	Cured
2007 (*13*)	84/F	*M. fortuitum*	PPM	1 mo	No/no	No	Yes	Yes	LVX × 3 mo	Cured
2007 (*15*)	78/F	*M. fortuitum*	PPM	3 mo	Yes/yes	No	NR	Yes	CLR + LVX + LZD × 2 wk, CLR + LVX × 6 mo	Cured
2007 (*16*)	78/F	*M. fortuitum*	PPM	<4 mo	Yes/NR	NR	NR	Yes	CLR + LVX + LZD × 2 wk, CLR + LVX × 22 wk	Cured
	77/F	*M. mageritense*	PPM	3 wk	NR/NA	NR	NR	No	FQ × 6 mo	Cured
2009 (*18*)	15/F	*M. fortuitum*	PPM	7 wk	Yes/yes	No	No	Yes	CLR + CIP × 6 mo	Cured
2010 (*20*)	78/M	*M. fortuitum*	PPM	NR	Yes/NR	NR	NR	Yes	CLR + CIP × 26 wk	Cured
2012 (*23*)	43/M	*M. fortuitum*	ICD	4 y	Yes/yes	Yes	No	Yes	CLR + CIP + AG	Died
2012 (*22*)	75/M	*M. peregrinum*	PPM	1 y	Yes/yes	No	NR	Yes	CLR + CIP × mo	Cured
2015#	60/M	*M. fortuitum* group	ICD	6 wk	Yes/no	No	Yes	Yes	CLR + CIP + FOX	Died
M. abscessus complex										
1998 (*5*)	68/M	*M. abscessus*	PPM	19 y	NR/yes	NR	No	Yes	CLR + AG + FOX × 5 wk	Died
2005 (*7*)	53/M	*M. abscessus*	ICD	2 wk	NR/NR	NR	Yes	Yes	CLR × 24 wk	Cured
2007 (*14*)	43/F	*M. massiliense*	PPM	11 mo	NR/yes	NR	No	Yes	CLR × 6 mo	Cured
M. smegmatis complex										
2006 (*12*)	86/M	*M. goodii*	PPM	16 d	Yes/NR	NR	NR	Yes	Multiple, ending with MIN + AG × 2 wk	Cured
2008 (*17*)	85/M	*M. goodii*	ICD	<7 d	No/NR	No	NR	Yes	TMP/SXT × 8 wk	Cured
2009 (*19*)	23/M	*M. goodii*	PPM	8 d	No/NA	No	Yes	No	DOX + FQ × 6 mo	Cured
M. chelonae complex										
2014 (*24*)	63/M	*M. chelonae*	PPM	NR	No/yes	Yes	NR	Yes	CLR + LVX + AG × >2 mo	Cured
Ungrouped rapidly growing species										
2011 (*21*)	73/M	*M. phlei*	ICD	1 mo	No/NR	NR	No	Yes	SXT + DOX × 12 mo	Cured

*AG, aminoglycoside; CIP, ciprofloxacin; CLR, clarithromycin; DOX, doxycycline; FOX, cefoxitin; FQ, fluoroquinolone other than CIP or LVX; ICD, implantable cardioverter-defibrillator; IE, infective endocarditis; LVX, levofloxacin; LZD, linezolid; MIN, minocycline; NA, not available; NR, not reported; PPM, permanent pacemaker; ref, reference; TMP/SXT, trimethoprim/sulfamethoxazole.
†Time since most recent device manipulation.
‡Defined as positive lead culture, acid fast stain, or presumptive diagnosis on the basis of imaging or operative findings.
§Transthoracic or transesophageal echocardiographic findings as defined by the Duke criteria (*25*).
¶No end date for therapy was specified, but patient had at least 1 year of treatment.
#The patient described in this article.

soft tissue infections caused by RGM, particularly after surgical or nonsurgical trauma, result from *M. fortuitum* (*3*), this organism is not considered a normal skin commensal. Sources of these infections are instead thought to be largely environmental (*3*). Nevertheless, among all skin and soft tissue infections caused by RGM, those on the chest or back seem more likely to result from *M. fortuitum* than from other RGM species (*36*).

Another trend we observed was that 11 (48%) of the 23 patients with a RGM infection had associated mycobacteremia (5 had no reported blood culture results). This finding indicates that the infection had spread beyond the device pocket to the intravascular component of the CIED system, suggesting endovascular infection. In the 13 (57%) patients for which both blood culture results and echocardiographic findings were reported, 4 (31%) had device-related endocarditis, as defined by the Duke criteria (*9,18,23–25*). Three of these 4 patients fulfilled clinical criteria for infective endocarditis on the basis of echocardiographic findings; the fourth had no echocardiographic abnormalities but fulfilled pathologic criteria on the basis of isolation of the organism in an operative culture. This patient was the only one with valvular endocarditis among all the reports in our review (*18*).

Conversely, in 4 patients, including the patient described in this article, mycobacteremia was detected in the absence of echocardiographic abnormalities. The patient we describe had an unremarkable transthoracic echocardiogram, but a transesophageal echocardiogram could not be performed for definitive evaluation of CIED-related endocarditis. Overall, the low rates of valvular endocarditis or disseminated infection suggest that CIED infections caused by an RGM behave similarly to catheter-related bloodstream infections caused by these organisms (*37*), although severe complications of CIED infections associated with bacteremia have rarely been described (*18,23*).

The Clinical and Laboratory Standards Institute recommends routine broth microdilution susceptibility testing of all RGM isolates against amikacin, cefoxitin, ciprofloxacin, clarithromycin, doxycycline, imipenem, and sulfamethoxazole (or trimethoprim/sulfamethoxazole), but newer agents (e.g., linezolid, moxifloxacin, and tigecycline) also frequently show in vitro activity against these organisms (*2*). Of all RGMs, the *M. fortuitum* group is traditionally considered the most susceptible to antimicrobial drugs, with isolates frequently being susceptible to many agents tested. However, current guidelines by the American Thoracic Society and the Infectious Diseases Society of America recommend therapy with ≥2 active drugs for several months for optimal results. The isolate of the patient in this report showed resistance to several tested agents, including clarithromycin (Table 1). Among patients with CIED infections caused by *M. fortuitum* group organisms for which susceptibility data were reported, only 4 (including the patient reported in this article) had macrolide resistance. A previous review of the RGM similarly noted that most *M. fortuitum* clinical isolates were macrolide susceptible by in vitro methods (*3*).

Genetic studies published after that review revealed that most, if not all, *M. fortuitum* isolates also harbor an *M. fortuitum* rRNA methylase gene, termed *erm*(39), that, if active, can confer macrolide resistance (*38,39*). Although the clinical significance of this potential mechanism of inducible macrolide resistance is unclear, particularly in strains in which the gene is inactive at baseline, this finding has led many experts to advise caution to clinicians who consider prescribing macrolide-based regimens for serious *M. fortuitum* infections, even when the isolate is reported as susceptible to macrolides on the basis of broth microdilution methods (*2*). Nevertheless, in most reported CIED infections caused by macrolide-susceptible *M. fortuitum* group organisms, a macrolide and fluoroquinolone combination has been used successfully. We speculate that the patient described in this article experienced delayed wound healing resulting from inadequate activity of the empirical antimicrobial regimen against his isolate.

Given the recommended antimicrobial drug regimens for infections caused by an RGM, cardiac device infections resulting from these organisms can pose a unique therapeutic dilemma. On the one hand, the propensity to biofilm formation makes these organisms difficult to eradicate with antimicrobial drug therapy alone. Consequently, most experts advocate an extended course of antimicrobial drugs combined with device removal (*37*), the strategy used in all but 3 (13%) of the 23 previously described case-patients with infections caused by an RGM. On the other hand, some of the most active agents against this group of organisms belong to the macrolide or fluoroquinolone classes, which are both types of antimicrobial drugs with potential proarrhythmic effects (*39,40*). An antimicrobial drug combination that has the potential to precipitate arrhythmias becomes problematic in patients being considered for device removal and having a preexisting risk for conduction abnormalities (caused by an underlying conduction disease, cardiomyopathy, or concomitant proarrhythmic medications). The patient we describe was noted to have a prolonged corrected QT interval before the start of macrolide or fluoroquinolone therapy, a circumstance that made selection of an appropriate empirical antimicrobial regimen challenging. This patient highlights the importance of expedited antimicrobial drug susceptibility testing for managing these infections, including evaluation of newer antimicrobial drugs with fewer direct arrhythmogenic effects than those resulting from macrolides or fluoroquinolones.

Infections occurring after implantation of cardiac devices and caused by nontuberculous mycobacteria are

uncommon, but as more devices are implanted, such infections are likely to be more frequently reported. Our patient illustrates many of the common clinical features of postimplantation CIED infections caused by RGMs, including early onset (<6 months from the most recent manipulation of the device) of disease, initial identification of the organism as a gram-positive bacillus, and isolation of a *M. fortuitum* group organism as the causative pathogen. In addition, this patient highlights several unique issues that warrant further investigation, such as reliability of macrolide therapy for *M. fortuitum* group infections and safety of long-term macrolide and fluoroquinolone use in patients with a preexisting high risk for serious arrhythmias.

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Dr. Phadke is an infectious diseases fellow at Emory University in Atlanta. His research interests include vaccine-preventable diseases.

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Address for correspondence: Varun K. Phadke, 49 Jesse Hill Jr. Dr, Atlanta, GA 30303, USA; email: vphadke@emory.edu

Tuberculosis Caused by *Mycobacterium africanum*, United States, 2004–2013

Aditya Sharma, Emily Bloss, Charles M. Heilig, Eleanor S. Click

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Release date: February 17, 2016; Expiration date: February 17, 2017

Learning Objectives

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

- Distinguish the prevalence of *Mycobacterium africanum* tuberculosis in the United States
- Analyze the phylogenetics and epidemiology of *M. africanum* tuberculosis in the United States
- Compare the clinical characteristics of infection with *M. africanum* vs. *M. tuberculosis*
- Assess risk factors for *M. africanum* infection

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CME Author

Charles P. Vega, MD, Clinical Professor of Family Medicine, University of California, Irvine. *Disclosure: Charles P. Vega, MD, has disclosed the following financial relationships: served as an advisor or consultant for Allergan, Inc.; McNeil Consumer Healthcare; Takeda Pharmaceuticals North America, Inc.; served as a speaker or a member of a speakers bureau for Shire Pharmaceuticals.*

Authors

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Mycobacterium africanum is endemic to West Africa and causes tuberculosis (TB). We reviewed reported cases of TB in the United States during 2004–2013 that had lineage assigned by genotype (spoligotype and mycobacterial interspersed repetitive unit variable number tandem repeats). *M. africanum* caused 315 (0.4%) of 73,290 TB cases with lineage assigned by genotype. TB caused by *M. africanum* was associated more with persons from West Africa (adjusted odds ratio [aOR] 253.8, 95% CI 59.9–1,076.1) and US-born black persons (aOR 5.7, 95% CI 1.2–25.9) than with US-born white persons. TB caused by *M. africanum* did not show differences in clinical characteristics when compared with TB caused by *M. tuberculosis*. Clustered cases defined as ≥2 cases in a county with identical 24-locus mycobacterial interspersed repetitive unit genotypes, were less likely for *M. africanum* (aOR 0.1, 95% CI 0.1–0.4), which suggests that *M. africanum* is not commonly transmitted in the United States.

Author affiliation: Centers for Disease Control and Prevention, Atlanta, Georgia, USA

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Tuberculosis (TB) is an infectious disease caused by a group of highly-related organisms comprising the *Mycobacterium tuberculosis* complex (MTBC), which includes *M. tuberculosis*, *M. africanum*, and *M. bovis*. Although all members of MTBC might cause disease in humans, *M. tuberculosis* and *M. africanum* are the primary cause of disease in humans globally, whereas *M. bovis* primarily causes disease in cattle (*1*,*2*). Like *M. tuberculosis, M. africanum* is spread by aerosol transmission (*3*).

Phylogenetic analysis has suggested there are 7 major lineages of MTBC, designated L1–L7 (*4*,*5*). *M africanum* was traditionally identified by using biochemical methods. However, molecular methods have shown that *M. africanum* is composed of 2 distinct lineages: L5 (also known in other nomenclature systems as *M. africanum* West African 1 [MAF1], West African lineage I), which is genetically part of *M. tuberculosis* sensu stricto, and L6 (also known as *M. africanum* West African 2 [MAF2], West African lineage II), which is genetically more similar to *M. bovis* (*4*–*9*).

Among lineages that primarily infect humans, *M. africanum* lineages are considered phylogenetically more ancient relative to the modern lineages of *M. tuberculosis* (Euro-American, East African Indian, East Asian). *M. africanum* has been described as endemic to equatorial Africa, with specimens isolated from countries such as Nigeria, Côte d'Ivoire, Benin, Senegal, Cameroon, Burkina Faso, The Gambia, Sierra Leone, and Uganda (*8*,*10*–*21*). *M. africanum* has also been isolated from patients with TB in countries in Europe (*22*–*25*), Brazil (*26*), and the United States (*27*). It is likely that TB caused by *M. africanum* in non-African countries is secondary to human migration from disease-endemic areas in equatorial Africa (*25*).

Several studies have explored whether there are clinical differences between TB caused by *M. africanum* and TB caused by *M. tuberculosis*. These studies demonstrated variable findings with regard to associations of *M. africanum* with HIV status and findings on chest radiography (*8*,*28*–*30*). Contacts of persons with TB caused by *M. africanum* appeared to have a lower rate of progression to active TB compared with contacts of persons with TB caused by *M. tuberculosis*, and a lower rate of genotype clustering has been described for *M. africanum* than for *M. tuberculosis* in relatively small studies from West Africa (*14*,*29*).

Although bacterial strains causing TB from all over the world can be found among cases of TB in the United States, analysis of routinely collected genotyping data for 2005–2009 showed that 179 (0.5%) of 36,458 TB cases reported nationally were caused by *M. africanum* (*31*). We sought to further expand knowledge of *M. africanum* in the United States by reviewing all cases of TB reported nationally during 2004–2013. The objectives of this study were to ascertain the proportion of TB cases caused by *M. africanum* in the United States; compare clinical and epidemiologic characteristics between *M. africanum* and *M. tuberculosis*; and determine the extent to which *M. africanum* strains in the United States might be related by transmission on the basis of genotype clustering.

Methods

Genotype data from the Centers for Disease Control and Prevention (CDC; Atlanta, GA, USA) National TB Genotyping Service for 2004 through 2013 were linked to routine demographic and clinical data from all culture-confirmed cases in the CDC National TB Surveillance System from all 50 US states and the District of Columbia (*32*). As described previously (*33*), phylogenetic lineage (*M. africanum* and *M. tuberculosis*) for TB cases was assigned on the basis of spoligotype by using a set of rules correlating spoligotype to lineages defined by large sequence polymorphisms; for cases that did not meet a full rule for assignment on the basis of spoligotype, 12-locus mycobacterial interspersed repetitive unit variable number tandem repeats (MIRU-VNTRs) was used in addition to spoligotype to assign lineage. Cases reported during 2004–2008 only had 12-locus MIRU-VNTR data available, and cases reported during 2009–2013 had 24-locus MIRU-VNTR data available. To identify cases that could be caused by ongoing transmission in the United States, clusters of cases were defined as ≥2 cases with the same spoligotype and 24-locus MIRU-VNTR pattern in a given county. Cases that were caused by organisms other than *M. africanum* or *M. tuberculosis* were excluded from analysis.

All analyses were conducted by using R statistical software version 3.0.1 (R Core Group, Vienna, Austria). Statistical test results were considered significant at $p<0.05$. We examined patient attributes, genotype clustering, clinical characteristics (e.g., disease site), and social risk factors (e.g., homelessness) associated with *M. africanum* and *M. tuberculosis*. Odd ratios (ORs) and 95% CIs were calculated. Differences in proportions of cases were detected by using Fisher exact and Pearson χ^2 tests.

Factors identified as statistically significant by bivariable analysis at $p<0.05$ were entered into a multivariable logistic regression model to assess whether these factors were independently associated with *M. africanum* and *M. tuberculosis*. Tolerance <0.10 was used to detect colinearity, and the likelihood ratio test was used to test for interaction. To address collinearity between race/ethnicity and origin of birth, variables for race/ethnicity, country of origin, and West African origin were combined into a single variable and included in selection of the multivariable regression model. West African origin was defined as having been born in any of the following countries in West Africa: Nigeria, Liberia, Sierra Leone, Guinea, The Gambia, Ghana, Mali, Senegal, Côte d'Ivoire, Togo, Cameroon, Mauritania, Niger, and Guinea-Bissau.

Ethics Statement

Data for this study were collected as part of routine TB surveillance by CDC. Thus, this study was not considered research involving human subjects, and institutional review board approval was not required.

Results

A total of 125,038 cases were reported to the National TB Surveillance System during 2004–2013 (Figure 1). Of these cases, 95,836 (76.6%) had a culture result positive for MTBC. Of cases with positive culture results, 73,290 (76.5%) had available lineage identification on the basis of genotype data. Of the cases for which lineage identification was available, the causative agent was determined to be *M. africanum* for 315 (0.4%) and *M. tuberculosis* for 71,727 (97.9%) cases: 1,248 (1.7%) cases had an isolated organism other than *M. africanum* or *M. tuberculosis* and were excluded from further analysis (Figure 1).

M. africanum was assigned as the causative agent of TB for isolates with a genotype-assigned lineage of L5 or L6. All isolates designated as *M. africanum* met the conventional spoligotype rule of the absence of spacers 8, 9, and 39 or the absence of spacers 7–9 and 39 (*7*). *M. tuberculosis* was assigned as the causative agent of TB for isolates with a genotype-assigned lineage of L1, L2, L3, L4, or L7.

Of the 315 case-patients with TB caused by *M. africanum*, 155 (49.2%) had the L5 lineage and 160 (50.8%) had the L6 lineage. Case-patients with the L5 lineage were most commonly born in Nigeria (n = 76), Liberia (n = 12), and Ghana (n = 12), and case-patients with the L6 lineage were most commonly born in Liberia (n = 27), Sierra Leone (n = 22), Guinea (n = 17), and The Gambia (n = 16).

Among case-patients with *M. africanum* as the causative agent of TB, 276 (87.6%) had country of birth other than the United States (online Technical Appendix Table 1, http://wwwnc.cdc.gov/EID/article/22/3/15-1505-Techapp1.pdf). Of the 276 foreign-born persons with *M. africanum*, most (254, 92.0%) persons were born in countries in West Africa, such as Nigeria (79, 31.1%), Liberia (39, 15.4%), and Sierra Leone (24, 9.4%).

Among all US states, 35 reported ≥1 case of TB caused by *M. africanum* (Figure 2). States that reported more than >10 cases of *M. africanum* TB during the study were New York (n = 77), Maryland (n = 41), Texas (n = 26), Virginia (n = 19), Georgia (n = 15), and California (n = 14). Across the United States, many reported cases of *M. africanum* TB appeared to be near major metropolitan areas, such as Atlanta, Georgia; Chicago, Illinois; Detroit, Michigan; Houston, Texas; Los Angeles, California; New York, New York; and Washington, DC.

The annual number of reported TB cases identified with *M. africanum* in the United States during 2004–2013 ranged from 18 to 40 (median 34 annual cases) (Figure 3). During this period, the proportion of *Mycobacterium* spp. TB isolates from persons born in West Africa with culture-

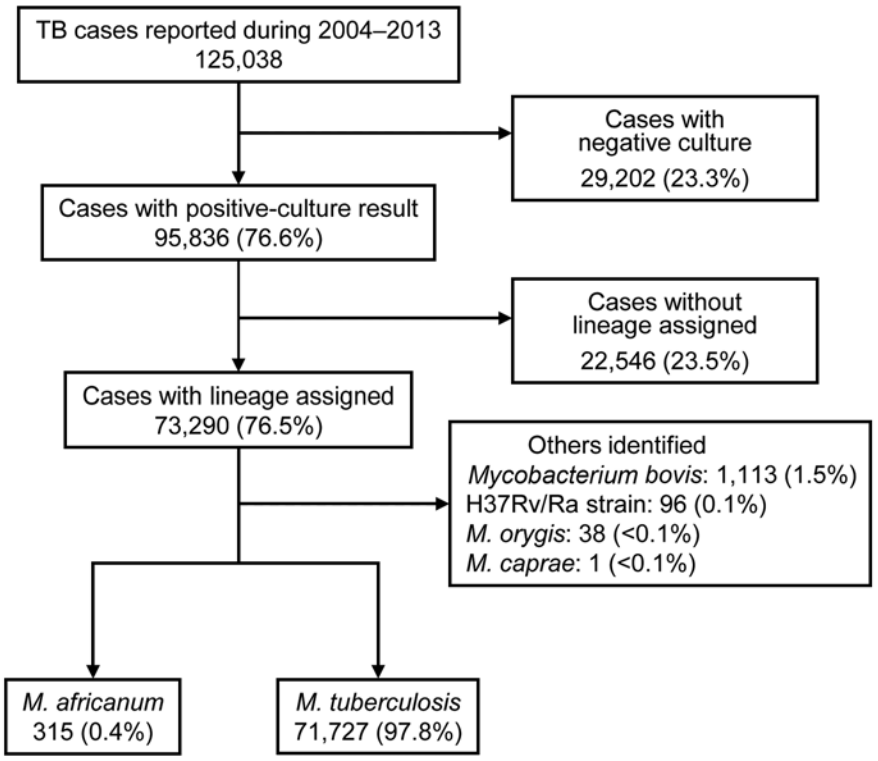

Figure 1. Selection of cases included in analysis of tuberculosis (TB) caused by *Mycobacterium africanum*, United States, 2004–2013.

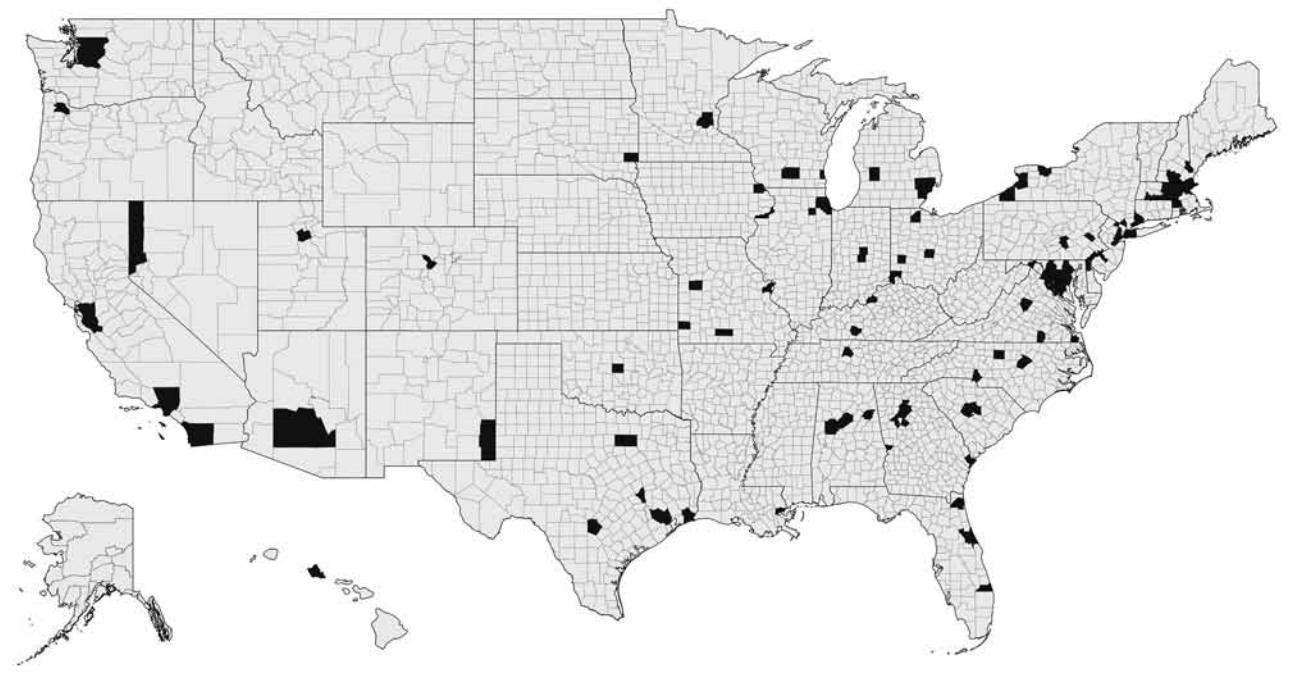

Figure 2. Counties in the United States with *Mycobacterium africanum* infections identified among tuberculosis (TB) cases (black) reported during 2004–2013.

confirmed TB that were genotyped ranged from 68.0% to 97.1%, which was comparable with the overall proportion of culture-confirmed TB cases that were genotyped nationally.

On the basis of the genotype cluster definition of ≥2 cases in the same county with identical spoligotype and 24-locus MIRU-VNTR patterns, only 1 cluster of *M. africanum* cases was identified during 2009–2013. The cluster consisted of 2 case-patients with the L5 lineage: 1 foreign-born person and 1 US-born person.

Among 315 cases of *M. africanum* TB, 183 distinct genotypes were identified (spoligotype and 12-locus MIRU-VNTR available for cases reported during 2004–2013; online Technical Appendix Table 2). Of these 183 genotypes, 139 (76.0%) were found in a single case only; the remaining 44 (24.0%) caused 176 cases. Among 141 *M. africanum* cases reported during 2009–2013 with spoligotype and 24-locus MIRU-VNTR data available, 123 distinct genotypes were identified (online Technical Appendix Table 3). Of these 123 genotypes, 113 (91.9%) were found in isolates from 1 case only, and 10 (8.1%) were found in >1 case.

Bivariable analysis showed that *M. africanum* and *M. tuberculosis* TB cases had major differences for several characteristics (online Technical Appendix Table 1). When compared with *M. tuberculosis* TB cases, *M. africanum* TB cases had higher odds of being in foreign-born persons (odds ratio [OR] 4.8, 95% CI 3.4–6.7), being in non-Hispanic black or multiracial non-Hispanic persons (OR 27.0, 95% CI 17.1–42.5), originating from countries in West Africa (OR 318.4, 95% CI 239.0–424.2), being in persons positive for HIV (OR 2.8, 95% CI 2.0–3.7), and being in persons with only extrapulmonary disease (OR 1.8, 95% CI 1.4–2.4) or in persons with pulmonary and extrapulmonary disease (OR 1.6, 95% CI 1.1–2.2).

M. africanum TB cases had lower odds than *M. tuberculosis* TB cases of being in a cluster (defined by spoligotype and 24-locus MIRU) of cases (OR 0.1, 95% CI 0.1–0.2), being in persons ≥65 years of age (OR 0.2, 95% CI 0.1–0.5), being in persons with an abnormal chest radiographic result and cavitation (OR 0.6, 95% CI 0.5–0.9) and in persons without cavitation (OR 0.5, 95% CI 0.4–0.7), being in a resident of a correctional facility (OR 0.2, 95% CI 0.0–0.6), being in a homeless person (OR 0.4, 95% CI 0.2–0.8), being in persons reporting excessive drug (OR 0.2, 95% CI 0.1–0.5) or alcohol use (OR 0.2, 95% CI 0.1–0.4), and being in persons who died during treatment (OR 0.3, 95% CI 0.2–0.7). Among foreign-born persons, *M. africanum* TB cases had lower odds than *M. tuberculosis* TB cases of being in persons who had been in the United States for >5 years before reporting TB (OR 0.3, 95% CI 0.3–0.5).

Multivariable analysis restricted to cases reported during 2009–2013 that had 24-locus MIRU-VNTR data available showed that foreign-born West African origin (OR 253.8, 95% CI 59.9–1076.1) and US-born non-Hispanic black race (OR 5.7, 95% CI 1.2–25.9) were independently associated with TB caused by *M. africanum* but not with TB caused by *M. tuberculosis* (Table). Clustered cases (OR 0.1, 95% CI 0.1–0.4) had lower adjusted odds of TB caused

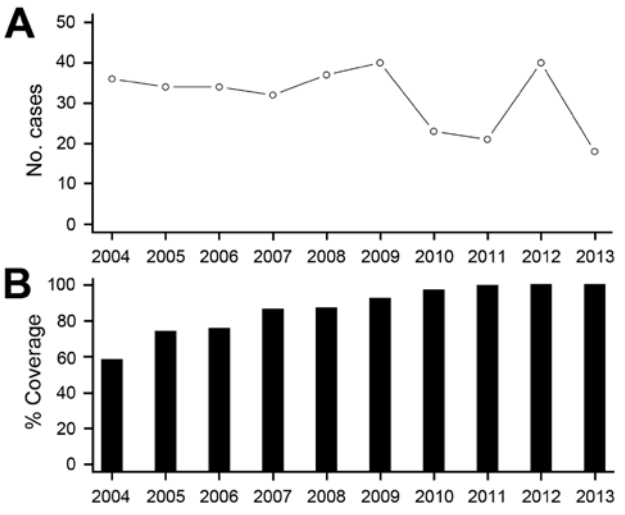

Figure 3. A) Annual number of reported *Mycobacterium africanum* tuberculosis cases and B) corresponding percentage of national genotype surveillance coverage, United States, 2004–2013.

by *M. africanum* than TB caused by *M. tuberculosis*. Other risk factors were not independently associated with *M. africanum* versus *M. tuberculosis*. No significant interaction terms were identified.

To control for possible host differences in larger analysis, we conducted a subanalysis of cases among foreign-born persons from West Africa. In this subanalysis, clustering was the only significant variable at the bivariable level, and *M. africanum* TB cases had lower odds of being in a cluster of cases than *M. tuberculosis* TB cases (OR 0.1, 95% CI 0.1–0.9). Among foreign-born persons with West African origin, we found no significant differences in clinical characteristics (e.g., HIV status, cavitary disease, sputum smear results) between TB cases caused by *M. africanum* versus those caused by *M. tuberculosis*. *M. africanum* TB cases with L5 and L6 lineages had similar proportions of HIV positivity (18.1% vs. 17.5%; p = 0.9) and cavitary disease by chest radiography (25.4% vs. 42.5%; p = 0.051). We found no significant differences in clinical characteristics or social risk factors for TB caused by L5 or L6 lineages.

Discussion

This study used nationally reported data on TB cases linked to genotype data to describe the epidemiology of *M. africanum* in the United States. The findings from this analysis indicate that *M. africanum* is a rare cause of TB in the United States and represents 315 (0.4%) of 73,290 cases with available genotype data reported during 2004–2013. Most cases were identified in large metropolitan areas throughout the United States. Although *M. africanum* is an infrequent cause of TB, most states reported ≥1 case of TB caused by *M. africanum* during the study period, which suggested that *M. africanum* is broadly distributed.

In this study, TB caused by *M. africanum* was more likely to occur in foreign-born West Africans and US-born non-Hispanic blacks and less likely in foreign-born persons originating from countries not in West Africa. These associations suggest that the epidemiology of *M. africanum* in the United States is driven primarily by migration of persons from West Africa. We also identified cases of *M. africanum* in US-born persons, primarily in non-Hispanic blacks. This finding suggests that transmission of *M. africanum* might

Table. Multivariable analysis of risk factors associated with tuberculosis caused by *Mycobacterium africanum* and *M. tuberculosis*, United States, 2009–2013

Risk factor	Adjusted OR (Wald 95% CI)
Combined race/ethnicity and origin	
Foreign born, non–West African	0.4 (0.1–1.9)
Foreign born, West African	253.8 (59.9–1076.1)
US born, non-Hispanic black	5.7 (1.2–25.9)
US born, Hispanic or other non-Hispanic race	1.1 (0.2–8.0)
US born, non-Hispanic white	Referent
Clustered case	
Yes	0.1 (0.1–0.4)
No	Referent
Age, y	
0–14	Referent
15–24	1.0 (0.3–3.5)
25–44	0.8 (0.2–2.5)
45–64	0.6 (0.2–2.0)
≥65	0.3 (0.1–1.3)
Sex	
F	0.9 (0.6–1.4)
M	Referent
Reported HIV status	
Negative	Referent
Positive	0.9 (0.5–1.4)
Unknown/not determined	1.7 (0.9–3.3)
Primary disease site	
Pulmonary	Referent
Extrapulmonary	1.9 (1.0–3.6)
Pulmonary and extrapulmonary	1.1 (0.6–2.3)
Chest radiography finding	
Abnormal, cavitary	2.1 (1.0–4.5)
Abnormal, noncavitary	0.9 (0.5–1.7)
Normal	Referent
Homeless in year before diagnosis	
Yes	1.0 (0.3–3.0)
No	Referent
Resident of correctional facility in year before diagnosis	
Yes	0.6 (0.1–4.8)
No	Referent
Any drug use	
Yes	0.4 (0.1–1.9)
No	Referent
Excessive alcohol use	
Yes	0.8 (0.3–2.4)
No	Referent
Reason therapy stopped	
Completed treatment	Referent
Died during treatment	0.2 (0.1–1.6)
Other reason	1.3 (0.5–3.2)

occur in the United States, but the possibility of acquisition of TB during travel (e.g., to West Africa) cannot be excluded because travel history was not available in national surveillance data. In an initial report of 5 *M. africanum* cases in the United States, several case-patients did not report a history of travel to West Africa (*34*).

The low proportion of TB cases attributed to *M. africanum* suggests decreased transmissibility in the United States. Reasons for decreased transmission of *M. africanum* are unknown but could include decreased infectiousness or decreased progression to disease compared with *M. tuberculosis*, as was previously reported (*8*).

Our findings support the observation that *M. africanum* is highly restricted to West Africa, where it has been estimated to cause up to 50% of all TB cases, although the reason for this restriction remains unclear (*8*). A recent study from Ghana reported an association between *M. africanum* and patient ethnicity, which suggests specificity of host–pathogen interaction could be 1 factor in limiting the spread of *M. africanum* to West Africa (*35*).

Most *M. africanum* TB cases were not part of genotype clusters, which suggested that transmission of *M. africanum* in the United States is not common. *M. africanum* TB cases were less likely to be associated with genotype clustering than *M. tuberculosis* TB cases by analyses of all cases reported in the United States and in a subanalysis of persons born in West Africa. This lower association of clustering is consistent with investigations from Ghana and The Gambia, which found *M. africanum* less likely to be in spoligotype-defined clusters (*30*,*36*).

After controlling for other factors, we found that TB cases in the United States caused by *M. africanum* and *M. tuberculosis* were similar regarding clinical presentation, social risk factors, and treatment outcomes. These findings are consistent with those of studies that compared treatment outcomes among cases of *M. africanum* and *M. tuberculosis* TB in West Africa, but contrast with studies describing differential associations with HIV and chest radiography findings (*8*,*14*,*28*,*29*). Unlike several reported studies, we could not compare specific chest radiographic findings for *M. africanum* versus *M. tuberculosis* because detailed radiographic information is not available in US surveillance data (*8*). Our study demonstrated similar clinical characteristics of TB caused by L5 and L6 lineages of *M. africanum*, which is consistent with that of a previous report (*29*).

Our results should be interpreted in light of the incomplete availability of genotype data. Nationwide coverage of genotyping has increased over time (*37*), but genotype data were not available for all culture confirmed cases. Although it is possible that our study underestimates the true burden of *M. africanum*, we expect that changes in system coverage do not substantially affect the main findings of the study. In addition, *M. africanum* and *M. tuberculosis* were identified by spoligotype and MIRU-VNTR, rather than by more phylogenetically robust methods, such as large-sequence polymorphism analysis. Therefore, some misclassification might have occurred, but there is no reason to assume any bias was introduced. Finally, our definition of clustered cases was based solely on identical spoligotype and 24-locus MIRU-VNTR in the same county during 2009–2013 and therefore probably overestimates the extent of transmission that might be occurring at the county level. More robust methods for identifying clustered cases rely on a narrower time interval between cases and evidence of epidemiologic links between cases (*38*). Even with the direction of bias toward overestimation of clustering, we found only 1 cluster.

Although the annual number of reported TB cases in the United States has decreased in the past decade, the proportion of TB contributed by foreign-born persons has increased to >60% in recent years (*39*). Similar to this trend, TB caused by *M. africanum* is highest among foreign-born persons, which is consistent with the understanding that spread of *M. africanum* in countries outside Africa is driven by human migration from West Africa. Given the low burden of TB caused by *M. africanum* in the United States, the similarity in clinical features of TB caused by *M. africanum* and *M. tuberculosis*, and the lower odds of clustered cases of *M. africanum* than those of *M. tuberculosis*, routine reporting of TB caused by *M. africanum* above standard reporting for general TB does not appear warranted at this time.

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Dr. Sharma is an Epidemic Intelligence Service Officer at the National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention, Centers for Disease Control and Prevention, Atlanta, Georgia. His primary interests are the epidemiology and control of infectious diseases, including TB, and drug-resistant bacteria.

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Address for correspondence: Aditya Sharma, Centers for Disease Control and Prevention, 1600 Clifton Rd NE, Mailstop E10, Atlanta, GA 30329-4027, USA; email: asharma4@cdc.gov

Methylotroph Infections and Chronic Granulomatous Disease

E. Liana Falcone, Jennifer R. Petts, Mary Beth Fasano, Bradley Ford, William M. Nauseef, João Farela Neves, Maria João Simões, Millard L. Tierce IV, M. Teresa de la Morena, David E. Greenberg, Christa S. Zerbe, Adrian M. Zelazny, Steven M. Holland

Chronic granulomatous disease (CGD) is a primary immunodeficiency caused by a defect in production of phagocyte-derived reactive oxygen species, which leads to recurrent infections with a characteristic group of pathogens not previously known to include methylotrophs. Methylotrophs are versatile environmental bacteria that can use single-carbon organic compounds as their sole source of energy; they rarely cause disease in immunocompetent persons. We have identified 12 infections with methylotrophs (5 reported here, 7 previously reported) in patients with CGD. Methylotrophs identified were *Granulibacter bethesdensis* (9 cases), *Acidomonas methanolica* (2 cases), and *Methylobacterium lusitanum* (1 case). Two patients in Europe died; the other 10, from North and Central America, recovered after prolonged courses of antimicrobial drug therapy and, for some, surgery. Methylotrophs are emerging as disease-causing organisms in patients with CGD. For all patients, sequencing of the 16S rRNA gene was required for correct diagnosis. Geographic origin of the methylotroph strain may affect clinical management and prognosis.

Chronic granulomatous disease (CGD) is a primary immunodeficiency characterized by recurrent infections of the lung, skin, lymph nodes, and liver, as well as granulomatous inflammation affecting those organs and hollow viscera. The immunodeficiency results from deficiencies in any 1 of the 5 subunits forming the NADPH (nicotinamide adenine dinucleotide phosphate) oxidase 2 (Nox2)–based complex, which leads to impaired production of reactive oxygen species in phagocytes. Defects in the Nox2 (gp-91^{phox}) enzymatic subunit (*CYBB* [cytochrome b-245, β polypeptide]) are inherited in an X-linked manner, whereas defects in subunits p47^{phox} (*NCF1* [neutrophil cytosolic factor 1]), p22^{phox} (*CYBA* [cytochrome b-245, α polypeptide]), p67^{phox} (*NCF2* [neutrophil cytosolic factor 2]), and p40^{phox} (*NCF4* [neutrophil cytosolic factor 4]) are inherited in an autosomal recessive manner (*1*,*2*).

CGD infections are often caused by a characteristic group of pathogens, including *Staphylococcus aureus*, *Serratia marcescens*, *Burkholdheria cepacia* complex, *Nocardia* spp., and *Aspergillus* spp. (*1*). However, new pathogens are emerging, and some reportedly are found almost exclusively in patients with CGD. Methylotrophs are bacteria that can use single-carbon organic compounds as their sole source of energy, the widespread availability of which makes these organisms versatile environmental inhabitants. However, they rarely cause disease in immunocompetent persons (*3*).

We previously reported 7 methylotroph infections in patients with CGD and here describe 5 more (Table). From these 12 infections, we have isolated the methylotrophs *Granulibacter bethesdensis*, *Acidomonas methanolica*, and *Methylobacterium lusitanum*. These infections were difficult to diagnose and required prolonged courses of antimicrobial drugs and sometimes surgery for complete resolution.

Patient 1

In 2008, a 1-year-old girl from Mexico who had p67^{phox}-deficient CGD was examined for fever, weight loss, and enlarged cervical lymph nodes; she had been receiving ceftriaxone, clindamycin, itraconazole, and interferon-γ for treatment of CGD. CGD was diagnosed when she was 6 months of age, at which time she had *Penicillium* sp. pneumonia and an abnormal dihydrorhodamine oxidation assay result. At 3 weeks of age, she had had a methicillin-sensitive *S. aureus* labial abscess, followed at 8 months of age by 3 episodes of pneumonia and an *S. marcescens* buttock abscess.

A computed tomographic (CT) scan performed at the time of admission showed cervical, mediastinal, and mesenteric lymphadenopathy and a right middle lung lobe infiltrate and hepatosplenomegaly. Excisional cervical lymph node and lung biopsy samples were processed for bacterial,

Author affiliations: National Institutes of Health, Bethesda, Maryland, USA (E.L. Falcone, C.S. Zerbe, A.M. Zelazny, S.M. Holland); University of Iowa Hospital and Clinics, Iowa City, Iowa, USA (J.R. Petts, M.B. Fasano, B. Ford, W.M. Nauseef); Veterans Administration Medical Center, Iowa City (W.M. Nauseef); Centro Hospitalar de Lisboa Central, Lisbon, Portugal (J.F. Neves); Instituto Nacional de Saúde Dr. Ricardo Jorge, Lisbon (M.J. Simões); Detroit Medical Center, Detroit, Michigan, USA (M.L. Tierce IV); University of Texas Southwestern Medical Center, Dallas, Texas, USA (M.T. de la Morena, D.E. Greenberg)

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nocardial, fungal, and mycobacterial cultures and staining. Direct Gram staining revealed moderate mononuclear cells but no organisms. After 11 days of incubation on chocolate agar at 37°C, the lung biopsy culture grew 1 pink colony. Species level identification conducted by full 16S rRNA gene sequencing (≈1,500 bp) showed a 99.8% match to the *M. lusitanum* type strain (*7*). Etest (bioMérieux Diagnostics, Marcy l'Etoile, France) showed the following MICs (in μg/mL): amikacin (MIC = 4), cefepime (MIC = 8), ceftriaxone (MIC = 2), ciprofloxacin (MIC = 16), piperacillin-tazobactam (MIC = 2), imipenem (MIC = 2), meropenem (MIC ≥32), trimethoprim/sulfamethoxazole (MIC >32), and aztreonam (MIC >256). Culture of the cervical lymph node biopsy sample grew *S. marcescens*.

After 7 months of treatment with ceftriaxone, clindamycin, and itraconazole, the patient completely recovered from

Table. Summary of methylotroph infections in patients with chronic granulomatous disease*

Patient no., reference	Patient age, y/sex	CGD genetics†	Clinical findings	Microbiological findings	Treatment‡	Outcome
1 (this study)	1/F	Autosomal recessive (*NCF2* c.304C>T; p.R102X)	Pneumonia, cervical lymphadenitis	*Methylobacterium lusitanum* (lung), *Serratia marcescens* (lymph node)	CRO/CLI/ITZ, HSCT	Recovered
2 (this study)	19/M	X-linked (*CYBB* intragenic deletion)	Necrotizing cervical lymphadenitis	*Granulibacter bethesdensis; Staphylococcus epidermidis*	VAN/CRO, CFD/DOX/RIF	Recovered
3 (this study)	16/M	X-linked (*CYBB* 13-exon deletion)	Meningitis	*G. bethesdensis*	MEM/CIP/AMK/DOX/TEC/VCZ, MEM/CIP/AMK/DOX/CSP/ LAMB/LZD/RIF/INH/CLR	Died
4 (this study)	9/M	X-linked (*CYBB* point mutation; exon 10)	Cervical abscess, lymphadenitis	*Acidomonas methanolica*	TZP/VAN/LAMB/CIP, MEM/VAN/LAMB/CIP, CIP/VCZ/TMPSMX/ IFN-γ, HSCT	Recovered
5 (this study)	36/M	X-linked (*CYBB* c.1139 G>A; p.W380X)	Multifocal lymphadenitis	*G. bethesdensis; S. epidermidis*	VAN/CRO, CRO/DOX/TMPSMX/ITZ	Still receiving treatment as of 2015
6 (*4*)	10/M	X-linked (*CYBB* p.Arg226X)	Necrotizing cervical lymphadenitis	*A. methanolica*	TMPSMX/CRO/DOX, TMPSMX/RFB/GEN	Recovered
7 (*5*)	10/M	X-linked	Bacteremia	*G. bethesdensis*	TMPSMX/CAZ/MTZ/LZD/VCZ	Died
8 (*6*)	39/M	X-linked	Necrotizing cervical, mediastinal, axillary lymphadenitis	*G. bethesdensis*	MEM/DOX, CRO/DOX	
9 (*6*)	36/M	X-linked	Multifocal necrotizing lymphadenitis, splenic lesions, ascites	*G. bethesdensis*	MEM/TMPSMX/ITZ, MEM/TMPSMX/VCZ/TOB, CRO/TMPSMX/IFN-γ, CPD/DOX/TMPSMX/IFN-γ, splenectomy/TGC	Recovered
10 (*6*)	13/M	X-linked	Necrotizing thoracic lymphadenitis	*G. bethesdensis*, *S. epidermidis*, *Candida glabrata*, *Streptococcus mitis group*	MEM/VCZ, CRO/TOB/DOX/VCZ, CRO, DOX, CRO, CFD	Recovered
11 (*6*)	17/M	X-linked	Necrotizing cervical and mediastinal lymphadenitis	*G. bethesdensis*	LVX, DOX, lymph node excision	Recovered
12 (*6*)	37/M	X-linked	Necrotizing supraclavicular lymphadenitis, splenic and liver lesions	*G. bethesdensis*	CRO/GEN/VAN, CRO/DOX/TMPSMX, CFD/DOX/TMPSMX, DOX/TMPSMX	Recovered

*AMK, amikacin; CAZ, ceftazidime; CFD, cefdinir; CGD, chronic granulomatous disease; CLI, clindamycin; CLR, clarithromycin; CIP, ciprofloxacin; CPD, cefpodoxime; CRO, ceftriaxone; CSP, caspofungin; *CYBB*, cytochrome b-245, β polypeptide; DOX, doxycycline; GEN, gentamicin; HSCT, hematopoietic stem cell transplant; IFN-**γ**, interferon- γ; INH, isoniazid; ITZ, itraconazole; LAMB, liposomal amphotericin B; LVX, levofloxacin; LZD, linezolid; MEM, meropenem; MTZ, metronidazole; *NCF2*, neutrophil cytosolic factor 2**;** RFB, rifabutin; RIF, rifampin; TEC, teicoplanin; TGC, tigecycline; TMPSMX, trimethoprim/sulfamethoxazole; TOB, tobramycin; VAN, vancomycin; VRZ, voriconazole.
†Information in parentheses indicates, when known, the mutation that led to CGD.
‡Slashes separate drugs in the same regimen; commas separate regimens.

the pneumonia and cervical lymphadenitis. She subsequently underwent successful hematopoietic stem cell transplant.

Patient 2

In 2011, a 19-year-old white man from Ohio, USA, who had X-linked CGD was examined for right neck swelling and tenderness (2 weeks' duration), a yellow ulcerated lesion on the right side of the hard palate, and an enlarged right tonsil with copious exudate. He had been receiving prophylactic trimethoprim-sulfamethoxazole and posaconazole. Erythrocyte sedimentation rate (ESR) was 28 mm/h, and C-reactive protein (CRP) concentration was 82 mg/L.

CGD had been diagnosed at birth on the basis of a positive family history. The patient had had hydrocephalus, catheter-associated fungal meningitis, and *Aspergillus fumigatus* pneumonia. When he was 15 years of age, CGD proctitis developed. Fourteen months before hospital admission, he had undergone right neck dissection for *Rothia aeria* infection, which was successfully treated with β-lactams (*8*).

CT images showed new bulky lymphadenopathy in the right neck, involving all nodal planes, and increased thickening and asymmetry of the right oropharynx with hypoattenuation of the right palatine tonsils. Culture of the right tonsillar exudate and empirical treatment with meropenem were not helpful. Antimicrobial therapy was switched to ceftriaxone and high-dose penicillin for empirical coverage of *G. bethesdensis* and *Actinomyces* spp. Right neck dissection with tonsillectomy yielded *Staphylococcus epidermidis*, and full 16S rRNA gene sequencing (≈1,500 bp) of 1 colony of a gram-negative bacillus showed a 99.8% match to the *G. bethesdensis* type strain. Nine weeks of vancomycin and ceftriaxone followed by 8 weeks of cefdinir, doxycycline, and rifampin led to complete resolution of the lymphadenitis.

Patient 3

In 2012, a 16-year-old boy from Portugal who had X-linked CGD was examined for fever, cervical lymphadenopathy, and elevated inflammatory markers. CT images showed a deep cervical abscess, from which nothing grew on culture but which completely resolved after 5 weeks of intravenous ceftriaxone, doxycycline, and ciprofloxacin and 6 weeks of oral amoxicillin/clavulanate, ciprofloxacin, and doxycycline, along with prophylactic itraconazole and interferon-γ.

Immediately after completion of that course of antimicrobial drugs, pneumonia with pleural effusions developed. Results of all cultures (blood, lymph node, bronchoalveolar lavage, and pleural fluid) were negative, but full 16S rRNA gene sequencing (≈1,500 bp) of pleural fluid showed a >99% match to *Cupriavidus* spp. The patient received meropenem, ciprofloxacin, amikacin, doxycycline, teicoplanin, and voriconazole. Two weeks later, fever with splenomegaly, pancytopenia, low fibrinogen levels, and elevated ferritin and soluble CD25 levels were noted. Interferon-γ prophylaxis was discontinued and the patient was administered dexamethasone and intravenous immunoglobulin, after which the presumed exuberant inflammatory response quickly resolved. CT images of the neck and lung were unremarkable, as were positron emission tomography images.

A month later, the boy was examined for fever, cough, and altered mental status; he required intubation and transfer to the intensive care unit. Magnetic resonance imaging revealed bilateral pneumonia and multiple intraparenchymal brain abscesses. Cerebrospinal fluid (CSF) was unremarkable. A lung biopsy sample, collected while the patient was receiving meropenem, ciprofloxacin, amikacin, doxycycline, teicoplanin, and voriconazole, was sterile. Voriconazole was switched to caspofungin and liposomal amphotericin B, and teicoplanin was switched to linezolid. The patient eventually recovered and was transferred out of the intensive care unit.

One month later, fever with focal neurologic deficits developed. CSF examination confirmed persistent pleocytosis with low glucose and elevated protein levels, and CT images indicated leptomeningitis. A 4-day culture of CSF on chocolate agar showed brownish colonies 1–2 mm in diameter. Full 16S rRNA gene sequencing (≈1,500 bp) performed on the isolate from CSF showed a 99.7% match to the *G. bethesdensis* type strain. Etest showed the following MICs (in μg/mL): tobramycin (MIC = 12), ceftriaxone (MIC >32), doxycycline (MIC = 24), and trimethoprim/sulfamethoxazole (MIC = 0.25). Isoniazid, clarithromycin, and rifampin had already been added to the patient's treatment regimen. Despite the above interventions, the patient died of obstructive hydrocephalus and multiorgan failure.

Patient 4

In 2013, a 9-year-old multiracial boy from Iowa, USA, who had X-linked CGD was examined for a 1-day history of fever, fatigue, decreased appetite, headache, and neck pain. He had been receiving oral trimethoprim/sulfamethoxazole, voriconazole and interferon-γ for CGD prophylaxis. When he was 1 month of age, he had had disseminated *Candida lusitaniae* infection with retropharyngeal, parapharyngeal, hepatic, and splenic abscesses. At 6 months of age, he was hospitalized for a progressively enlarging left posterior neck mass. A lymph node biopsy sample showed necrotizing granulomata and rare yeast forms suggestive of *Histoplasma*, but no specific organism was identified. He also had recurrent otitis media, tonsillitis, and aphthous stomatitis. Two months before the visit reported here, he had had *Aspergillus versicolor* pneumonia complicated by granulomatous appendicitis.

At the time of this hospital admission, he had 2 enlarged right anterior cervical nodes, which were soft, mobile, and not tender. ESR was 41 mm/h, and CRP concentration was 76 mg/L. CT images showed a 23 × 9 × 18-mm abscess adjacent to the right sternocleidomastoid muscle, extensive left supraclavicular lymphadenopathy, and left-sided pneumonia and pleural effusion.

Excisional biopsy of the right cervical lymph nodes yielded pus but no organisms. After 3 days of culture on chocolate agar, ≈20 tan colonies of an aerobic gram-negative bacillus were seen. The organism was oxidase-positive, catalase-positive, and indole-negative. After 6 days, abundant growth of a morphologically identical organism was seen on potato dextrose agar without antimicrobial agent and on Mycosel agar with chloramphenicol and cycloheximide but not on brain heart infusion agar with chloramphenicol and gentamicin (all media from Remel, Lenexa, KS, USA). Matrix-assisted laser desorption/ionization–time of flight mass spectrometry (Biotyper system version 3.1; Bruker Daltonics Inc., Billerica, MA, USA) from directly smeared colonies with and without formic acid overlay (*9*) yielded no identification, and growth was insufficient for biochemical identification or susceptibility testing. Species-level identification conducted by 16S rRNA gene sequencing (ABI MicroSeq 500 kit; Thermo Fisher Scientific, Grand Island, NY, USA, and the IDNS SmartGene system, version 3.6.10; SmartGene Inc., Raleigh, NC, USA) was interpreted as *A. methanolica* (100% identity >388 bp with type strain CGDAM1) (*7*). No other pathogens were grown or amplified from any specimen.

The patient initially received piperacillin/tazobactam and vancomycin; liposomal amphotericin B was administered in view of his recent *A. versicolor* pneumonia. After 4 days, the piperacillin/tazobactam was switched to meropenem and ciprofloxacin was added. Levels of inflammatory markers eventually returned to reference values, and the lymphadenopathy improved after 5 weeks of intravenous meropenem and intravenous and oral ciprofloxacin. The patient was discharged with ciprofloxacin, voriconazole for *A. versicolor* pneumonia, prophylactic trimethoprim/sulfamethoxazole, and interferon-γ. He subsequently underwent successful transplant of matched unrelated hematopoietic stem cells.

Patient 5

In 2014, a 36-year-old white man from Georgia, USA, who had X-linked CGD was hospitalized with cervical and abdominal lymphadenopathy; he had 1-year history of fever, malaise, and weight loss. X-linked CGD had been diagnosed when the patient was 6 months of age, when he had had hepatosplenomegaly, cervical lymphadenitis, and recurrent *S. aureus* infections. *Aspergillus* spp. pneumonia developed when he was 2 years of age, a liver abscess required incision and drainage when he was 4 years of age, and multiple abdominal abscesses required surgery when he was 6 years of age. Prophylactic trimethoprim/sulfamethoxazole for CGD had been effective until the hospitalization reported here.

A year before admission, the patient had experienced sudden onset of fever, cough, and shortness of breath. Right-sided pleural effusion was treated with antimicrobial drugs and thoracentesis. Cough and dyspnea improved, but fever, fatigue, malaise, myalgia, and weight loss of >15 kg were refractory to hydroxychloroquine and interferon-γ. At 2 months before admission, he had had cervical and abdominal lymphadenopathy with ascites. Peritoneal fluid examination, esophagogastroduodenoscopy, and colonoscopy were not informative. Excisional biopsy of a right posterior cervical lymph node, tuberculin skin testing, and QuantiFERON–TB Gold (QIAGEN, Valencia, CA, USA) testing produced negative results. Ciprofloxacin and metronidazole did not abate symptoms and fever.

At admission, the patient had right supraclavicular and left axillary lymphadenopathy and hepatosplenomegaly. ESR was 88 mm/h, and CRP concentration was 131 mg/L. CT images showed lung scarring, splenomegaly, pericardial effusion, and multifocal adenopathy of the left axilla, mediastinum, celiac, periaortic, retroperitoneal, and mesenteric regions. Culture of excised axillary lymph node grew *S. epidermidis,* and *G. bethesdensis* (100% match to the *G. bethesdensis* type strain by full 16S rRNA gene sequencing, ≈1,500 bp). Etest of *G. bethesdensis* isolate showed the following MICs in μg/mL: tobramycin (MIC = 4), ceftriaxone (MIC = 32), doxycycline (MIC = 8), trimethoprim/sulfamethoxazole (MIC = 2), and tigecycline (MIC = 16).

The patient was empirically administered vancomycin and ceftriaxone and was discharged with ceftriaxone, doxycycline, prophylactic trimethoprim/sulfamethoxazole, and itraconazole. His medication was eventually switched to cefdinir along with CGD prophylaxis. After 15 months, inflammatory markers and left neck and supraclavicular lymphadenopathy had improved but had not yet normalized.

Discussion

We have identified a total of 12 infections caused by 3 methylotroph bacteria in patients with CGD: 2 *A. methanolica*, 1 *M. lusitanum*, and 9 *G. bethesdensis* infections. Infections caused by *A. methanolica* and *G. bethesdensis* have been reported only for patients with CGD, whereas *M. lusitanum* in a patient without CGD undergoing chemotherapy for leukemia has been reported (*10*). These observations suggest that Nox2-based complex activity (superoxide production) is critical for protection against methylotroph infections. Consistent with this hypothesis, previous studies have demonstrated that *G. bethesdensis* persists in Nox2-based complex-deficient myeloid cells

and is largely resistant to oxygen-independent microbicidal activity (*11,12*).

Methylotroph infections in CGD patients typically result in elevated inflammatory markers and lymphadenopathy, which may progress to necrotizing lymphadenitis with or without abscess formation. The clinical course may be protracted because of infection persistence, antimicrobial drug resistance, and relapse. Culturing these bacteria is difficult, requiring atypical media and prolonged incubation. These infections have not been fatal for patients in North and Central America, but *G. bethesdensis* infections in patients from Portugal and Spain (*5*) have caused fatal meningitis and bacteremia, respectively. The different clinical courses and outcomes for these patients compared with those in North and Central America suggest that the *G. bethesdensis* strain from Europe may be more virulent; animal studies are needed to explore this possibility. Furthermore, the strains isolated in Spain and Portugal showed only a 99.7% match with the 16S rRNA sequence of the type strain from North America, whereas most strains from North and Central America showed a 100% match with the type strain. Moreover, the strain from Europe displayed more in vitro resistance to antimicrobial agents and was resistant to colistin, most β-lactams, and quinolones (*5*). Although the value and accuracy of in vitro susceptibility testing for *G. bethesdensis* are unknown and may lack predictive value, the clinical and laboratory differences between *G. bethesdensis* strains from the United States and Europe may have substantial implications for therapy.

The facultative methylotrophs we report were difficult to culture; their correct identification required non–culture-based techniques, such as 16S rRNA gene sequencing, and a high index of suspicion. Application of 16S rRNA sequencing and molecular probes to target tissues may identify previously unrecognized bacteria, which may accompany and possibly facilitate methylotroph infections.

G. bethesdensis is a gram-negative, aerobic, oxidase-negative, and catalase positive bacillus. Culture is facilitated by specimen centrifugation and plating on buffered charcoal yeast extract agar or solid mycobacterial media; incubation takes up to 2 weeks (*6*). *A. methanolica* is a gram-negative, aerobic, acid-tolerant, catalase-positive, urease-positive, oxidase-positive, non–spore-forming, and nonmotile rod-shaped bacterium (*13*). It forms tan colonies in <5 days on chocolate agar and grows well on potato dextrose agar. *M. lusitanum* is a vacuolated gram-negative aerobic rod that is positive for indophenol oxidase, catalase, and urease and produces a pink pigment. It also reduces nitrate and assimilates malate (*10*). Methanol dehydrogenase serology is currently under investigation as a potential supportive diagnostic or prognostic tool for tracking methylotroph infections, particularly those caused by *G. bethesdensis* (*14*).

Management of methylotroph infections is often prolonged and may require combination antimicrobial drug therapy and surgery. Drug susceptibilities are difficult to determine and interpret. In vitro, *G. bethesdensis* is typically resistant to most penicillins, cephalosporins, carbapenems, and quinolones, but it is sometimes susceptible in vitro to ceftriaxone, aminoglycosides, doxycycline, and trimethoprim/sulfamethoxazole; combinations of these drugs have helped achieve initial resolution (*6*). Although antimicrobial drug susceptibilities for *A. methanolica* are not well defined, infections seemed to respond to combinations including meropenem, ciprofloxacin, gentamicin, doxycycline, and trimethoprim/sulfamethoxazole. *M. lusitanum* seems to be susceptible to aminoglycosides, cephalosporins, ciprofloxacin, piperacillin/tazobactam, and imipenem but not to meropenem, trimethoprim/sulfamethoxazole, or aztreonam. The role of surgery in treating methylotroph infections has not been defined, but its successful use in several cases is noteworthy.

In conclusion, methylotrophs are environmental organisms that can cause necrotizing infections in patients with CGD. Infectious prodromes and clinical courses may be prolonged. Diagnosis requires a high index of suspicion so that appropriate culture conditions and culture-independent techniques can be established for diagnosis. The difficulty of growing methylotrophs from infected lesions gives pause for the use of the label "sterile inflammation" with regard to CGD patients. Methylotrophs should be aggressively sought as the cause of chronic necrotizing infections in patients with CGD.

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Dr. Falcone is a clinical fellow and an Oxford-Cambridge–National Institutes of Health scholar in the Laboratory of Clinical Infectious Diseases, National Institute of Allergy and Infectious Diseases, at the National Institutes of Health. Her primary research interests include host–pathogen interactions in patients with CGD and the role of the microbiome in CGD colitis.

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Address for correspondence: E. Liana Falcone, NIH–LCID/NIAID, Rm B3-4141, 9000 Rockville Pike Blvd 10/CRC, Bethesda, MD 20892, USA; email: emilia.falcone@nih.gov

etymologia

Methylotroph [meth'il-o-trof"]

From the Greek, *methy*, "wine," plus *trophē*, "food," methylotrophs are a diverse group of bacteria that can synthesize all their cell constituents from reduced single-carbon compounds, such as methanol or methane, or multicarbon compounds with no carbon–carbon bonds. The first methylotroph, *Methylomonas methanica*, was described (as *Bacillus methanicus*) grown aerobically on methane by Söhngen in 1906.

"**Metba**" by DOE

http://genome.jgi.doe.gov/finished_microbes/metba/metba.home.html

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Address for correspondence: Ronnie Henry, Centers for Disease Control and Prevention, 1600 Clifton Rd NE, Mailstop E03, Atlanta, GA 30329-4027, USA; email: boq3@cdc.gov

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Mortality Rates during Cholera Epidemic, Haiti, 2010–2011

Francisco J. Luquero, Marc Rondy, Jacques Boncy, André Munger, Helmi Mekaoui, Ellen Rymshaw, Anne-Laure Page, Brahima Toure, Marie Amelie Degail, Sarala Nicolas, Francesco Grandesso, Maud Ginsbourger, Jonathan Polonsky, Kathryn P. Alberti, Mego Terzian, David Olson, Klaudia Porten, Iza Ciglenecki

The 2010 cholera epidemic in Haiti was one of the largest cholera epidemics ever recorded. To estimate the magnitude of the death toll during the first wave of the epidemic, we retrospectively conducted surveys at 4 sites in the northern part of Haiti. Overall, 70,903 participants were included; at all sites, the crude mortality rates (19.1–35.4 deaths/1,000 person-years) were higher than the expected baseline mortality rate for Haiti (9 deaths/1,000 person-years). This finding represents an excess of 3,406 deaths (2.9-fold increase) for the 4.4% of the Haiti population covered by these surveys, suggesting a substantially higher cholera mortality rate than previously reported.

On October 22, 2010, the first cholera case in a century was confirmed in Haiti (*1*), one of the poorest countries in Latin America and the Caribbean. The ensuing cholera epidemic progressed rapidly, affecting all departments in the country within 1 month. Haiti's Ministère de la Santé Publique et de la Population (MSPP) led a large intervention to combat the epidemic (*2*). Médecins Sans Frontières (MSF) was one of the first nongovernmental relief organizations to respond to the epidemic and became the main organization supporting the MSPP in providing case management; more than half of all cholera patients nationwide received treatment in MSF-supported facilities (*3*,*4*).

The surveillance systems in place at the onset of the epidemic were unable to provide accurate and timely information (*5*); thus, on November 1, 2010, the MSPP launched a dedicated national cholera surveillance system based on daily collection of data about cholera cases and cholera-related deaths recorded in healthcare facilities across the country and of community cases and deaths reported by community members. Information about cholera-related deaths -in the community was collected through a variety of channels including reports from physicians, community health workers, and community leaders (*6*). In addition, in November 2010 an alert and response surveillance system was implemented to complement the national cholera surveillance system and to better monitor the spread of the epidemic and guide prevention and control activities. The alert and response surveillance system collected broad information about any cholera event requiring immediate response (*7*). By mid-April 2011 (end of the first wave of the cholera epidemic), 283,362 cases of cholera had been reported to the national cholera surveillance system, including 152,816 hospitalizations and 4,856 deaths (*6*). Although large, this number of deaths implies a small (≈1.1-fold) increase in the crude mortality rate for Haiti, where ≈90,000 deaths are expected to occur annually (*8*). According to the national cholera surveillance system data, by mid-January 2011, the case-fatality rate within healthcare facilities dropped to <1%, indicating improved cholera case management (*6*,*9*).

However, a rapid assessment of cholera-related deaths, conducted by active case finding in Artibonite Department in November 2010, estimated that 87% of deaths were not recorded in the hospital records (*10*). These findings raised the possibility that a substantial number of cases and deaths across the country were not reported during the first wave of the epidemic, a prospect supported by subsequent assertions that the existing surveillance systems at the onset of the epidemic were unable to fully capture the amount and type of data needed to monitor the rapid evolution of the epidemic (*6*,*11*). If true, this assertion would imply that the public health consequences of this epidemic were underestimated and would raise questions about ways to improve the implementation and accuracy of cholera surveillance during epidemics so that these vital data are rapidly available to help first responders implement the most effective public health interventions possible.

For this reason, MSF conducted 4 retrospective community-based surveys (1 in each of 4 locations where MSF intervened) to assess the extent of deaths during the first phase of the epidemic in Haiti (mid-October 2010 through mid-April 2011). We present the findings of these surveys and new estimates of the magnitude of the death

Author affiliations: Epicentre, Paris, France (F.J. Luquero, M. Rondy, A.-L. Page, B. Toure, M.A. Degail, S. Nicolas, F. Grandesso, M. Ginsbourger, J. Polonsky, K.P. Alberti, K. Porten); Ministry of Health, Port-au-Prince, Haiti (J. Boncy); Médecins Sans Frontières, Paris (A. Munger, M. Terzian, D. Olson); Médecins Sans Frontières, Geneva, Switzerland (H. Mekaoui, E. Rymshaw, I. Ciglenecki)

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toll and crude mortality rates for this first epidemic wave of cholera in Haiti.

Methods

The study was conducted in collaboration with the MSPP after obtaining permission to conduct the survey. The study protocol was approved by the National Ethical Review Board of Haiti. Written consent was obtained from all study participants.

Study Setting and Design

Of the 4 survey sites (Gonaives, Cap-Haïtien, North Department, and Gaspard and Zabricots), 2 were urban and 2 were remote rural areas (Figure 1). In Gonaives, the main town of Artibonite Department, the survey covered the entire town. In Cap-Haïtien, the capital of North Department, the study was conducted in a densely populated slum. In Gaspard and Zabricots, the survey was conducted in a small, hilly section where poor road quality made road access difficult. The North Department rural site combined remote, isolated areas with areas of better access. These 4 sites were selected because of the large number of cases reported from them to the national cholera surveillance system during the first wave of the epidemic and because MSF had implemented a large intervention at each of them. These settings also represented diverse contexts (urban vs. rural, high vs. low population density, good vs. poor access to healthcare) where cholera could have evolved in different ways.

We used a core generic protocol for the 4 sites and then adapted the sampling approach to the different settings. At Gonaives and North Department, we conducted a 2-stage, household-based cluster survey. At the first stage of sampling, clusters were allocated to communal sections (administrative subdivisions of the source population) proportionally to their selected population size. At the second stage, we randomly selected the first household of each cluster through spatial sampling by using the R statistical package (*12*). The starting household of each cluster in the field was identified by use of a global positioning system. We then selected subsequent households by proximity, until the cluster was complete. At Cap-Haïtien and at Gaspard and Zabricots, every household was surveyed because of the small total populations (exhaustive surveys).

The cluster-based surveys were conducted during March 29–April 7, 2011, in Gonaives and during April 23–May 13, 2011, in North Department. The exhaustive surveys were conducted during April 11–29, 2011, in Gaspard and Zabricots and during April 1–14, 2011, in Cap-Haïtien.

Sample Size

The study design called for sampling 16,000 persons at each site. This sample size was sufficient to estimate a crude mortality rate of 18 deaths/1,000 person-years (95% CI 14–22), which represents twice the expected crude mortality rate for 2010 in Haiti (9 deaths/1,000 person-years) by the United Nations World Population Prospects (*8*) (online Technical Appendix, http://wwwnc.cdc.

Figure 1. Study sites used to determine mortality rates during cholera epidemic, Haiti, 2010–2011: entire town of Gonaives, urban slum in Cap-Haïtien, rural communal sections in North Department, and communal section of Gaspard. Red circles, rural sites; blue circles, urban sites. Circle size is proportional to the estimated population of each site.

gov/EID/article/22/3/14-1970-Techapp1.pdf). The study involved a recall period of 170 days and a hypothetical design effect (loss of variance because of intracluster homogeneity) of 2.

Data Collection

The same questionnaire was used at each of the 4 sites. Survey teams recorded data about deaths that occurred during predefined recall periods starting on October 17, 2010 (epidemiologic week 42 in 2010) and ending on the date of the interview in 2011 (no later than May 13, 2011). At all sites, death recall periods included the first (main) wave of the cholera epidemic in Haiti.

Trained interviewers administered the questionnaires to the head of the household or the most senior adult responsible for the household present at the time of the interview. The questionnaire asked about deaths in the household that occurred within the recall period. For each death, we reported the date and the age of the deceased (in years) and coded the reported cause of death. In addition, we asked about episodes of watery diarrhea during the recall period and the outcome of these episodes.

To facilitate recall of the survey period, we used a calendar of locally important events. We also asked about family members who were absent and about persons who were visiting the household; we excluded from the denominator periods when household members were absent for >2 weeks and visitors stayed for <1 month. We asked all respondents for the age and sex of living household members.

Estimated Deaths

The crude mortality rate and watery diarrhea–specific mortality rate were each expressed as deaths per 1,000 person-years; we used as the denominator each person's time at risk during the recall period. To estimate the diarrhea-specific mortality rate, we counted those for whom death was the reported outcome of a watery diarrhea episode.

We also calculated mortality rates per epidemiologic week (*13*) by dividing deaths that occurred in these periods by the total person-time spent by the surveyed population in each week. The number of days at risk for each person was determined as the difference between his/her date of entry into the household (birth, moving in, or October 17, 2010) and date of exit (death, moving out, or interview date).

The expected number of deaths in the absence of an epidemic was computed by using as a baseline the expected mortality rate for Haiti in 2010 provided by United Nations World Population Prospects (*8*), which was based on a combination of nationally representative household surveys, census reports, and death registries (*8*,*14*). The expected number of deaths was obtained by multiplying the expected mortality rate by the estimated person-years lived in the study areas. The number of excess deaths was calculated by subtracting the expected number of deaths in the absence of an epidemic from the estimated number of deaths during the study period.

Statistical Analyses

Data were entered by using EpiData version 3.0 (EpiData Association, Odense, Denmark) and analyzed with Stata 10 software (StataCorp LP, College Station, TX, USA). Crude mortality rate point estimates were obtained by using a Poisson regression model; the design effect was estimated by using the STATA command "svy" to obtain 95% CIs.

Results

For the cluster-based surveys in Gonaives and North Department, we randomly selected 105 and 138 clusters from which we included, respectively, 3,201 and 3,187 households. The total population surveyed and the household size was similar for all 4 sites (i.e., for the cluster-based and the exhaustive surveys, varying from 5.3 members in North Department to 6.2 in Gaspard and Zabricots). Median age varied from 19 years in Gaspard and Zabricots to 21 in North Department and Cap-Haïtien (Table 1).

A total of 983 deaths were reported from the 4 sites (Table 2), corresponding to crude mortality rates (deaths/1,000 person-years) ranging from 19.1 in Gonaives to 35.4 in Gaspard and Zabricots. The most frequently reported cause of death was diarrhea. The second most frequently reported cause was respiratory tract infection.

Overall, 1,800 deaths were expected during the study period (average recall period 176 days) in the target

Table 1. Sites and participants in study of mortality rates during cholera epidemic, Haiti, 2010–2011

Variable	Study site			
	Gonaives	Cap-Haïtien	North Department	Gaspard
Estimated population	228,725	16,000	173,904	17,000
No. clusters	105	Not applicable	138	Not applicable
No. households sampled	3,201	2,682	3,187	3,379
No. survey participants present on survey date	18,363	14,694	16,900	20,946
Average recall period, d	162	170	195	174
Median age (interquartile range), y	20 (11–30)	21 (12–32)	21 (11–40)	19 (9–36)
No. (%) children younger <5 y of age	1,921 (10.5)	1,482 (10.4)	1,690 (10.0)	2,574 (12.3)
Male-to-female ratio	0.84	0.87	0.91	0.99
Average household size (SD)	5.7 (2.5)	5.5 (3.1)	5.3 (2.3)	6.2 (2.8)
No. births	155	106	110	309

Table 2. Crude and diarrhea-specific mortality rates during cholera epidemic, Haiti, 2010–2011

Variable	Study site			
	Gonaives	Cap-Haïtien	North Department	Gaspard
Study population	18,363	14,694	16,900	20,946
Person-years	8,121	6,230	9,027	10,004
No. deaths	159	194	275	355
Crude mortality rate (95% CI)*	19.1 (14.9–24.4)	28.4	30.2 (23.5–38.8)	35.4
No. diarrhea-related deaths	105	166	224	277
Diarrhea-specific mortality rate (95% CI)*	12.4 (8.9–17.2)	24.3	24.5 (18.5–32.6)	27.7

*No. deaths per 1,000 person-years, calculated by using Poisson regression taking into account the survey design.

population (438,505 persons), but we estimated that 5,296 deaths occurred. The difference between these numbers (i.e., 3,406 deaths) represents the excess deaths and corresponds to a 2.9-fold overall increase (ranging from a 2-fold increase in Gonaives to a 4-fold increase in Gaspard and Zabricots) for the 4.4% of the Haiti population covered by these surveys. Overall, we estimated that 3,999 diarrhea-related deaths occurred in the study population (Table 3).

The excess deaths were not distributed equally over time. The highest number of deaths occurred in 2010 during epidemiologic weeks 44–52 (October 17, 2010–1 January 1, 2011) in the 4 sites, reaching 127 deaths per 1,000 person-years in North Department—a 14-fold increase compared with baseline mortality rates in Haiti (Figure 2). After January 1, 2011, the crude mortality rate started to decrease, and by the end of the recall period, the rate returned to the baseline crude mortality rate expected for Haiti (Figure 2).

Discussion

From October 2010 through April 2011 at the 4 study sites in Haiti (which covered 4.4% of the Haiti population), the crude mortality rate increased by an estimated 2.9-fold (2.1–4.0-fold across sites) compared with baseline data, which corresponds with 3,406 excess deaths. However, the official number of cholera deaths reported for the entire country during the study period was 4,856 (*6*,*7*), which would represent an ≈1.1 fold increase in the crude mortality rate. In Gonaives, where a direct comparison between the number of deaths calculated in our study and the number of cholera deaths reported by national cholera surveillance was possible, we estimated 1,254 watery diarrhea–related deaths and 1,028 excess deaths, whereas the national cholera surveillance system reported only 132 cholera deaths during the same period (*6*,*7*). Considering the high attack rates reported throughout most of the country during this period (*6*,*7*), our results suggest a larger effect of the epidemic on the mortality rates than previously reported in Haiti.

Most of the deaths we recorded occurred during the first weeks of the epidemic and were attributed by survey respondents to watery diarrhea. We found that during January 2011, the crude mortality rate in the 4 study areas decreased to baseline rates (i.e., similar to estimates for 2010 that do not account for the epidemic), indicating that the largest effect on mortality rates occurred during the first 6 weeks of the epidemic.

Several limitations should be considered when interpreting our findings. First, because the study assessed deaths retrospectively, recall bias might have occurred. Although recall bias associated with ascertaining death events per se is unlikely, considering the proximity between the events and the surveys, recall bias might have influenced the accuracy of reported dates, cause of death, or both. In particular, overreporting of diarrhea as cause of death is possible, considering the strong psychological effects of the cholera epidemic on the Haiti population during this period. We tried to minimize the effect of this possible bias in our calculation of the diarrhea-specific mortality rate by including in this calculation death reported as an outcome of a clearly identified diarrhea episode, rather than death with diarrhea cited as a cause of death. However, this effort may have been insufficient to entirely correct this bias.

Another limitation is that, despite efforts to be exhaustive in Gaspard and Zabricots and in Cap-Haïtien, 12% of the estimated population of the Cap-Haïtien slum was not included in the survey. This lack of coverage might be

Table 3. Excess deaths during cholera epidemic, Haiti, 2010–2011

Variable	Study site			
	Gonaives	Cap-Haïtien	North Department	Gaspard
Population	228,725	14,931	173,903	20,946
Person-years	101,167	6,936	93,026	10,028
Expected total no. deaths*	905	62	833	90
Estimated total no. deaths (95% CI)†	1,933 (1,512–2,472)	197	2,810 (2,186–3,612)	355
Risk ratio	2.1	3.2	3.4	4.0
No. excess deaths (95% CI)	1,028 (606–1,567)	132	1,978 (1,354–2,780)	265
Estimated no. diarrhea-related deaths (95% CI)†	1,254 (900 – 1,740)	169	2,279 (1,721–3,033)	278

*Calculated by multiplying the expected baseline mortality rate in Haiti for the year 2010 in the absence of an epidemic (8.95 deaths/1,000 person-years) by the population (person-years).
†Estimated according to answers to the questionnaire.

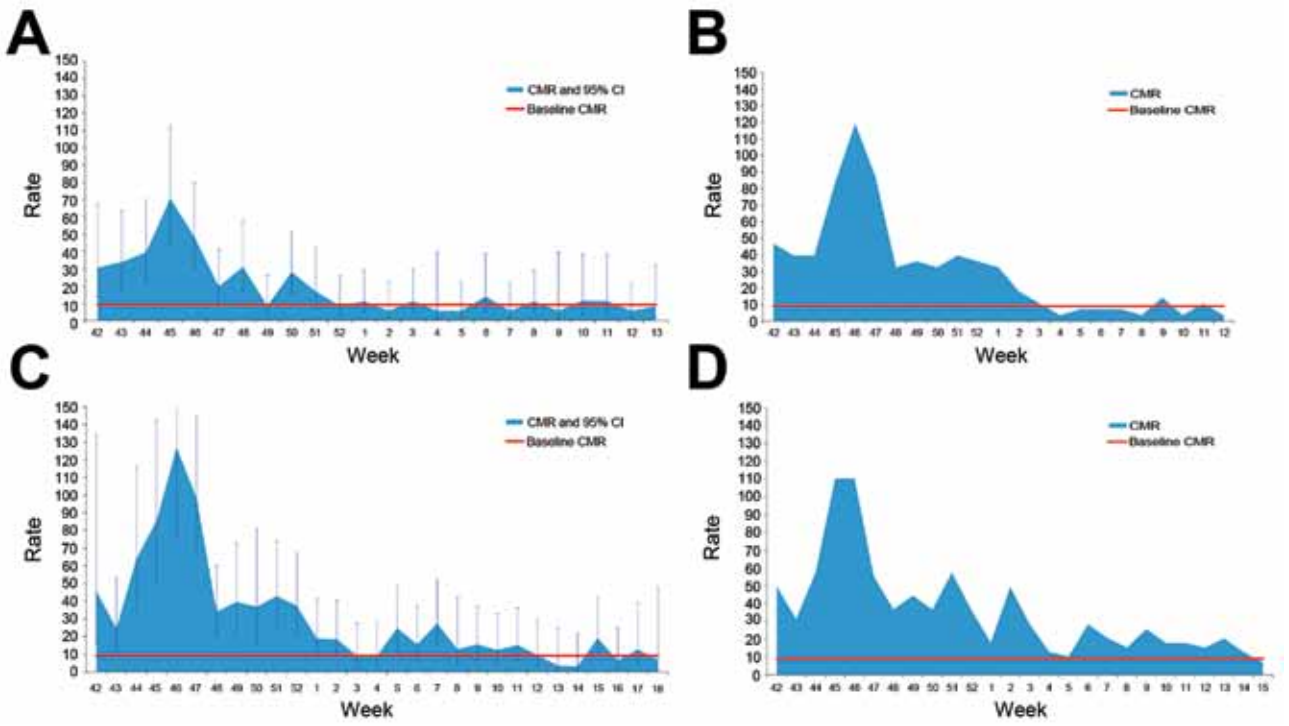

Figure 2. Crude mortality rate (CMR; no. deaths/1,000 person-years), by week, at study sites used to determine mortality rates during cholera epidemic, Haiti, 2010–2011. A) Gonaives; B) Cap-Haïtien; C) North Department; D) Gaspard. Red line indicates the expected crude mortality rate for Haiti in 2010 in the absence of an epidemic. Error bars indicate 95% CIs.

explained by an inaccurate estimate of the official population size, by missed households, or both. If the excluded population differed from the study population in terms of deaths, the Cap-Haïtien estimates would be biased.

In the absence of exceptional events, mortality rates generally follow stable trends (online Technical Appendix). The baseline mortality rates that we used for calculation of excess deaths in Haiti are internationally accepted as valid indicators of the rate of death occurrences (*8*,*14*). Variations in completeness and accuracy of the data sources might bias these baseline estimates. As an example, for Haiti during 2010–2015, the United Nations World Population Prospects provides a low variant of 8.5 deaths and a high variant of 8.7 deaths per 1,000 person-years. To assess the possible bias associated with the baseline mortality rate assumption, we conducted a sensitivity analysis (online Technical Appendix), which showed a low effect of these variations. Seasonal variation of morality rate could also partially explain the excess deaths; however, the strong correlation between the crude mortality rates and the epidemic curve suggest a true association between the excess deaths and the peak of the epidemic. In addition, the high proportion of deaths attributed to diarrhea relative to the expected proportion (≈85% vs. 5% 16%; [*15–18*]) is consistent with the existence of high numbers of deaths from cholera.

As expected, the overall crude mortality rates varied by region; however, the only baseline available was a national average, not regionally specific crude mortality rates for Haiti. The site-specific estimates of excess deaths may be less accurate because the local baseline crude mortality rates may be higher or lower than the national average. Because our surveys included a range of contexts (e.g., urban, rural, good and poor access to healthcare), pooled comparisons are probably largely representative of the excess deaths caused by the cholera epidemic in areas with high incidence of cholera. However, cholera incidence rates at the study sites may have been higher than those in other regions because these sites were selected for their large number of reported cases.

Before the establishment of the national cholera surveillance system and the alert and response surveillance system, health surveillance relied on 2 syndrome-based disease-surveillance systems that were implemented after an earthquake occurred near the capital of Port-au-Prince in January 2010 (*19*,*20*). However, these systems were insufficient for handling the amount and type of data needed to monitor the evolution of the cholera epidemic (*6*). Because surveillance is a cornerstone in any epidemic response intervention providing essential information to guide prevention and control strategies, a comprehensive cholera surveillance system was required. However, if the estimates presented here are correct, then many deaths in Haiti were never counted in the official

statistics during the first wave of the cholera epidemic despite the commendable effort to promptly implement a national cholera surveillance system.

Prior cholera epidemics have shown the limits of traditional response strategies for reducing the spread of the epidemics (*21*); but for many epidemics, the response interventions have been considered successful to limit the number of deaths (*22*). In Haiti, the national cholera surveillance system showed similar trends; however, our study results suggest that that the cholera-associated mortality rates have been substantially underestimated. These results imply that the outbreak response strategy was insufficient for avoiding a high number of deaths in the first weeks of the epidemic despite the enormous effort made by the MSPP, the World Health Organization, MSF, and other agencies to improve access to appropriate treatment for cholera patients.

The high mortality rate documented in our study during the first weeks of the epidemic might be associated with different factors. The healthcare system in Haiti had been severely strained by the 2010 earthquake (*23*). Although none of the areas included in our survey were directly affected by the earthquake, the national health services were still rebuilding when the cholera epidemic began (*6*,*19*,*24*). In addition, cholera was an unknown disease for the Haiti population, including medical staff who were not accustomed to treating the rapid clinical evolution of dehydration associated with severe cholera. Likewise, members of the population were unaware of how to prevent cholera and the value of promptly seeking care at the onset of signs and symptoms.

Because most cholera-associated deaths occur on the first day of sickness (*10*,*25*), early access to care is critical for improving survival rates. Thus, among the crucial steps for reducing cholera-associated deaths are decentralizing medical care and creating public awareness about cholera and where to seek care. However, decentralization of healthcare structures in Haiti was and remains difficult in very remote areas such as some villages in North Department that require a 10-hour walk to get to the nearest healthcare facility. This link between healthcare access and cholera deaths is consistent with our observation of large differences in mortality rates across the 4 study sites; for example, the mortality rate for the most remote area of Gaspard was almost twice as high as that for urban Gonaives (35.4 vs. 19.1 deaths/1,000 person-years, respectively). Although further investigations are required to fully interpret these figures, distance and ease of accessing care are most likely contributing factors (*26*). Innovation is needed to improve the promptness of establishing access to healthcare, especially in remote areas. Involving communities in preparedness plans for epidemics might be a promising approach (*27*,*28*). New tools for preventing cholera should be considered, such as innovative water treatment systems, new sanitation solutions, and vaccines. At the onset of the cholera epidemic in Haiti, a limited number of vaccine doses were available, but they were not used in the control strategy. Since 2010, vaccine supply and use have increased worldwide, including in Haiti, and vaccination is becoming an additional tool that should be considered for outbreak prevention and control (*29*,*30*), especially where good access to healthcare cannot be made rapidly available or guaranteed over time. We also consider essential the provision of clear guidance on ways to improve current epidemic response plans from the World Health Organization and the Global Task Force for Cholera Control (*31*).

Our study findings offer some implications for surveillance and the response strategy in future epidemics. The results suggest that relying on surveillance based primarily in healthcare facilities provides a biased picture of an epidemic and underestimates illness and death from the disease, especially if the surveillance system has weaknesses and requires adaptation during the first phase of the epidemic. This limitation has been documented in Zimbabwe, where community-based studies showed underreporting of cholera-related deaths (*32*). Rigorous assessments at the community level, including surveys and community-based surveillance, are essential for accurately estimating the true extent of cholera illness, death, and socioeconomic cost. In the absence of better estimates, cholera will remain a neglected problem for less-developed countries if the attention, innovation, and funding allocated are insufficient for improving the current control efforts.

In conclusion, our study findings suggest that the mortality rate during the cholera epidemic in Haiti was larger than that reported in the official statistics (*6*). Cholera epidemics are primarily surveyed through information collected in healthcare facilities; however, this type of surveillance might not be enough to describe the true extent of cholera, especially in places where the healthcare systems are weak. Community-based systems should be reinforced to complement healthcare facility–based systems. Affected communities should also be more involved in preparedness and response strategies because they can effect timely provision of oral rehydration therapy, promote prompt seeking of healthcare, and integrate new preventive tools into local practices. Clear leadership and international consensus are required to improve current epidemic response strategies, which should ultimately stop cholera from causing a large and avoidable number of deaths.

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Dr. Luquero is a medical epidemiologist who researches neglected health problems in vulnerable populations. His primary research interest is vaccine-preventable diseases, including cholera, rotavirus infection, and measles.

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Address for correspondence: Francisco J. Luquero, Epicentre, 8 Rue Saint Sabin, 75011 Paris, France; email: francisco.luquero@epicentre.msf.org

Use of Transnational Services to Prevent Treatment Interruption in Tuberculosis-Infected Persons Who Leave the United States[1]

Cynthia A. Tschampl, Deborah W. Garnick, Edward Zuroweste, Moaven Razavi, Donald S. Shepard

A major problem resulting from interrupted tuberculosis (TB) treatment is the development of drug-resistant TB, including multidrug-resistant TB (MDR TB), a more deadly and costly-to-treat form of the disease. Global health systems are not equipped to diagnose and treat the current burden of MDR TB. TB-infected foreign visitors and temporary US residents who leave the country during treatment can experience treatment interruption and, thus, are at greater risk for drug-resistant TB. Using epidemiologic and demographic data, we estimated TB incidence among this group, as well as the proportion of patients referred to transnational care–continuity and management services during relocation; each year, ≈2,827 visitors and temporary residents are at risk for TB treatment interruption, 222 (8%) of whom are referred for transnational services. Scale up of transnational services for persons at high risk for treatment interruption is possible and encouraged because of potential health gains and reductions in healthcare costs for the United States and receiving countries.

Drug-resistant tuberculosis causes tremendous suffering and high death rates, as well as disruption to public health budgets and TB control efforts (*1*,*2*). Multidrug-resistant TB (MDR TB), defined as TB resistant to the 2 main TB drugs, is a growing concern, and current global health systems are inadequate to deal with this airborne, deadly pandemic disease (*3*,*4*). Mobile populations are more likely to have TB because of various risk factors (e.g., crowded housing and stress of relocating) and to spread TB in the absence of timely and effective intervention (*5*,*6*). Most TB cases in high-income nations are in persons born outside those nations (*7*,*8*). Mobility also contributes to a risk for treatment interruption, a key cause of drug resistance (*5*,*6*,*9*).

An understanding of the magnitude and dynamics of treatment interruption among mobile populations is essential for public health surveillance and policymaking. To elucidate this problem, we used epidemiologic and demographic data from organizations such as the World Health Organization (WHO), US Department of Homeland Security (DHS), and Pew Hispanic Center to estimate the incidence of TB in a population at elevated risk for drug resistance, namely foreign-born persons who depart the United States before clinically recommended TB treatment was completed. We then estimated the proportion of those persons who received transnational care–continuity services by using case management data from the provider organizations (the nonprofit Migrant Clinicians Network [MCN], Austin, Texas, USA, and the County of San Diego TB Control Program, San Diego, CA, USA).

Methods

Population

The study population included any nonimmigrant, nonrefugee, nonnative visitor to the United States during 2008–2012 who had TB and left the country before treatment completion (Table 1). Because persons visit the United States from many countries and via many routes, both legal and illegal, the study population was categorized into subgroups. Sufficient data were available to calculate person-years among those temporarily in the United States with authorization. This subpopulation included all nonimmigrant visitors and temporary residents because they had been in the country long enough to receive a diagnosis of TB but had visa restrictions that nearly assured TB treatment would not be finished before they left.

We classified authorized visitors into 7 categories (online Technical Appendix Table 1, http://wwwnc.cdc.gov/EID/article/22/3/14-1971-Techapp1.pdf): tourists and business travelers, students and exchange visitors, temporary workers, diplomats and other representatives, persons with any other visa class, persons with unknown visa class, and Canada and Mexico nationals not requiring an entry–exit (I-94) card. The remaining persons within the study population were in the country without authorization and were

Author affiliations: Brandeis University, Waltham, Massachusetts, USA (C.A. Tschampl, D.W. Garnick, M. Razavi, D.S. Shepard); Migrant Clinicians Network, Austin, Texas, USA (E. Zuroweste)

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divided into 4 data-driven groupings: persons detained and then removed by US officials (nonexpedited), all other nonexpedited removals, persons from Mexico who voluntarily left, and all other persons who voluntarily left.

Six subgroups, including an expedited removal subgroup, were excluded (Table 1). Exclusion criteria comprised permanent US residency and no US entry or exit during the study period. MCN and Brandeis University (Waltham, Massachusetts, USA) Institutional Review Boards approved this study.

Data

To estimate incident TB cases, we needed TB incidence rates and number of person-years for each subgroup. We obtained person-years by combining an appropriate measure of time at risk for active TB with a measure of magnitude (e.g., number of nonimmigrant visa admissions) (Table 2). We obtained country-specific TB incidence rates per 100,000 person-years from WHO (*10*). As in other studies (*11*), we defined countries with high, medium, and low TB incidence as ≥100, 15–99, and 0–14 cases per 100,000 person-years, respectively.

Table 1. Study population inclusion and exclusion criteria, data sources, and estimation equations used to determine number at risk of treatment interruption among TB-infected, authorized and unauthorized visitors to the United States, 2008-2012*

Subgroup	Justification	References	Calculation method
Included in study			
Resided in the United States with authorization†			
Tourist or business travelers	Left United States after <2 mo	(*10*–*15*)	PY × (country-specific TB incidence rate)‡
Student or exchange visitors	Left United States after <9 mo	(*10*–*15*)	PY × (country-specific TB incidence rate)‡
Temporary workers	Left United States after <5 mo	(*10*–*15*)	PY × (country-specific TB incidence rate)‡
Diplomat or other representatives	Left United States after <3 mo	(*10*–*15*)	PY × (country-specific TB incidence rate)‡
Persons with all other visa types	Left United States after <1 mo	(*10*–*15*)	PY × (country-specific TB incidence rate)‡
Persons with unknown visa type	Left United States after <2 mo	(*10*–*15*)	PY × (country-specific TB incidence rate)‡
Visitors from Canada and MX without I-94 card	Left United States after <1 mo	(*10*–*15*)	PY × (country-specific TB incidence rate)‡
Resided in United States without authorization			
Detained first and then removed by US officials	Left United States; subgroup in this category for which most data was available	(*12*,*16*,*17*)	No. detainees × (183/365) × TB incidence rate for detainees × proportion removed§
All other removals meeting inclusion criteria	Left United States	(*10*,*12*,*16*,*17*)	(No. nonexpedited removals × estimated no. detained before removal) × (183/365) × (country-specific TB incidence rate)¶
MX nationals leaving United States of own volition	Left United States	(*10*,*12*,*16*,*18*,*19*)	No. MX nonexpedited removals × estimated % left voluntarily × (183/365) × (MX TB incidence rate)#
All other nationals leaving United States of own volition	Left United States	(*10*,*12*,*16*,*18*,*19*)	(Total who left voluntarily − MX left voluntarily) × (183/365) × (57/100,000 PY)**
Excluded from study			
Resided in the United States with authorization			
Immigrants	Permanent residents; no requirement to leave United States	(*12*–*15*)	NA
Refugees	Permanent residents	(*12*–*15*)	NA
Asylees	Permanent residents	(*12*–*15*)	NA
Resided in the United States without authorization			
Currently residing in the United States	Did not leave United States during study period	(*12*,*16*,*18*)	NA
Returnees and expedited removals††	Did not officially enter United States	(*12*,*16*,*18*)	NA
Detained but not removed	Did not leave United States during study period	(*12*,*16*,*18*)	NA

*Study population is defined as those who were born outside the United States, had active tuberculosis while in the United States, and then left the United States before treatment completion was possible. I-94 card, the entry/exit form that all nonimmigrant visitors (except certain ones from MX and Canada) must fill out; MX, Mexico/Mexican; NA, not applicable; PY, person-years; TB, tuberculosis.
†These subgroups included family members. See online Technical Appendix Table 1 (http://wwwnc.cdc.gov/EID/article/22/3/14-1971-Techapp1.pdf) for a complete list of visas for each subgroup and their corresponding mean and median length of stay.
‡Calculated for all countries, 2008–2012. PY = no. of admissions × (weighted mean length of stay in days/365). Weighted median length of stay was used for all these groups in sensitivity analyses, except those without an I-94 card, as only the mean was available. See online Technical Appendix Tables 3–6 for results.
§183 d, or 6 mo, of risk was assumed as the minimum amount of time for TB to be diagnosed, treatment started, and a treatment interruption caused by leaving the United States.
¶Calculated for top 12 receiving countries by using World Health Organization country-specific TB incidence rates. All other countries grouped together and multiplied by the midpoint TB incidence rate of 57 cases/100,000 PY.
#Calculated for MX nationals; they make up the majority (assumed at 90%) of this subgroup.
**All other countries' nationals assumed to make up 10% of this subgroup; the midpoint incidence rate of TB burden was 57 cases/100,000 PY.
††These are 2 immigration enforcement categories with specific definitions used by US Department of Homeland Security (*16*).

We obtained the number of nonimmigrant visas issued in 2008–2012 from the US Department of State (*15*) and the number of nonimmigrant visa admissions with median and mean lengths of stay (LOS) for each country from DHS (*12*,*13*). We categorized nonimmigrant visa admissions into 7 groups, including a group of nonimmigrant visitors from Canada and Mexico without an I-94 card. DHS also provided data on the proportion of these admissions from Canada (28.5%) and Mexico (71.5%) (*12*,*14*).

We used DHS data (reported in aggregate and categorized by top receiving countries) on the number of compulsory and confirmed departures from the United States (*12*,*16*). To extrapolate the number of voluntary exits for persons from Mexico, we used previously estimated percentages (*18*) of Mexican nationals involuntarily returning home and mean LOS before removal. We used data reported by Schneider and Lobato (*17*) on TB case rates and removal rates for persons detained by US immigration officials.

We estimated the number of persons served by transnational care coordination services by using published case management data from the 2 existing referral programs, Health Network (previously known as TBNet) and CureTB. MCN operates Health Network, which began in 1998 and provides bridge case management, care continuity, patient education and navigation, and bidirectional communication between providers on behalf of patients for high-value interventions. In 2011, Health Network managed patients returning to >50 countries and achieved an 84.7% treatment completion rate (*20*). CureTB, operated by the County of San Diego, started managing binational (United States and Mexico) TB cases in 1997 and recently expanded to manage cases in persons moving to Central America; CureTB reported a 79% treatment completion rate (*21*).

Statistical Analysis

Some subgroups had better data available for estimating incident TB cases; therefore, we present the analyses in order of increasing complexity (Table 1) and then discuss calculations regarding the transnational care–continuity services. First, we estimated incident TB cases for authorized vis-

Table 2. Admissions, person-years, incident tuberculosis cases, and case rates stratified by visa group and tuberculosis burden level for persons temporarily in the United States, with authorization, 2008–2012*

			Tuberculosis		
Visa group†	Admissions (%)	PY (%)	Total no. cases (%)	No. cases/100,000 PY (95% CI)	No. cases/100,000 admissions (95% CI)
Tourist and business traveler	201,578,207 (25)	14,431,062 (47)	6,161 (48)	43 (36–49)	3 (3–4)
High-burden countries	13,858,503 (2)	1,277,466 (4)	2,614 (20)	205 (174–235)	19 (16–22)
Medium-burden countries	126,042,138 (15)	10,733,970 (35)	3,342 (26)	31 (26–36)	3 (2–3)
Low-burden countries	61,677,566 (8)	2,419,625 (8)	205 (2)	8 (7–10)	0
Student/exchange visitor‡	9,417,888 (1)	6,293,260 (21)	3,675 (28)	58 (50–67)	39 (33–45)
High-burden countries	1,862,032	1,244,255 (4)	2,040 (16)	164 (139–189)	110 (93–126)
Medium-burden countries	4,932,913 (1)	3,296,292 (11)	1,516 (12)	46 (39–53)	31 (26–35)
Low-burden countries	2,622,943	1,752,714 (6)	118 (1)	6 (5–7)	5 (4–5)
Temporary worker‡	12,904,847 (2)	4,948,262 (16)	2,319 (18)	47 (40–54)	18 (15–21)
High-burden countries	2,154,566	826,151 (3)	1,604 (12)	194 (165–223)	74 (63–86)
Medium-burden countries	5,252,984 (1)	2,014,215 (7)	587 (5)	29 (25–34)	11 (10–13)
Low-burden countries	5,497,297 (1)	2,107,895 (7)	128 (1)	6 (5–7)	2 (2–3)
Diplomat and other representative‡	1,761,901	381,343 (1)	243 (2)	64 (54–73)	14 (12–16)
High-burden countries	332,182	71,897	167 (1)	232 (198–267)	50 (43–58)
Medium-burden countries	819,393	177,348 (1)	66 (1)	37 (31–42)	8 (7–9)
Low-burden countries	610,326	132,098	10	8 (7–9)	2 (1–2)
All other classes	2,267,465	119,836	107 (1)	90 (76–103)	5 (4–5)
High-burden countries	905,522	38,206	89 (1)	232 (197–267)	10 (8–11)
Medium-burden countries	1,107,955	46,747	18	38 (32–44)	2 (1–2)
Low-burden countries	253,988	34,884	0.8	2 (2–3)	0
Unknown visa class	1,123,438	90,579	52	57 (49–66)	5 (4–5)
High-burden countries	71,316	6,643	16	236 (200–271)	22 (19–25)
Medium-burden countries	792,676	73,838	35	47 (40–54)	4 (4–5)
Low-burden countries	259,446	10,098	2	17 (14–20)	1 (1–1)
Canada and Mexico nonimmigrant without I-94 card	592,645,430 (72)	4,266,235 (14)	371 (3)	9 (7–10)	0
Total	821,699,176	30,530,577	12,928	NA	NA
Annual average	164,339,835	6,106,115	2,586	NA	NA

*I-94 card, the entry/exit form that all nonimmigrant visitors (except certain ones from Mexico and Canada) must fill out; NA, not applicable; PY, person-years.
†High-burden countries were defined as having ≥100 TB incident cases/100,000 PY; medium-burden countries were defined as having 15–99 cases/100,000 PY, and low-burden countries were defined as having 0–14 cases/100,000 PY.
‡Corresponding spouses and children are also included in each of these categories; see online Technical Appendix Table 1 (http://wwwnc.cdc.gov/EID/article/22/3/14-1971-Techapp1.pdf) for full list of visas included in each subgroup.

itors in the United States stratified by visa group, country, and year and subsequently aggregated across levels of TB burden before final summation. We started by calculating a weighted mean LOS for each visa group (online Technical Appendix Table 1) and then applied the following equation (Equation 1):

> incident TB cases = (person-years for visitors with authorization) × (country-specific TB incidence)

where person-years = (no. of admissions) × (mean LOS in days/365 days per year). For example, in 2008 there were 163,845 persons from South Korea in the students and exchange visitors subgroup who stayed a mean of 244 days, resulting in 109,485 person-years (*12*). As a sensitivity analysis, we substituted available weighted median LOS and found 79,005 person-years (online Technical Appendix).

For the group with unknown visa type, we used mean LOS (34 days) for all visas (*13*). Persons from Canada and Mexico without an I-94 card had a mean LOS of 3.7 and 1.1 days, respectively (*13*). We used birth-country TB case rates because past studies suggested TB activation rates among non–US-born persons most closely match their TB risk at home (*22,23*). For admissions with no country, we applied the midpoint rate from the medium-incidence category (i.e., 57 cases/100,000 person-years) after testing it against the global average rate of 122 cases/100,000 person-years (*24*).

We further calculated TB cases per 100,000 person-years and 100,000 admissions, along with 95% CIs, assuming a Poisson distribution (online Technical Appendix). Another sensitivity analysis, using I-94 visa counts from US Department of State (*15*), provided an alternative to the 95% CI. We calculated the range within which the actual number of cases should fall by adapting equation 1. For the lower bound, we assumed 1 admission per visa (despite multiple-entry visas) and replaced admissions with visa counts. For the upper bound, we assumed each visitor had 12 months of risk, the highest possible value.

Second, we estimated TB cases for persons in the United States without authorization whom US officials removed. We began by adapting Equation 1 and multiplying by proportion (*17*) of persons removed postdetention (Table 1). We assumed a 6-month risk for all unauthorized subgroups because that is the minimum amount of time required to receive a diagnosis of TB infection, begin treatment, and still leave the United States before treatment completion. Sensitivity analyses included varied parameters of time at risk, TB case rate, and proportion removed (online Technical Appendix). We then estimated, again adapting Equation 1, TB cases for all remaining persons who were in the country without authorization. For these person-years, we separately calculated removals for each year among the group of top receiving countries (i.e., Brazil, China, Colombia, Dominican Republic, Ecuador, El Salvador, Guatemala, Honduras, India, Jamaica, Mexico, Nicaragua) and among the all other countries group. For the all other countries group, we used the midpoint TB case rate (57 cases/100,000 person years). In sensitivity analyses, we varied the time at risk for TB from a maximum of 9 months to a minimum represented by a weighted mean LOS in the United States before removal (i.e., 140 days) (*18*). This calculation was done for all 4 subgroups of persons in the United States without authorization.

Third, we estimated TB cases for persons in the United States without authorization who subsequently voluntarily left. Because most of this subpopulation consists of persons from Mexico, which is also the group for which most data were available (*18*), we began with the DHS-reported numbers of total nonexpedited removals of Mexican nationals (*16*). We applied equation 1 to the following unique person-years (Equation 2):

> person-years of unauthorized Mexican nationals leaving US on own = ([total unauthorized Mexican nationals leaving] – [Mexican, nonexpedited removals]) × (183/365)

where total unauthorized Mexican nationals leaving = Mexican nonexpedited removals/35%. We used the highest proportion of involuntary to voluntary departures (35:65) (*18*) because of an increase in removals in the past decade (*25*). A report from Mexico on migratory flows provided corroborative evidence for our estimate of total departures of Mexican nationals (*19*).

To obtain the final estimate of TB cases among subgroups without authorization, we assumed that persons from Mexico made up 90% of those who voluntarily left the United States because they are the documented majority of migrants (*18*), Mexico is a bordering nation, and local antiimmigration laws tend to target unauthorized visitors from Mexico (*19*,*26*). We then adapted Equation 2 and applied the 90% assumption.

Next, we estimated the number in the study population who were referred for transnational care–continuity services by extrapolating from and adding previously reported provider data (*20,21*,*27*,*28*). No evidence was found that any of these persons met 1 of 4 relevant exclusion criteria.

Last, we calculated the proportion of the study population who received transnational services to mitigate drug resistance and other negative consequences of interrupted TB treatment. To do this, we divided the number of persons receiving services by the estimated number of incident TB cases. We also estimated the proportion of referred cases included in the detained-then-removed subgroup.

Table 3. Estimated number of incident tuberculosis cases for all subgroups at risk for treatment interruption due to voluntary or involuntary departure from the United States, 2008–2012*

Study subgroup	No. cases, by year					Yearly average (%)
	2008	2009	2010	2011	2012	
Resided in United States with authorization						
Tourist and business traveler	1,099	987	1,219	1,403	1,454	1,232 (44)
Student and exchange visitor†	696	657	785	791	745	735 (26)
Temporary worker†	474	394	473	503	475	464 (16)
Diplomat and other representative†	47	46	50	50	49	49 (2)
All other NIV classes	24	22	21	21	20	21 (1)
Unknown NIV class	10	9	15	10	8	10
Canada residents, no I-94 card	21	19	15	15	15	17 (1)
Mexico residents, no I-94 card	64	60	54	52	55	57 (2)
Resided in United States without authorization						
Detained then removed	173	175	166	196	218	186 (7)
Nondetained, removed	6	6	6	6	6	6
Mexico resident, voluntary departures	35	42	39	40	39	39 (1)
All other voluntary departures	10	12	11	11	11	11
Total	2,659	2,430	2,853	3,099	3,094	2,827

*I-94 card, the entry/exit form that all nonimmigrant visitors (except certain ones from Mexico and Canada) must fill out; NIV, nonimmigrant visa.
†Corresponding spouses and children were included in each of these categories; see online Technical Appendix Table 1 (http://wwwnc.cdc.gov/EID/article/22/3/14-1971-Techapp1.pdf) for full list of visas included in each subgroup.

Results

The cumulative number of incident TB cases among the study population was 14,134, and the annual average incidence was 2,827 cases (95% CI 2,440–3,213; Table 3) among an estimated annual population of 6.9 million. The sensitivity analysis using available median LOS resulted in 1,544 annual cases (95% CI 1,249–1,840; online Technical Appendix Tables 3–6). Further sensitivity analysis using visa count data produced an annual range of 1,352–4,637 cases.

For the authorized subpopulations, we calculated a total of 30,530,577 person-years and 12,928 cases during 2008–2012. Tourist and business travelers represented 47% (14,431,062) of these person-years; students and exchange visitors, 21% (6,293,260); temporary workers, 16% (4,948,262); diplomats, 1.2% (381,343); and persons from Canada and Mexico without an I-94 card 14% (4,266,235). Tourist and business travelers from medium-incidence countries accounted for most cases (3,342; 26%). However, students and exchange visitors from countries with a high TB incidence had the highest number of cases per 100,000 admissions (110, 95% CI 93–126), followed by temporary workers from high-incidence countries (74, 95% CI 63–86), diplomats from high-incidence countries (50, 95% CI 43–58), and students and exchange visitors from medium-incidence countries (31, 95% CI 26–35).

Among the subpopulations without authorization, we calculated a total of 1,206 incident TB cases, representing an annual average of 241 (Table 3). Persons removed by US officials and those who left voluntarily represented 958 and 259 cases, respectively. These subpopulations represented 8.5% (241/2,827) of annual cases (Figure). Sensitivity analyses showed an annual range of 180–324 cases (6.4%–11.5% of total).

We estimated CureTB and Health Network managed 510 and 599 TB cases, respectively, for a collective annual average of 222 cases during the study period (Table 4). Thus, 7.9% (222/2,827) of persons leaving the United States before treatment completion received transnational care–continuity services. We further estimated that 67% (124/186) of persons who received transnational services belonged to the subgroup that was detained before removal.

Discussion

We estimated that, during 2008–2012, a substantial number of TB-infected persons were at risk for drug resistance

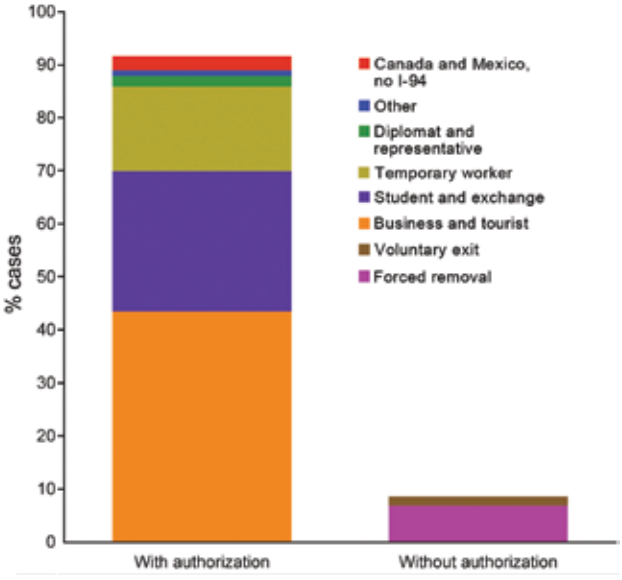

Figure. Estimated percentage of incident tuberculosis cases among authorized and unauthorized visitors to the United States who were at risk for treatment interruption due to voluntary or involuntary departure from the country, 2008–2012. Key indicates subgroups of visitors by visa status.

Table 4. Estimated number of persons with tuberculosis referred for transnational care–continuity services to prevent treatment interruption due to voluntary or involuntary departure from the United States, 2008–2012

Variable	Total no. estimated cases among study population*	No. cases managed by CureTB†	No. cases managed by Health Network
Year			
2008	2,659	90	106
2009	2,430	111	95
2010	2,853	108	109
2011	3,099	111	134
2012	3,094	90	155
Annual average (% referred)	2,827 (8)	102	120
Total incident cases from detained and removed subgroup (% referred)	928 (67)	180	442
Annual average for subgroup	186	36	88

*Study population was defined as nonimmigrants, nonrefugees who were born outside of the United States had active tuberculosis while in the United States, and then left the United States before treatment completion was possible.
†Numbers for 2008 and 2009 were extrapolated by using previously reported data from 2010–2012 (*27*,*28*).

because of treatment interruption due to departure from the United States. During that time, 14,134 cases of incident TB occurred among visitors to the United States, representing a yearly average of 2,827 cases (2,586 and 241, respectively, among persons with and without authorization). Approximately 10% of these persons received transnational care–continuity services (from Health Network or CureTB). Thus, ≈90% of infected persons departed the country without such services, a finding that highlights a neglected public health area and the feasibility of scaling up intervention.

Pathogens that cause TB are transmitted via breathing, and the disease has a high death rate if untreated (*29*), thereby incurring severe negative externalities for the public's health and economic wellbeing (*30*). A single untreated case can lead to hundreds of new infections (*31*,*32*). If treatment is interrupted, the situation is worsened because of the risk for poorer outcomes (*29*). Our findings contribute to TB control efforts by elucidating characteristics of an understudied population at risk for acquiring and spreading drug-resistant TB (*6*) and by highlighting opportunities to prevent this serious threat to the public's health and the corresponding fiscal consequences. Moreover, our findings contribute to previously identified needs for improving screening practices for migrants (*33*) and for understanding how best to target TB prevention and control efforts (*7*). Our findings build on those of Liu et al. (*11*), particularly the finding that temporary residents contribute appreciably to illness in the United States caused by TB; the reported number of TB cases in 2012 was 9,945, of which 6,274 were among foreign-born persons (*8*). Our estimate of 2,827 yearly cases among visitors to the United States does not entirely overlap with the number from that report because we counted persons with <90 days of treatment (*34*) and we captured undiagnosed cases.

Little is known about TB cases among subpopulations living in the United States without authorization. The attribution of only 8.5% of cases to this subgroup contradicts widespread opinion that TB in the United States is primarily due to illegal immigration. Moreover, 8.5% is consistent with the finding in a multinational study (*35*). A county-level study found 25% of TB cases in the unauthorized population (*36*), but it is difficult to generalize from a single county's data. A related and somewhat encouraging finding was that 67% (124/186) of persons receiving transnational services were among the most vulnerable subgroup (those detained before removal). Ideally, no one would be forcibly relocated until after treatment completion (*35*), but assuring all who are removed receive transnational services is another way to avoid treatment interruption and development of drug-resistant TB. Our findings suggest that scaling up transnational care-continuity services is feasible and desirable, given the likely return on investment (*9*,*30*). Furthermore, removal of unauthorized visitors from the United States has been increasing over the past decade (*25*), suggesting incident TB cases among this subgroup will remain at estimated levels or decrease in future years. The Obama administration's executive action in November 2014 to provide immigration relief to specific persons without authorization to enter the United States may slightly reduce this estimate because it temporarily halts deportation.

The authorized subgroups differ from each other, just as the unauthorized subgroups differ in risk and migratory profiles. Therefore, here we consider program and policy implications separately by subgroup. First, we concur with the suggestion by Liu et al. (*11*) to prescreen only subgroups that have the highest case rates per 100,000 admissions and are in the United States long enough to make postarrival medical follow-up feasible and worthwhile. This policy would affect students, exchange visitors, and temporary workers from countries with high TB incidences and expand the successful prescreening–plus–follow-up policy for immigrants and refugees (*37*). If persons in these subgroups do not stay in the United States long enough to complete treatment, they should be referred for transnational care–continuity services. Any compulsory screening program must be accompanied by regard for civil liberties and medical ethical principles (*6*). In addition, some persons with TB who leave the country complete treatment

without the aid of transnational services; however, case management increases the likelihood of completion, and US-based providers would have more data should a patient return, a probable occurrence for many (*9*).

Second, diplomats and other representatives from high-incidence countries also had a relatively high TB case rate, but the number of admissions was not sufficient to make prescreening a high-yield activity. Political calculus also weighs heavily for this group of visitors, and diplomats tend to have preexisting mechanisms for health emergencies. Therefore, further intervention is impractical or unnecessary.

Third, when a large volume of admissions to the United States and relatively low TB case rates are combined, referral to transnational care–continuity services after TB diagnosis is more rational than prescreening. Subgroups falling into this category are tourist and business travelers; persons from Canada and Mexico entering without an I-94 card; and any authorized visitor from a country with medium or low TB incidence, except for diplomats.

Last, subgroups without authorization to enter the United States have little interaction with formal systems that would help to identify and treat their TB infections in a timely manner. This situation is especially true in the wake of the Affordable Care Act of 2010, which prohibited such persons from purchasing private health insurance (*38*). The best option in this circumstance is to refer unauthorized visitors for transnational services immediately after they are diagnosed with TB. Persons who are detained by immigration officials are typically screened for TB (*17*); this practice should continue, as it increases the chances of referral for transnational care–continuity services.

Our study had limitations. First, there were time lags in DHS data (*16*), thus, where available, we used postadjustment numbers for removal totals. Also, in 2010, DHS started counting all visa admissions separately rather than counting multiple entries for 1 person as 1 admission. An increase resulted, particularly among admissions from Canada and Mexico (*12*), suggesting that estimates from 2009 and earlier were biased toward undercounting. This change also represents the second biggest factor in the difference between our estimate and those from previous studies (*11*). Nevertheless, given the affected subgroups, the policy implications do not change.

Second, there was uncertainty around the time at risk for TB. However, our sensitivity analyses varied this input in both directions for the unauthorized subpopulations, and the findings remained robust. For the authorized subpopulations with an I-94 card, substituting median LOS for mean LOS dramatically reduced time at risk. The overall estimate was nearly halved, but the order of magnitude was the same, as do intervention recommendations, with the exception that prescreening for temporary workers from high-incidence countries might no longer be a high-yield intervention. Furthermore, the available LOS data are highly suggestive of smooth skews rather than random outliers with problematic influence (*13*); thus, the best way to statistically account for those days at risk is by using mean LOS.

Third, a conservative bias was introduced by global TB underreporting (*39*), which affected the estimated number of cases and corresponding CIs. A countervailing bias was introduced by not adjusting for visitor socioeconomic status or age upon US entry because of insufficient data. Moreover, data from our sources were consistent with those in similar studies (*11*). Additional bias toward overcounting occurred due to lack of data on visitors who adjusted status to permanent residency, for whom TB screening is required. Because most of those who adjust status come from the group for whom we recommended preentry screening and postentry follow up, our recommendation remains unchanged and would aid visitors who adjust their status, because they will have completed their TB screening early.

The 2,827 annual cases would include some drug-resistant TB cases, depending on the strain contracted. Drug-resistant and MDR TB lend urgency to achieving treatment completion; however, without additional mechanisms besides the international referral form, US clinicians and health departments rarely know outcomes for patients exiting the country. In contrast, CureTB and Health Network have documented completion rates, approaching the WHO target of 85% (*20*,*21*,*24*). Therefore, our recommendation to refer these patients for transnational services is justified in order to reduce the number and spread of these deadly and costly conditions.

In summary, TB in mobile persons in the United States is not well understood and represents a particular challenge to global TB control (*6*), as well as a key opportunity to reduce development and spread of drug-resistance. Our findings provide new epidemiologic evidence that will inform an effective TB control strategy (*6*). Because many mobile persons with TB may return to the United States (*9*) and the global prevalence of MDR TB is increasing (*4*,*24*), scaling up transnational care–continuity services would benefit the US directly and bolster international TB control efforts (*40*). Use of such services of would reduce suffering, save lives, build goodwill with receiving countries, improve global TB surveillance data, and bolster economic productivity. Access to healthcare varies among subgroups of mobile, TB-infected persons; however, programs like CureTB and Health Network are able to serve all subgroups. The most complete policy response may be to make these services available to public and private clinicians alike.

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Dr. Tschampl is a senior research associate at the Heller School's Schneider Institutes for Health Policy at Brandeis University in Massachusetts. Her research interests include health disparities, public health delivery and finance systems, cost-effectiveness evaluation, immigrant health, healthcare for the homeless, and health policy.

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Address for correspondence: Cynthia A. Tschampl, Heller School for Social Policy and Management, Brandeis University, MS035, Waltham, MA 02454, USA; email: Tschampl@brandeis.edu

Encephalitis, Ontario, Canada, 2002–2013

Alyssa S. Parpia,[1] Ye Li, Cynthia Chen, Badal Dhar, Natasha S. Crowcroft

Encephalitis, a brain inflammation leading to severe illness and often death, is caused by >100 pathogens. To assess the incidence and trends of encephalitis in Ontario, Canada, we obtained data on 6,463 Ontario encephalitis hospitalizations from the hospital Discharge Abstract Database for April 2002–December 2013 and analyzed these data using multiple negative binomial regression. The estimated crude incidence of all-cause encephalitis in Ontario was ≈4.3 cases/100,000 persons/year. Incidence rates for infants <1 year of age and adults ≥65 years were 3.9 and 3.0 times that of adults 20–44 years of age, respectively. Incidence peaks during August–September in 2002 and 2012 resulted primarily from encephalitis of unknown cause and viral encephalitis. Encephalitis occurred more frequently in older age groups and less frequently in women in Ontario when compared to England, but despite differences in population, vector-borne diseases, climate, and geography, the epidemiology was overall remarkably similar in the two regions.

Encephalitis is a brain inflammation that over the long term can reduce neurologic health and cause disability and even death (*1*,*2*). More than 100 infectious, post-infectious, and immune-mediated conditions can cause encephalitis, which occurs most often in infants and in adults ≥65 years of age (*3*–*5*). Studies worldwide indicate that cause is unknown for 37%–85% of encephalitis cases and that recorded causes differ by region and implementation of systematized diagnostic algorithms (*3*,*5*–*9*).

Vaccination has reduced the incidence of encephalitis caused by measles, mumps, rubella, and varicella. However, efforts to prevent and reduce infectious and immune-mediated causes of encephalitis must be maintained because the number of possible causes is increasing (*7*). Climate change and increased mobility of humans have contributed to the spread of infectious diseases to newly supportive environments to which such infections are not endemic, ultimately changing the regions in which vectors can transmit various infectious forms of encephalitis (*10*,*11*). Additionally, the increased survival and life expectancy of persons with immunocompromising conditions contribute to the increased incidence of encephalitis. Several studies have identified herpes simplex virus as responsible for the greatest proportion of encephalitis-associated hospitalizations (*3*,*5*,*6*,*8*,*12*), followed by varicella zoster virus (*6*–*8*), or in some studies, *Mycobacterium tuberculosis* (*12*) or *Toxoplasma* meningoencephalitis (*6*).

During 1994–2008, the estimated annual incidence of encephalitis in Ontario, Canada, was ≈4.6 (95% CI 4.5–4.7) cases per 100,000 persons, according to codes recorded based on the International Classification of Diseases (ICD), Ninth and Tenth Revisions (*4*). Encephalitis is a reportable disease according to Ontario Public Health Standards, as are many diseases that can cause encephalitis, such as West Nile virus illness, rabies, and measles (*13*,*14*). However, little is known about the various causes of encephalitis in particular and their category-specific incidence rates and proportions in Ontario. Given the severity of encephalitis, hospitalization data have been found to be reliable for identifying encephalitis incidence, unlike notification data, which yield underestimates due to underchildren-reporting, despite the status of encephalitis as a reportable disease (*4*,*15*). In England, studies have helped identify gaps in understanding and have shown that length of hospital stay varies among categories of encephalitis cause (*7*). England is similar to Ontario in terms of socioeconomic makeup, yet has a starkly different geography. Both have publicly funded healthcare and comparable data available for analysis. Thus, comparison of the incidence of encephalitis in these 2 regions might be telling of region-specific causes. The extent to which hospitalization duration and other measures of illness burden vary among encephalitis causes in Ontario is unknown.

Our objective was to estimate the annual incidence of encephalitis in Ontario by cause category for 2002–2013, compare incidence rates between Ontario and England, and identify whether an association exists between encephalitis cause category and length of hospitalization. Public Health Ontario (Ontario Agency for Health Protection and Promotion) Research Review Board provided ethics approval for this study.

Methods

Data Source

We extracted hospital discharge diagnoses data from the Canadian Institute for Health Information (http://www.cihi.ca),

Author affiliations: Public Health Ontario, Toronto, Ontario, Canada (A.S. Parpia, Y. Li, C. Chen, B. Dhar, N.S. Crowcroft); University of Toronto, Toronto (A.S. Parpia, Y. Li, N.S. Crowcroft)

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[1]Current affiliation: Yale School of Public Health, New Haven, Connecticut, USA.

Ontario Discharge Abstract Database, for April 2002–December 2013 through Ontario Ministry of Health and Long-Term Care's IntelliHEALTH Ontario. The Ontario Discharge Abstract Database used ICD-10 during this period. We obtained ICD-10 codes for encephalitis diagnoses by reviewing similar studies (*4*,*15*,*16*). An encephalitis-associated hospitalization was defined as a hospitalization for which an encephalitis diagnostic code or specified combination of encephalitis codes were recorded in any of the diagnostic fields, including the field for the most responsible diagnosis (most responsible for the length of hospitalization), as done elsewhere (*15*).

We categorized ICD-10 codes into 8 categories of encephalitis cause: viral, bacterial, amebic, fungal, immune-mediated, parasitic, other, and unknown. We used a ninth category for cases that could not be categorized because of contradictory encephalitis-related ICD codes attributed to a single case. Multiple encephalitis hospitalizations for the same patient that occurred within 6 months (e.g., ≤6 months between the first discharge and second admission with an encephalitis ICD code in any diagnostic field) were considered 1 admission (*15*,*17*). In this situation, lengths of stay for the 2 hospitalizations were totaled into a single length of stay for the encephalitis patient. If the time between the first discharge and second admission was >6 months, the hospitalizations were considered unique visits and unique cases of encephalitis. Thus, we counted incident encephalitis-associated hospitalizations for a given patient with multiple admissions when the hospitalizations occurred >6 months apart. ICD-10 codes for immunosuppression were identified through a review of other studies and were related to having HIV, organ transplantation, immunodeficiency, or cancer (*7*,*18*).

Data Extraction

We selected ICD-10 codes using the first 3 characters (e.g., B00) in any diagnostic field corresponding to encephalitis conditions. Filters were then implemented to extract specific 4-character (e.g., B004) encephalitis ICD-10 codes, both single codes and code combinations, that were recorded upon diagnosis of an encephalitis case (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/22/3/15-1545-Techapp1.pdf).

Analysis

Data were analyzed by using SAS version 9.3 (SAS Institute Inc., Cary, NC, USA). Incident cases of encephalitis were stratified by year of patient hospital admission; sex; age at admission (<1, 1–4, 5–19, 20–44, 45–64, ≥65 years of age); and geography (patient Local Health Integration Network [LHIN]). Hospitalization rates for incident all-cause encephalitis were calculated overall, by year and patient sex, age group, and LHIN by using yearly Ontario population estimates from Statistics Canada (http://www.statscan.ca) CANSIM tables. We calculated 95% CIs for incidence densities through bootstrap resampling with 4,000 repetitions. We also calculated incidence rates and 95% CIs by category of encephalitis cause, stratified by year, sex, and age. These values were compared with incidence rates from studies conducted in England (*15*). We calculated proportions and frequency counts of discharges by specific encephalitis cause for their respective cause categories and for encephalitis in Ontario as a whole.

After applying incidence estimates for England from April 1, 2005, through March 31, 2009 (2005–2008 fiscal years), to Ontario population data, we determined the expected case counts for each sex and age group if the age/sex incidence of encephalitis in Ontario was the same as in England. We compared these expected case counts on the basis of incidence rate data in England with the actual case counts of encephalitis in Ontario during these fiscal years.

Yearly and seasonal trends in hospital discharges from incident all-cause encephalitis were investigated by regression analyses adjusted for age and sex. The outcome variable was the number of incident encephalitis-associated hospitalizations in Ontario. We applied negative binomial regression with an overdispersion parameter that captured the heterogeneity among observations that could not be accounted with Poisson model. The logarithm of the population at risk, the Ontario population, was included as an offset in this model. Single predictor and multivariable negative binomial regression models were performed; the latter was adjusted for age, sex, and year.

We used multiple linear regression to assess the association between length of hospital stay for a patient with an encephalitis-associated admission (continuous variable) and encephalitis cause (a 7-category variable for type of encephalitis cause: viral, bacterial, immune-mediated, amebic/parasitic/fungal, other, unknown, and unable to classify). The length of hospitalization outcome variable was natural log transformed to ensure it was normally distributed in this linear regression model. To enable the log transformation, we recorded all hospitalizations of <1 day (0 days) as 0.5 days because of a lack of precise information about admission and discharge times. Using descriptive analysis, we explored the mean and median length of hospitalization for the different groups of encephalitis cause. Unadjusted associations and associations adjusted for sex and age were calculated. We then adjusted for the baseline model that included age and sex by clinically relevant predictors of the outcome and confounders of the association.

Results

Incidence

During April 2002–December 2013, incidence of all-cause encephalitis was ≈4.3 (95% CI 4.2–4.4) cases/100,000 persons

per year in Ontario. Encephalitis occurred more frequently among male than female Ontario residents in all age groups except children 1–4 years of age (Table 1, http://wwwnc.cdc.gov/EID/article/22/1/15-1545-T1.htm). The youngest and oldest age groups had the highest incidence of encephalitis; for infants <1 year of age, incidence was 10.7 (95% CI 9.1–12.1) cases/100,000 population, and for persons ≥65 years of age, incidence was 8.1 (95% CI 7.9–8.6) cases/100,000 population. These trends were consistent during the entire 12-year study period; encephalitis peaked in infants in 2004 (18.7 [95% CI 12.0–26.2] cases/100,000 persons) and in elderly persons in 2002 (14.1 [95% CI 12.1–16.4] cases/100,000).

The incidence of all-cause encephalitis peaked for both male and female residents in August and September 2002 (96 and 140 cases/100,000 persons, respectively) and 2012 (101 and 85 cases/100,000 persons, respectively). Otherwise, we observed no linear time trend during the 12-year study period (p = 0.9). In general, during July–October, incidence rates were higher by age group for infants and for persons ≥65 years of age; for other age groups, encephalitis incidence remained relatively constant throughout the year.

The incidence of immune-mediated encephalitis was highest in children 1–4 years of age (0.7 cases/100,000 persons) (Figure). The incidence of viral encephalitis and encephalitis of unknown cause was highest in infants <1 year of age, followed by adults ≥65 years of age.

Immunocompetent and Immunocompromised Persons with Encephalitis

The 938 immunocompromised patients with encephalitis received the following ICD-10 codes at hospital discharge: 65.4%, a code indicating cancer; 27.9%, a code indicating HIV infection; 12.4%, a code indicating transplantation; and 3.4%, a code indicating immunodeficiency (Table 2). Fifty-one percent of encephalitis patients with HIV, 40.6% with immunodeficiency, 44.8% who had undergone transplantation, and 28.1% with cancer had viral encephalitis. Sixty (22.9%) of encephalitis cases among persons with HIV were amebic/parasitic/fungal encephalitis, which was more than twice the proportion of these causes among other immunocompromised persons. Among encephalitis patients with cancer, 32.1% had immune-mediated encephalitis; for 28.2%, encephalitis cause was unknown. Among immunocompromised persons with HIV, immunodeficiency, or a transplantation, the most common encephalitis cause, other than viral, was unknown cause.

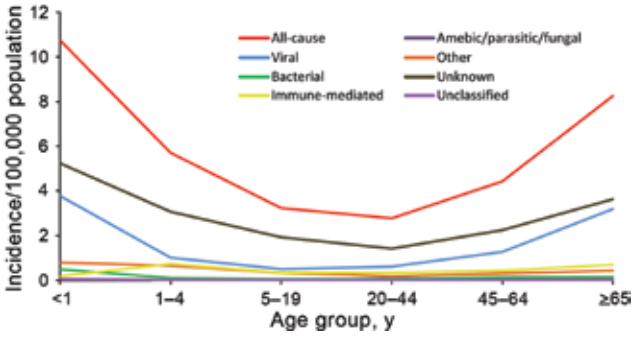

Figure. Incidence rate (cases per 100,000 persons) for all-cause encephalitis and categories of encephalitis causes, by age group, Ontario, Canada, 2002–2013.

Encephalitis cause was unknown for 55.2% of immunocompetent patients and for 26.6% of immunocompromised patients. A total of 35.6% of immunocompromised persons and 26.3% of immunocompetent persons had viral encephalitis, a difference of 9.3%. For immune-mediated encephalitis, the difference was 13.6% (21.8% for immunocompromised vs. 8.2% for immunocompetent patients); for amebic/parasitic/fungal causes, the difference was 7.1% (7.5% for immunocompromised vs. 0.4% for immunocompetent patients).

The mean log-transformed length of hospitalization for encephalitis, as determined by discharge data, was significantly longer for immunocompromised than immunocompetent patients (p<0.0001). The 32 persons in whom immunodeficiency was diagnosed had the widest range of hospitalization stay, and the 116 persons who had an organ transplant had the longest median hospitalization stay (22.5 days), of all subcategories of persons with immunocompromising conditions. For both immunocompromised and immunocompetent persons, bacterial encephalitis resulted in the longest hospital stays (34.5 and 16.5 days, respectively). Among encephalitis cases we were able to classify, encephalitis of unknown cause resulted in the shortest hospital stays for both immunocompromised (18 days) and immunocompetent (9 days) patients, even though stay was twice as long for immunocompromised patients.

Overall, during 2002–2013, age- and year-adjusted encephalitis incidence was 15% higher for male patients (4.6 [95% CI 4.4–4.8] cases/100,000 persons) than for female patients (4.0 [95% CI 3.8–4.1] cases/100,000 persons) (Table 3). Sex- and year-adjusted encephalitis incidence for infants was 3.9 (95% CI 3.3–4.5) times greater than for adults 20–44 years of age (considered the referent category because this group had the lowest incidence), and sex- and year-adjusted encephalitis incidence for adults ≥65 years of age was 3.0 (95% CI 2.8–3.2) times that of adults 20–44 years of age (p<0.0001). Incidence rate ratios of Ontario and England by age and sex did not appear to differ substantially, except for the oldest age group. In multivariable models, compared with adults in the 20–44-year age category, persons ≥65 years of age in Ontario had an incidence rate ratio of 3.0 (95% CI 2.8–3.2) versus a significantly lower incidence rate ratio of 1.9 (95% CI 1.8–2.1) for this age group in England.

Table 2. Cause of encephalitis in immunocompromised patients, Ontario, Canada, 2002–2013

Encephalitis cause	Total encephalitis cases, no. (%), N = 6,463	Immunocompromising condition, no. (%), n = 938			
		HIV, n = 262	Other immunodeficiency, n = 32	Transplant, n = 116	Cancer, n = 613
Unknown	3,299 (51.0)	45 (17.2)	9 (28.1)	42 (36.2)	176 (28.7)
Viral	1,788 (27.7)	134 (51.2)	13 (40.6)	52 (44.8)	172 (28.1)
Immune mediated	657 (10.2)	3 (1.2)	5 (15.6)	7 (6.0)	197 (32.1)
Other	466 (7.2)	11 (4.2)	2 (6.3)	5 (4.3)	42 (6.9)
Bacterial	152 (2.4)	7 (2.7)	0	2 (1.7)	13 (2.1)
Amebic/parasitic/fungal	92 (1.4)	60 (22.9)	3 (9.4)	8 (6.9)	12 (2.0)
Unable to classify	9 (0.1)	2 (0.8)	0	0	1 (0.2)
Total	6,463	262 (27.9)	32 (3.4)	116 (12.4)	613 (65.4)

Comparison of Encephalitis Cases in Ontario and England

In Ontario, the annual total number of encephalitis cases fell within the 95% CIs for the England-derived Ontario expected case counts in the 2005, 2007, and 2008 fiscal years. During the 2006 fiscal year, the number of cases in Ontario was lower than the estimated number expected on the basis of incidence rates in England. Overall, during April 2005–March 2009, the actual average per year case count of encephalitis in Ontario was 494 cases, which is not significantly different from the number of cases that would occur if England incidence rates were applied to the Ontario population (550 [95% CI 476–631] cases). During this period, encephalitis occurred significantly less often in female patients in Ontario (220 cases) than in England (268 [95% CI 233–307] cases). For adults ≥65 years of age, encephalitis occurred significantly more often in Ontario (126 cases) than in England (102 [95% CI 89–113] cases). In England, the proportion of encephalitis cases in immunocompromised patients as identified by a population-based prospective study was 15.3%, and in Ontario, 14.5% (*7*).

Encephalitis Cause and Length of Hospitalization

The multiple linear regression model exploring the association between category of encephalitis cause and length of hospitalization was adjusted by sex, age, immune status, and co-morbidity level, all of which resulted in a >20% change in the parameter coefficients from the baseline model (Table 4). Season, year, and patient LHIN did not significantly change (>20%) in the parameter estimates for the baseline model (which included age and sex in addition to main exposure and outcome) and were thus excluded from the model. After adjusting for all significant covariates of interest, we found that patients with amebic/parasitic/fungal encephalitis had a 27.5% (95% CI 1.4%–60.4%) longer hospital stay than did patients with viral encephalitis (p = 0.038). In addition, after adjusting for all covariates of interest, we found length of hospitalization to be 22.1% (95% CI 17.0%–26.8%) shorter for patients with encephalitis of unknown cause than for patients with viral encephalitis.

Length of hospitalization did not differ significantly by patient sex (p = 0.3634) but was 25.3% longer for immunocompromised than for immunocompetent patients (p<0.0001). After adjustment, compared with results for adults 45–64 years of age, average hospitalization was 40.8% (95% CI 33.2%–47.5%) shorter for children 1–4 years of age, 16.9% (95% CI 12.2%–20.4%) shorter for children and youth 5–19 years of age, 12.6% (95% CI 5.9%–18.8%) shorter for adults 20–44 years of age, and 14.2% (95% CI 6.4%–22.7%) longer for adults ≥65 years of age. All levels of co-morbidity were associated with significantly longer hospitalization (p<0.0001) than was lack of any co-morbidities.

Table 3. Univariable and multivariable negative binomial regression model assessing variation in incident encephalitis hospitalizations, Ontario, Canada, and England*

Variable	No. (%) cases, N = 6,463	Incidence rate	Multivariable analysis			
			Ontario, 2002–2013		England, 2005–2009†	
			Adjusted IRR (95% CI)	p value	Adjusted IRR (95% CI)	p value
Sex						
M	3,417 (52.8)	4.6	Referent	<0.0001	Referent	0.002
F	3,046 (47.1)	4.0	0.9 (0.8–0.9)		0.9 (0.9–1.0)	
Age group, y						
<1	173 (2.7)	10.7	3.9 (3.3–4.5)	<0.0001	3.7 (3.2–4.2)	<0.001
1–4	377 (5.8)	5.7	2.1 (1.8–2.3)		1.9 (1.7–2.1)	
5–19	915 (14.2)	3.2	1.2 (1.1–1.3)		0.9 (0.8–1.0)	
20–44	1,486 (23.0)	2.8	Referent		Referent	
45–64	1,823 (28.2)	4.4	1.6 (1.5–1.8)		1.4 (1.3–1.5)	
≥65	1,689 (26.1)	8.3	3.0 (2.8–3.2)		1.9 (1.8–2.1)	

*Incidence is number of cases/100,000 persons. IRR, incident rate ratio.
†Reference (*1*).

Table 4. Multiple linear regression modeling association between log-transformed length of hospitalization and category of encephalitis cause, Ontario, Canada, 2002–2013

Variable*	No. (%) cases	Mean length of hospitalization, d (median)	Exponentiated β-coefficient (95% CI)	t	p value
Intercept			8.9 (8.0–9.8)	41.0	<0.0001
Encephalitis cause					
Viral	1,788 (27.7)	27.36 (14)			
Bacterial	152 (2.4)	27.45 (19)	1.2 (1.0–1.4)	1.5	0.128
Amebic/parasitic/fungal	92 (1.4)	43.17 (18)	1.3 (1.0–1.6)	2.1	0.038
Immune mediated	657 (10.2)	26.37 (14)	1.1 (1.0–1.2)	1.8	0.0804
Other	466 (7.2)	24.33 (11)	0.9 (0.8–1.0)	−1.7	0.0817
Unknown	3,299 (51.0)	19.79 (9)	0.8 (0.7–0.8)	−7.8	<0.0001
Unable to classify	9 (0.1)	38.78 (16)	2.1 (1.0–4.1)	2.0	0.0448
Sex					
M	3,417 (52.8)	23.07 (11)			
F	3,046 (47.1)	23.82 (12)	1.0 (1.0–1.1)	0.9	0.3634
Age group, y					
<1	173 (2.7)	22.83 (16)	1.0 (0.8–1.1)	−0.6	0.55
1–4	377 (5.8)	12.28 (5)	0.6 (0.5–0.7)	−8.6	<0.0001
5–19	915 (14.2)	14.54 (6)	0.8 (0.8–0.9)	−8.4	<0.0001
20–44	1,486 (23.0)	23.27 (9)	0.8 (0.8–0.9)	−3.6	0.0003
45–64	1,823 (28.2)	25.02 (13)			
≥65	1,689 (26.1)	29.19 (17)	1.1 (1.1–1.2)	3.7	0.0003
Immune status					
Immunocompetent	5,525 (85.5)	21.29 (10)			
Immunocompromised	938 (14.5)	35.99 (19)	1.3 (1.2–1.4)	5.6	<0.0001
Co-morbidity level†					
None	3718 (57.5)	14.05 (8)			
Low	564 (8.7)	26.08 (14)	1.7 (1.6–1.9)	11.0	<0.0001
Moderate	911 (14.1)	26.13 (15)	1.8 (1.7–2.0)	14.8	<0.0001
High	575 (8.9)	39.00 (25)	2.8 (2.6–3.1)	21.2	<0.0001
Very high	520 (8.1)	55.28 (36)	4.0 (3.6–4.4)	27.0	<0.0001
Missing data	175 (2.7)	14.05 (41)	4.4 (3.7–5.2)	17.9	<0.0001

*Season, year, and patient Local Health Integration Network were not found to cause a significant change (>20%) in the parameter estimates for the exposure variable (category of encephalitis cause) and were thus excluded from the mode.
†Case mix grouping plus comorbidity levels are based on cumulative cost impact of comorbidities on patient stay, where "none" represents no impact and "very high" represents the greatest impact.

Discussion

Our findings regarding the epidemiology of encephalitis in Ontario are similar to those identified in previous studies in Canada, the United States, and England and update the incidence of encephalitis in Ontario and its causal distribution (*3*,*4*,*17*,*19*). In particular, we found results similar to those from England, in relation both to the proportion of encephalitis cases of unknown cause and incidence by patient age and sex, despite the occurrence of zoonotic viral infections in Ontario that are not found in England. These findings imply that most infectious causes are likely to be globally distributed with similar epidemiology in both England and Ontario, not clustering in particular locations or in large outbreaks. Alternatively, a similarly broadly distributed noninfectious cause might be responsible, such as an immune-mediated cause that has been more recently discovered or that is yet unidentified. The shorter hospital stay for persons with encephalitis of unknown cause also might indicate that some cases are not actually encephalitis. This information will provide baselines for future studies, as new diagnostic methods become available, examining changes in the distribution of encephalitis cases by cause and studies evaluating trends in encephalitis incidence over time.

Limitations exist to the use of administrative data to describe epidemiology. We were unable to validate the diagnoses and did not have access to additional laboratory testing information or specimens, which prevented us from identifying and correcting any possible coding errors (*9*). In England, this limitation was addressed through a study of encephalitis, one of the largest population-based studies that exists (*20*). We also were unable to control the diagnostic testing methods used by physicians in Ontario and could only assume that physicians followed provincial standards to derive encephalitis diagnoses. Because of the use of administrative data, misclassification bias also is highly possible, particularly because specific causes of encephalitis often are difficult to diagnose, and whether cases identified are truly incident cases and not sequelae remaining long after infection is unclear. Because we used all diagnostic fields, not solely the primary diagnostic field, to identify encephalitis cases, we could be overestimating the number of cases in persons admitted for sequelae. In some cases, assigning a diagnostic code from information available in the administrative dataset is difficult. We found 329 encephalitis patients who had multiple hospitalizations <6 months apart that did not have the same ultimate encephalitis

diagnosis decision for each hospitalization. Of these cases, 320 had multiple encephalitis diagnoses from different hospitalizations that were in the same cause category as previously defined. The remaining 9 cases were categorized as "unable to classify." Last, our study included cases for which encephalitis was listed as the most responsible diagnosis and cases for which it was listed as a secondary reason for hospital admission. We were unable to test whether this measure confounded the association between encephalitis cause and length of hospitalization.

Several possible reasons explain why there are encephalitis patients with multiple hospitalizations that have different encephalitis cause diagnoses. First, we analyzed administrative data that might have ICD-10 coding errors, resulting in conflicting encephalitis diagnosis decisions for the same patient within a 6-month period. Second, given the difficult task of diagnosing encephalitis, and more specifically identifying the specific type of encephalitis, for patients rehospitalized for encephalitis within a 6-month period it is possible that the initial diagnosis was incorrect, and that the subsequent diagnosis was more accurate.

This study has several strengths. The study was not conducted solely during an outbreak, so it is not biased toward a particular cause. Data were collected and analyzed from the entire province, and geography was tested as an important confounder of the main association by the proxy variable of the LHIN in which the patient resides. Use of discharge data also prevented double counting of patients who were transferred between hospitals, an important and common occurrence for encephalitis patients who might need tertiary care facilities.

The results from this study increases understanding of encephalitis incidence in Ontario. These results can be used as a baseline for future studies to identify changes in encephalitis over time and changes in the distribution of causes of encephalitis to identify emerging diseases that are initially likely to be categorized as being of unknown cause. These findings also suggest that under-ascertainment of encephalitis cases is similar in Ontario and England or does not occur. Better understanding the association between encephalitis cause and length of hospitalization can help target interventions, and these data can be used to help advocate for increased use of personal protective devices against mosquitoes and ticks, which are major vectors of encephalitis in Ontario. An understanding of the epidemiology of encephalitis in Ontario is beneficial in public health surveillance of emerging infectious diseases. Similarities between the epidemiology of encephalitis in Ontario and England, despite differences such as the presence of West Nile virus in Ontario, imply that infectious causes of encephalitis are most likely to be widespread and non-epidemic pathogens, or alternatively, not infectious diseases at all.

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Ms. Parpia was a Master of Public Health student working in the Communicable and Infectious Diseases Department at Public Health Ontario, when this work was conducted, and currently is a research associate at the Yale School of Public Health. Her primary research interest is infectious disease epidemiology, specifically using mathematical modeling to evaluate disease transmission and burden.

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Address for correspondence: Natasha S. Crowcroft, Public Health Ontario, 480 University Ave, Office 922, Toronto, ON M5G 1V2, Canada; email: natasha.crowcroft@oahpp.ca

Effects of Response to 2014–2015 Ebola Outbreak on Deaths from Malaria, HIV/AIDS, and Tuberculosis, West Africa

Alyssa S. Parpia,[1] Martial L. Ndeffo-Mbah,[1] Natasha S. Wenzel, Alison P. Galvani

Response to the 2014–2015 Ebola outbreak in West Africa overwhelmed the healthcare systems of Guinea, Liberia, and Sierra Leone, reducing access to health services for diagnosis and treatment for the major diseases that are endemic to the region: malaria, HIV/AIDS, and tuberculosis. To estimate the repercussions of the Ebola outbreak on the populations at risk for these diseases, we developed computational models for disease transmission and infection progression. We estimated that a 50% reduction in access to healthcare services during the Ebola outbreak exacerbated malaria, HIV/AIDS, and tuberculosis mortality rates by additional death counts of 6,269 (2,564–12,407) in Guinea; 1,535 (522–2,8780) in Liberia; and 2,819 (844–4,844) in Sierra Leone. The 2014–2015 Ebola outbreak was catastrophic in these countries, and its indirect impact of increasing the mortality rates of other diseases was also substantial.

The 2014–2015 Ebola outbreak in West Africa debilitated the healthcare systems of affected countries, hampering diagnosis and treatment for endemic diseases such as malaria, HIV/AIDS, and tuberculosis (TB) (*1,2*). The deaths of several healthcare workers early in 2014, as well as the strain on healthcare facilities caused by increased numbers of patients and decreased staff, resulted in the closure of many clinics and the interruption of routine health delivery services, including HIV testing, childhood vaccinations, and maternity care. Fear of Ebola transmission decreased outpatient attendance to as low as 10% (*3,4*). Surveys conducted by the United Nations in Guinea, Liberia, and Sierra Leone found a substantial decline in the number of persons seeking healthcare because they feared nosocomial Ebola transmission (*1*). In addition, mandatory curfews, border closures, and disruption of transportation routes made obtaining medical services or continuing drug therapy challenging. The reduced demand for and availability of healthcare in the Ebola-affected regions exacerbated the severity of illness and number of deaths caused by malaria, HIV/AIDS, and TB.

Author affiliation: Yale School of Public Health, New Haven, Connecticut, USA

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Malaria, HIV/AIDS, and TB are 3 of the most prevalent infectious diseases in West Africa (5). Halting the spread of these pathogens is a 2015 Millennium Development Goal and the priority of the Global Fund (*5*). Among children <5 years of age, the annual prevalence of malaria is estimated to be 44% in Guinea, 45% in Liberia, and 43% in Sierra Leone (*6–8*). HIV/AIDS prevalence among persons 15–49 years of age is 1.7% in Guinea, 1.1% in Liberia, and 1.6% in Sierra Leone (*9,10*), and TB prevalence across all ages in the 3 countries is 0.24%, 0.44%, and 0.43%, respectively (*11*). Without treatment, annual mortality rates are reported to be as high as 80% for severe malaria, 25% for HIV/AIDS, and 40% for TB (*12–14*). In addition, HIV/AIDS and TB require long-term treatment, while malaria reinfections require repeated treatment. Thus, disruptions of healthcare services that interrupt treatment may substantially increase the number of deaths associated with malaria, HIV/AIDS, and TB.

To estimate the indirect health burden of the 2014–2015 Ebola outbreak in Guinea, Liberia, and Sierra Leone, we developed computational simulation models for malaria, HIV/AIDS, and TB. We used epidemiologic data obtained from the most recent reports of the World Health Organization (WHO) Demographic Health Surveys (DHS) (Table 1; online Technical Appendix Table 1, http://wwwnc.cdc.gov/EID/article/22/3/15-0977-Techapp1.pdf) (*15–23*) and the Global Burden of Disease study (*5,24*) on disease prevalence, incidence, and mortality rates to calibrate our models to the disease burden before the Ebola outbreak. We then projected the calibrated models to estimate the effect that the Ebola outbreak had on disease-related deaths through reduced access to treatment for varying reductions in treatment coverage.

Methods

We developed 3 computational simulation models from a population perspective of disease burden: a disease progression model for malaria and 2 decision tree models for HIV/AIDS and active TB cases. In all models, the decision node branches represent temporal intervals before and during the Ebola outbreak. For each disease, we conservatively considered deaths only for the highest risk groups: malaria

[1]These joint first authors contributed equally to this article.

among children >5 years of age, HIV/AIDS among persons 15–49 years of age, and active TB in all age groups.

We used these models to estimate the number of deaths from malaria, HIV/AIDS, and TB that would have occurred in Guinea, Liberia, and Sierra Leone from March 2014, the start of the Ebola outbreak, through March 2015. We compared this estimate with a scenario of reduced access to health services from June 2014, when Ebola was reported in the major cities of the 3 countries, through March 2015, when the outbreak was tapering (*1,25*). In other settings, this modeling approach has been used to assess the burden of illness for malaria (*18*), TB (*26*), and HIV/AIDS (*27*).

Malaria

To evaluate the death rate of malaria, we developed a model (Figure 1, panel A) that projects the impact of

Table 1. Parameter estimates and distributions for models of malaria in Guinea, Liberia, and Sierra Leone, measuring impact of response to the 2014–2015 Ebola outbreak on deaths*

Malaria-related parameter estimates	Value	Reference
Probability of death without treatment, range		
Uncomplicated malaria	0.005–0.02	(*15*)
Severe malaria	0.45–0.80	(*13*)
Probability of death while undergoing treatment, range		
Uncomplicated malaria	0.00024–0.00112	(*16*)
Severe malaria	0.05–0.2	(*17*)
Probability of progressing from uncomplicated to severe malaria given no treatment	0.03–0.13	(*13*,*18*)
Proportion of case-patients with fever attributable to Malaria	0.01–0.11	(*18*,*19*)
Probability of spontaneous recovery from uncomplicated malaria	0.10–0.20	(*18*)
Probability of treatment for severe malaria	0.60–0.80	(*20*)
Age-specific probabilities†		
Guinea		
Development of fever within 2 weeks (β distribution)		(*21*)
<1 y	376/1,453	
1–2 y	476/1,296	
2–3 y	406/1,192	
3–4 y	337/1,253	
4–5 y	301/1,252	
Receiving treatment for malaria before Ebola outbreak		
<1 y	0.128–0.221	(*21*)
1–2 y	0.194–0.334	
2–3 y	0.159–0.260	
3–4 y	0.198–0.309	
4–5 y	0.163–0.271	
Liberia		
Development of fever within 2 weeks (β distribution)		(*22*)
<1 y	391/1,333	
1–2 y	429/1,272	
2–3 y	309/1,085	
3–4 y	327/1,198	
4–5 y	273/1,159	
Receiving treatment for malaria before Ebola outbreak		
<1 y	0.296–0.381	(*22*)
1–2 y	0.461–0.603	
2–3 y	0.393–0.538	
3–4 y	0.449–0.618	
4–5 y	0.521–0.624	
Sierra Leone		
Development of fever within 2 weeks (β distribution)		(*23*)
<1 y	576/2,406	
1–2 y	706/2,169	
2–3 y	570/2,011	
3–4 y	493/2,237	
4–5 y	406/1,991	
Receiving treatment for malaria before Ebola outbreak		(*23*)
<1 y	0.301–0.395	
1–2 y	0.376–0.502	
2–3 y	0.354–0.484	
3–4 y	0.395–0.543	
4–5 y	0.376–0.501	

*See online Technical Appendix Table 1 (http://wwwnc.cdc.gov/EID/article/22/3/15-0977-Techapp1.pdf) for HIV/AIDS and tuberculosis parameter estimates and distributions.
†For fever, values are no. persons in that age group that had a fever 2 weeks before the survey/total no. persons in age group.

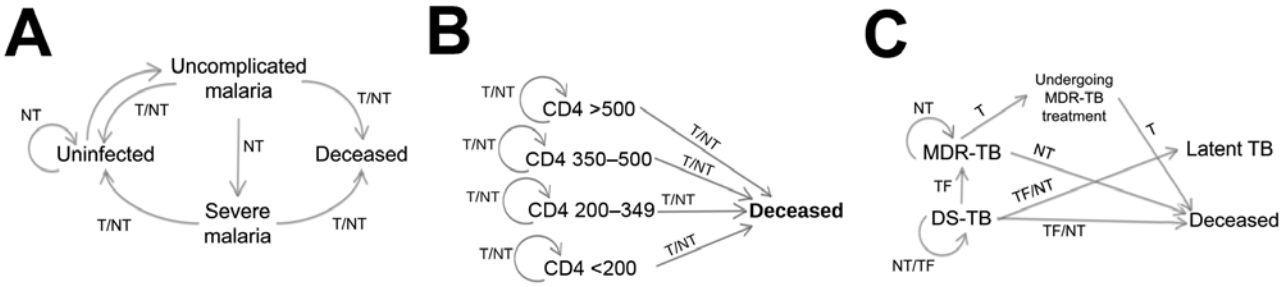

Figure 1. Health state transition diagrams for A) malaria, B) HIV/AIDS, and C) tuberculosis models for disease transmission and progression. T, patient was treated; NT, no treatment was provided; TF, treatment failure or default.

malaria among children <5 years of age with an 8-day time step using a Markov model. The 8-day time step corresponds to the average duration of a malaria episode among children (*28*). Our model tracks 4 health states: uninfected, infected with uncomplicated symptomatic malaria, infected with severe malaria, and deceased. Based on time-dependent probabilities of infection, treatment, or disease progression, persons may transition through these health states over the course of a specified time horizon. We used an age-specific infection probability to account for naturally acquired immunity (*29*). Upon infection, children transition to the health state of uncomplicated malaria, from which they can recover, progress to severe malaria, or die from their infection. Similarly, children with severe malaria may either recover or die. For simplicity, we assumed that severe malaria can only occur as a progressive state from uncomplicated malaria. Probabilities of recovery and death depend on whether malaria is uncomplicated or severe and on whether or not children receive treatment.

In sub-Saharan Africa, most cases of uncomplicated malaria are treated presumptively on the basis of symptoms rather than a positive test or formal medical diagnosis, and treatment medication is purchased from a local store or pharmacy (*30*). Because of this, we conservatively assumed that disruption of health services from the Ebola outbreak only affected treatment for severe malaria.

HIV/AIDS

We developed a decision tree model to estimate the impact of reduced antiretroviral treatment (ART) on HIV/AIDS-related deaths in Guinea, Liberia, and Sierra Leone (Figure 1, panel B). We measured the HIV/AIDS mortality rates in terms of related deaths among persons 15–49 years of age with HIV/AIDS. We took into account both the distribution of CD4 count (>500, 350–500, 200–349, <200 cells/mm^3), and probabilities dependent on CD4 counts of dying without ART, ART failure, and dying while receiving ART (online Technical Appendix Table 1). Following WHO recommendations for ART initiation that were in place during the pertinent time period (2013–2015), we assumed that persons did not initiate ART unless their CD4 count was <500 cells/mm^3 (*31*). We did not incorporate HIV progression because the progression rate is slow compared with the time frame of interest (*32*).

Tuberculosis

We developed a decision tree model to estimate the impact of reduced treatment coverage on the TB burdens in Guinea, Liberia, and Sierra Leone throughout the Ebola outbreak (Figure 1, panel C). We focused on persons with active TB at the start of the Ebola outbreak. We distinguished between infection with drug-susceptible TB and infection with multidrug-resistant TB (MDR TB). We assumed that without treatment, persons infected with DS-TB would either recover naturally, remain infected with drug-susceptible TB, or die at rates based on results of clinical studies. MDR TB case-patients were assumed to either remain infected or die from their infection over the course of the Ebola outbreak.

We assumed that persons with drug-susceptible TB who receive treatment can either be successfully treated, be unsuccessfully treated and remain alive, or die. Those who experience treatment failure or discontinue treatment before taking the full course may progress to MDR TB, and then may seek treatment for MDR TB. We assumed that persons who were infected with MDR TB at the start of the Ebola outbreak would remain infected or die during the 9-month time horizon of our analysis if they were not treated. Given that MDR TB requires 24 months of treatment (*33*), we assumed that persons who received treatment would remain under treatment throughout the 9 months regardless of whether they were still infected or had regressed to latent TB.

Model Calibration

We parameterized the model with a distribution of values for the epidemiologic parameters and population-at-risk

estimates from published literature (Table 1; online Technical Appendix Tables 1, 2). We calibrated our models by comparing annual deaths before the Ebola outbreak with estimates from the 2013 Global Burden of Disease studies for Guinea, Liberia, and Sierra Leone (*5,24*). Each input parameter distribution was sampled 5,000 times. The samples for which the death counts fell within the 95% CI of the Global Burden of Disease studies were selected for our uncertainty analysis (Table 2). Model iterations simulated the disease and treatment events of 5,000 persons for all diseases, resulting in a total of 25 million realizations. The incidence of malaria episodes in our model was 0.3 (95% CI 0.1–0.6) episode/person-year in Guinea, 0.3 (95% CI 0.1–0.6) episode/person-year in Liberia, and 0.3 (95% CI 0.2–0.5) episode/person-year in Sierra Leone, consistent with empirical estimates from West Africa (*29*). On the basis of reports regarding the reduction of routine healthcare services and access to treatment in Guinea, Liberia, and Sierra Leone during the Ebola outbreak (*1*), we considered a 50% reduction for malaria, HIV/AIDS, and TB treatment in our base case analysis.

Multivariate Sensitivity Analysis

To evaluate the effect of each epidemiologic parameter on the relative excess deaths from Ebola, we conducted a multivariate sensitivity analysis using partial rank correlation coefficient (PRCC). PRCC measures the strength of monotonic association between the input parameters and output variable (*28*). The larger the PRCC, the stronger the influence of the model parameter on mortality rates from the disease of interest: malaria, HIV/AIDS, or TB.

Results

Malaria

We estimated that a 50% reduction in treatment coverage during the Ebola outbreak would lead to the deaths of 12,825 (95% CI 4,845–21,945) children <5 years of age from malaria in Guinea, 2,573 (95% CI 735–5,040) in Liberia, and 4,860 (95% CI 2,700–9,450) in Sierra Leone. We estimated that malaria-attributable mortality rates increased by 48.0% (95% CI 4.9%–93.8%) in Guinea, 53.6% (95% CI 4.8%–145.5%) in Liberia, and 50.0% (95% CI 5.0%–118.8%) in Sierra Leone. This increase represents 4,275 (95% CI 570–9,405) additional deaths in Guinea, 788 (95% CI 105–1,890) in Liberia, and 1,755 (95% CI 135–2,970) in Sierra Leone (Table 3).

HIV/AIDS

Given a 50% reduction in ART coverage during the Ebola outbreak, we estimated that 5,151 (95% CI 3,099–7,333) adults 15–49 of age would have died in Guinea, 1,198 (95% CI 851–1,841) in Liberia, and 2,621 (95% CI 1,390–4,183) in Sierra Leone. The increase in HIV/AIDS deaths attributable to this reduction in ART coverage was estimated to be 16.2% (95% CI 1.3%–30.2%) in Guinea, 13.0% (95% CI 2.6%–25.4%) in Liberia, and 9.1% (95% CI 1.6%–19.1%) in Sierra Leone. This increase represents 713 (95% CI 58–1,528) additional deaths in Guinea, 155 (95% CI 23–297) in Liberia, and 223 (95% CI 29–504) in Sierra Leone (Table 3).

Tuberculosis

Using a 50% reduction in treatment coverage for both drug susceptible and multidrug-resistant TB, we estimated that 3,463 (95% CI 2,808–4,349) persons would have died from TB in Guinea, 1,553 (95% CI 1,216–1,875) in Liberia, and 2,164 (95% CI 1,815–2,548) in Sierra Leone. The increase in TB deaths attributable to this reduction in TB treatment coverage was estimated to be 51.1% (95% CI 44.7%–70.5%) in Guinea, 59% (95% CI 47.9%–77.4%) in Liberia, and 61.4% (95% CI 49.2%–87.6%) in Sierra Leone. This increase represents 1,281 (95% CI 877–1,474) additional deaths in Guinea, 592 (95% CI 394–691) in Liberia, and 841 (95% CI 680–1,010) in Sierra Leone (Table 3).

Variation in Treatment Coverage

We conducted a sensitivity analysis by varying the reduction of treatment coverage over a range of 10%–90% of the level before the Ebola outbreak for malaria, HIV/AIDS, and TB in Guinea, Liberia, and Sierra Leone. Because treatment coverage was varied, the additional deaths attributable to the Ebola outbreak in Guinea were estimated

Table 2. Model calibration results compared to empirical data from the 2013 GBD Study of situation before the start of the 2014–2015 Ebola outbreak, for deaths due to malaria, HIV/AIDS, and tuberculosis*

	Average no. deaths (range)					
	Malaria		HIV/AIDS		Tuberculosis	
Country	GBD Study	Model	GBD Study	Model	GBD Study	Model
Guinea	11,591 (4,817–19,932)	15,200 (4,370–20,330)	4,913 (2,774–7,956)	5,832 (2,916–7,920)	3,479 (2,696–4,378)	3,519 (2,698–4,382)
Liberia	2,111 (603–4,420)	2,100 (700–4,200)	1,741 (1,062–2,652)	1,548 (1,062–2,652)	1,394 (1,081–1,843)	1,400 (1,076–1,850)
Sierra Leone	7,011 (2,591–12,613)	5,400 (2,430–12,600)	3,419 (1,830–5,494)	3,132 (1,836–5,472)	1,986 (1,522–2,579)	1,978 (1,514–2,575)

*GBD, Global Burden of Diseases (*5,24*).

Table 3. Deaths from malaria, HIV/AIDS, and tuberculosis correlated with a 50% reduction in treatment coverage attributable to response to the Ebola outbreak, West Africa, 2014–2015

Country and disease	Total no. estimated deaths	No. deaths (95% CI) attributable to outbreak	% Change in attributable deaths (95% CI)	Total deaths attributable to outbreak
Guinea				6,269 (2,564–12,407)
Malaria	12,825 (4,845–21,945)	4,275 (570–9,405)	48.0 (4.9–93.8)	
HIV/AIDS	5,151 (3,099–7,333)	713 (58–1,528)	16.2 (1.3–30.2)	
Tuberculosis	3,463 (2,808–4,349)	1,281 (877–1474)	51.1 (44.7–70.5)	
Liberia				1,535 (522–2,878)
Malaria	2,573 (735–5,040)	788 (105–1,890)	53.6 (4.8–145.5)	
HIV/AIDS	1,198 (851–1,841)	155 (23–297)	13.0 (2.6–25.4)	
Tuberculosis	1,553 (1,216–1,875)	592 (394–691)	59.0 (47.9–77.4)	
Sierra Leone				2,819 (844–4,844)
Malaria	4,860 (2,700–9,450)	1,755 (135–2970)	50.0 (5.0–118.8)	
HIV/AIDS	2,621 (1,390–4,183)	223 (29–504)	9.1 (1.6–19.1)	
Tuberculosis	2,164 (1,815–2,548)	841 (680–1,010)	61.4 (49.2–87.6)	

to vary from 1,425–8,336 for malaria, 146–1,237 for HIV/AIDS, and 277–2,317 for TB. In Liberia, additional deaths attributable to Ebola outbreak varied from 210–1,502 for malaria, 50–314 for HIV/AIDS, and 100–987 for TB. Additional death counts in Sierra Leone varied from 630–3,172 for malaria, 70–630 for HIV/AIDS, and 143–1,723 for TB.

We estimated that, for a reduction of treatment coverage of >15% in Guinea, the indirect deaths from malaria, HIV/AIDS, and TB associated with repercussions of Ebola exceeded the 2,170 cumulative death toll from Ebola reported in Guinea through March 8, 2015 (Figure 2, panel A) (*34*). In Liberia, the reported 4,162 direct deaths from Ebola (*34*) was likely greater than its indirect repercussions on malaria, HIV/AIDS, and TB (Figure 2, panel B). In Sierra Leone, a reduction in treatment coverage by ≥65% resulted in higher numbers of indirect deaths from malaria, HIV/AIDS, and TB than the reported 3,629 direct deaths from Ebola (Figure 2, panel C) (*34*). Overall, in the 3 countries studied in West Africa,

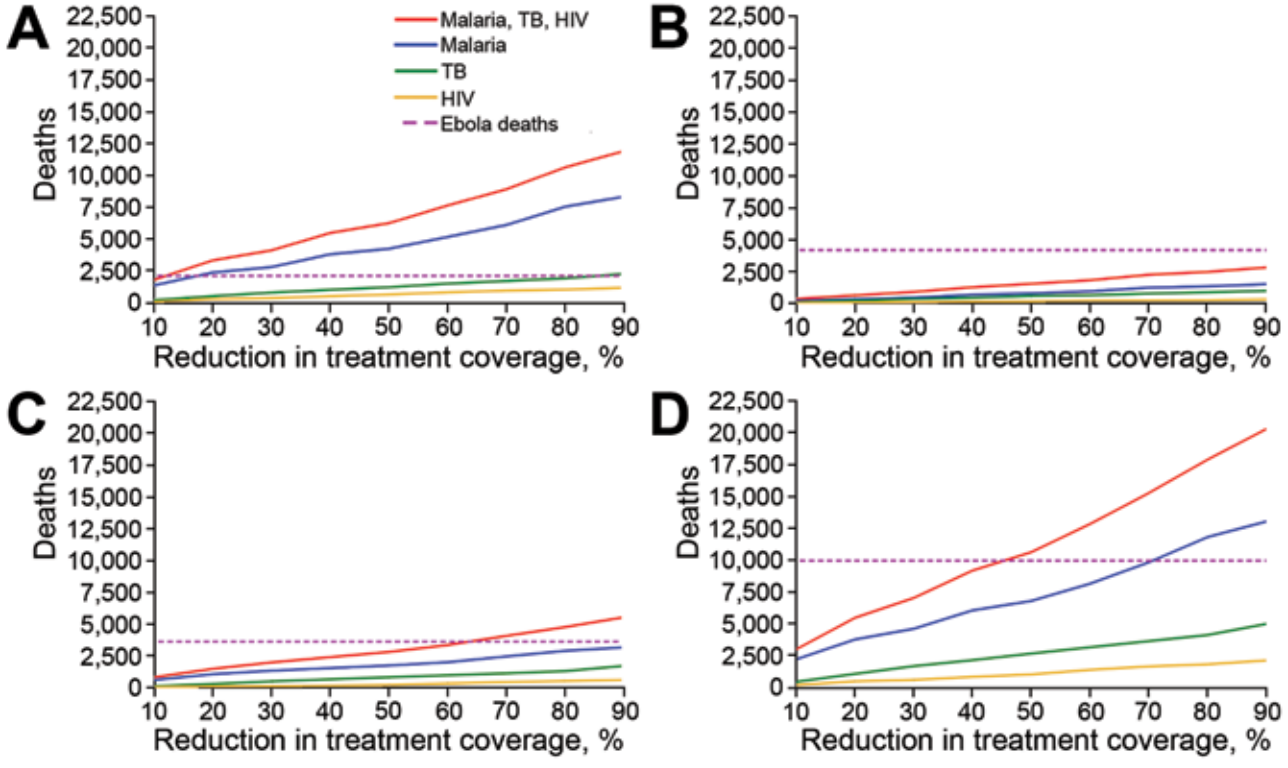

Figure 2. Sensitivity analysis of model outcomes to variation in treatment coverage during response to the 2014–2015 Ebola outbreak in West Africa. A) Guinea, B) Liberia, C) Sierra Leone, and D) all 3 countries. Treatment coverage of malaria, HIV/AIDS, and tuberculosis varied from 10% to 90% reduction compared with the coverage before the Ebola outbreak. Average additional attributable deaths from malaria, HIV/AIDS, and tuberculosis as well as total direct deaths from Ebola are shown. Estimates of additional attributable deaths were associated with considerable uncertainty and are not shown here.

a reduction in treatment coverage by 50% resulted in higher indirect deaths than direct deaths from Ebola (Figure 2, panel D).

Multivariate Sensitivity Analysis

Our PRCC analysis demonstrated that the probabilities of dying from severe malaria with and without undergoing treatment, the probability of progressing from uncomplicated to severe malaria, the probability of dying from uncomplicated malaria without receiving treatment, and the probability of a child with a fever developing malaria have the greatest effects on additional malaria deaths attributable to Ebola for these 3 countries (Table 4). We found that the ART coverage before the Ebola outbreak and the probabilities of dying with and without undergoing ART for persons with a CD4 count <200 cells/mm^3 were of most value in determining additional HIV/AIDS deaths attributable to Ebola (Table 4). Similarly, the treatment coverage for TB before the Ebola outbreak was found to be the principal parameter in determining deaths associated with TB (Table 4).

Discussion

Our analysis estimates the extent to which the 2014–2015 Ebola outbreak in West Africa exacerbated the number of deaths from malaria, HIV/AIDS, and TB through reduced access to treatment. As unprecedentedly catastrophic as the Ebola outbreak has been, we estimated that these indirect repercussions of the Ebola outbreak may have been even greater than the deaths directly attributable to Ebola in Guinea, Sierra Leone, and Liberia. As little as a 15% reduction in treatment would lead to greater indirect than direct deaths from Ebola in Guinea, underscoring the importance

Table 4. Sensitivity of epidemiologic parameters to mortality rates from malaria, HIV/AIDS, and tuberculosis during response to the 2014–2015 Ebola outbreak in Guinea, Liberia, and Sierra Leone

Parameter	PRCC*		
	Guinea	Liberia	Sierra Leone
Malaria			
Death from severe malaria with treatment	–0.594	–0.751	–0.587
Death from severe malaria without treatment	0.822	0.626	0.411
Progressing from uncomplicated to severe malaria given no treatment	0.556	0.854	0.738
Death from uncomplicated malaria without treatment	–0.529	–0.785	–0.477
Fever cases attributable to malaria	0.470	0.701	0.415
Spontaneous recovery from uncomplicated malaria	0.173	0.305	0.289
Developing fever within 2 weeks at age <1	0.333	0.085	–0.018
Developing fever within 2 weeks at age 1–2	–0.145	–0.107	0.182
Developing fever within 2 weeks at age 2–3	–0.044	–0.009	–0.059
Developing fever within 2 weeks at age 3–4	0.139	0.287	–0.045
Developing fever within 2 weeks at age 4–5	–0.071	–0.315	–0.342
Treatment coverage for severe malaria	0.260	–0.093	0.444
Death from uncomplicated malaria with treatment	–0.396	–0.344	–0.411
Treatment coverage for malaria before Ebola outbreak for age <1	0.038	–0.104	0.211
Treatment coverage for malaria before Ebola outbreak for ages 1–2	0.139	–0.013	–0.163
Treatment coverage for malaria before Ebola outbreak for ages 2–3	0.071	0.143	0.313
Treatment coverage for malaria before Ebola outbreak for ages 3–4	–0.149	–0.030	–0.061
Treatment coverage for malaria before Ebola outbreak for ages 4–5	–0.455	–0.662	–0.239
HIV/AIDS			
Death while receiving ART and CD4 count <200 cells/mm^3	–0.679	–0.734	–0.649
Treatment coverage for HIV/AIDS before Ebola outbreak	0.381	0.474	0.467
Death while receiving no treatment and CD4 count <200 cells/mm3	0.494	0.683	0.693
Population with CD4 count >500 cells/mm^3	0.244	0.604	0.397
Population with CD4 count 350 to 499 cells/mm^3	–0.168	–0.062	–0.165
Population with CD4 count 200 to 349 cells/mm^3	–0.220	0.042	0.084
Death while receiving ART with CD4 count 350 to 499 cells/mm^3	–0.204	–0.271	–0.313
Death while receiving ART with CD4 count 200 to 349 cells/mm^3	–0.329	–0.451	–0.320
Death while receiving no treatment and CD4 count 350 to 499 cells/mm^3	–0.295	–0.438	–0.367
Death while receiving no treatment and CD4 count 200 to 349 cells/mm^3	0.107	0.116	–0.088
Tuberculosis			
Treatment coverage for tuberculosis before Ebola outbreak	0.916	0.894	0.946
Death while receiving treatment for MDR-TB	0.134	–0.243	0.148
Death while receiving no treatment for tuberculosis	0.255	–0.138	–0.002
Death while receiving treatment for DS-TB	0.030	–0.211	0.075
Clearing infection after treatment default	0.148	0.463	0.174
MDR–TB cases out of all new TB cases	0.154	–0.057	–0.214
Unsuccessful tuberculosis treatment (failure or default) for DS-TB	–0.037	–0.521	0.009
Treatment failure for DS-TB out of all unsuccessful treatment	–0.226	–0.479	0.177

*Partial rank correlation coefficients (PRCCs) were used to determine the association between the probability of mortality from the studied diseases and input parameters of the model. The PRCC of each parameter was statistically significant ($p<0.001$).

of treating these endemic diseases and the fragility of the local healthcare system. A 65% reduction in treatment coverage would have been necessary to result in more deaths indirectly attributable than directly attributable to Ebola in Sierra Leone. In Liberia, although on average our estimates of indirectly attributable deaths due to Ebola were lower than directly attributable deaths, these estimates were subject to considerable uncertainty. For a 70% or more reduction in treatment, the upper range value of indirectly attributable deaths, 4,376, exceeded directly attributable Ebola deaths. At a more plausible reduction in treatment coverage of 50% for these 3 diseases, we estimated 6,269 (95% CI 2,564–12,407) additional deaths in Guinea, 1,535 (95% CI 522–2,878) in Liberia, and 2,819 (95% CI 844–4,844) in Sierra Leone.

The Ebola outbreak likely had the most detrimental effect on children with malaria, with an estimated 4,275 additional deaths among children <5 years of age in Guinea, 788 in Liberia, and 1,755 in Sierra Leone. Malaria is the most prevalent disease in West Africa and the primary cause of death among children. Although Ebola primarily affected young adults in West Africa (*35*), the indirect deaths were highest among young children who did not receive adequate treatment for malaria.

Our results are consistent with other studies that have indicated that the number of deaths caused by Ebola during his outbreak may have been surpassed by other viral diseases (*4,36*). For example, Walker et al. (*4*) found the number of additional deaths from malaria attributable to Ebola for a 50% reduction in healthcare capacity were estimated to be 2,700 (95% CI 1,400–5,200) in Guinea, 700 (95% CI 400–1,400) in Liberia, and 1,800 (95% CI 900–3,600) in Sierra Leone, which are consistent with our results.

For a 50% reduction in treatment coverage caused by healthcare deficiencies related to the Ebola outbreak, the percentage increase in malaria deaths was higher in Liberia (53.6%) compared with that of Guinea (48.0%) and Sierra Leone (50.0%). These differences are likely attributable to the higher pre-Ebola malaria treatment coverage in Liberia (51.1%); Guinea and Sierra Leone had 22.0% and 42.8% treatment coverage, respectively. The percentage increase in HIV/AIDS deaths attributable to Ebola was higher in Guinea (16.2%) than in Liberia (13.0%) and Sierra Leone (9.1%), consistent with higher pre-Ebola ART coverage in Guinea (51.3%), compared with Liberia (42.9%) and Sierra Leone (33.0%). For TB, the percentage increase in deaths attributable to Ebola was highest in Sierra Leone (61.4%) compared with Guinea (51.1%) and Liberia (59.0%), which corresponds with the high treatment coverage before the Ebola outbreak in Sierra Leone (66.5%) compared with Guinea (52.1%) and Liberia (54.8%).

Our analysis was conservative in several respects. Our base case estimates are conservative in the sense that some reports have cited much greater than 50% reduction in treatment accessibility due to the Ebola outbreak on healthcare systems in Guinea, Liberia, and Sierra Leone (*1*). We assumed no change in the transmission rate of malaria, HIV/AIDS, and TB during the course of the Ebola outbreak, due to a lack of data to estimate potential variation in disease transmission. This assumption may also be conservative because reduced treatment coverage may have elevated transmission; for example, viral loads in untreated HIV-positive persons would be expected to rise, concomitantly increasing risk for transmission (*3,4*). We also did not consider the effect of Ebola on reducing coverage of public health interventions such as bed nets and insecticide provision for malaria prevention, condoms and sexual health education to prevent HIV transmission, or Bacille Calmette-Guérin vaccination. In addition, we did not consider HIV/TB co-infection as a health state in our models. Although our study was conducted with the WHO recommendation for ART initiation at a CD4 count of 500 cells/mm^3 in place during the timeframe of our study, it is possible that these guidelines were not yet being fully implemented in West Africa. Furthermore, our model considers only a short time horizon, limiting long-term measurements of the impact of the Ebola outbreak on HIV/AIDS, TB, and malaria deaths.

Because malaria may be highly seasonal in some West Africa countries, future studies should account for seasonality in malaria transmission to capture transient dynamics of annual malaria incidence and mortality rates (*29*). In West Africa, malaria transmission predominantly occurs during the rainy season, during April–December, which coincides with the peak of the Ebola outbreak in West Africa. This temporal overlap between the rainy season and the peak of the 2014–2015 Ebola outbreak exacerbated the indirect effect of Ebola on malaria in the 3 most affected countries (*4*).

Fear of nosocomial Ebola transmission may have deterred persons from seeking treatment for malaria, which has symptoms similar to Ebola, including fever, dizziness, and body aches (*1*). This problem was compounded by the unprecedented strain on the health systems of Guinea, Liberia, and Sierra Leon, which starkly limited provision of routine health services, such as childhood immunizations for vaccine-preventable diseases, obstetric care, and screening for sexually transmitted infections (*1,37*), as well as public health efforts against neglected tropical diseases (*38*). Thus, the burden of illness of the 2014–2015 Ebola outbreak will inevitably continue to include repercussions beyond deaths related to malaria, HIV/AIDS, and TB. These repercussions will continue long after our study period, caused by, for example, potential development of drug resistance and loss of vital healthcare workers. The societal burden from these diseases, which are beyond the scope of our analyses, extends beyond their direct health

effect, yet is critical to perpetuating the vicious cycle of poverty and disease that leaves children unable to receive education and adults incapable of achieving their potential productivity and fully contributing to the development of their communities.

International donor organizations and governments, in combination with local community-based organizations, were instrumental to curtailing the Ebola outbreak in West Africa, without which more deaths directly attributable to Ebola, as well as further indirect devastation, would have occurred (*39*). Although public health officials rightfully focused efforts on curbing the Ebola outbreak, the long-term weakening of health systems related to the Ebola outbreak will require extensive investment directed at strengthening diffuse health systems for a plethora of diseases (*40*). Our analysis illustrates the need to invest resources in strengthening of health systems to mitigate vulnerability and reduce costs associated with health systems failing when stressed by acute events.

In conclusion, our results estimate that the 2014–2015 Ebola outbreak in West Africa has substantially impeded the fight against malaria, HIV/AIDS, and TB in the 3 countries most affected. As the Ebola outbreak wanes, it is essential for control strategies to include a comprehensive approach not only to stem the spread of Ebola, provide care for medical complications of recovered case-patients, and offer support for affected families but also to address the extensive repercussions of the outbreak that will continue long after Ebola elimination.

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Ms. Parpia received her Master of Public Health in Epidemiology from the University of Toronto, during which she completed a practicum placement at Yale University in the Center for Infectious Disease Modeling and Analysis under the supervision of Dr. Alison Galvani and Dr. Martial L. Ndeffo-Mbah. Her primary research interest is infectious disease epidemiology, specifically using mathematical modeling to evaluate disease transmission and burden.

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Address for correspondence: Martial L. Ndeffo-Mbah, Center for Infectious Disease Modeling and Analysis, 135 College St, Ste 200, New Haven, CT 06510, USA; email: martial.ndeffo-mbah@yale.edu.

Changes in Predominance of Pulsed-Field Gel Electrophoresis Profiles of *Bordetella pertussis* Isolates, United States, 2000–2012

Pamela K. Cassiday, Tami H. Skoff, Selina Jawahir, M. Lucia Tondella

To clarify the characteristics of circulating *Bordetella pertussis* isolates, we used pulsed-field gel electrophoresis (PFGE) to analyze 5,262 isolates collected in the United States during 2000–2012. We found 199 PFGE profiles; 5 profiles accounted for 72% of isolates. The most common profile, CDC013, accounted for 35%–46% of isolates tested from 2000–2009; however, the proportion of isolates of this profile rapidly decreased in 2010. Profile CDC237, first seen in 2009, increased rapidly and accounted for 29% of 2012 isolates. No location bias was observed among profiles during 2000–2010, but differences were observed among isolates from different states during 2012. Predominant profiles match those observed in recent European PFGE studies. PFGE profile changes are concurrent with other recent molecular changes in *B. pertussis* and may be contributing to the reemergence of pertussis in the United States. Continued PFGE monitoring is critical for understanding the changing epidemiology of pertussis.

Despite high coverage with *Bordetella pertussis* component–containing vaccines, the incidence of reported pertussis has been increasing in the United States, and notable pertussis outbreaks have occurred in recent years (*1*). More than 48,000 pertussis cases were reported in the United States in 2012, the highest number reported since 1955 (*2*). Multiple factors have likely contributed to this increase, including increased recognition of pertussis among the general population, increased diagnosis of healthcare providers, improved diagnostic testing and reporting, and waning immunity from pertussis vaccines (*3*–*5*).

Concerns over adverse reactions after receipt of vaccines containing whole-cell preparations (WCVs) of *B. pertussis* led to development of vaccines with less reactogenicity (*6*). Starting in the 1990s, vaccines with acellular pertussis components (ACVs) began replacing the use of WCVs in the United States, and in 2005 ACVs with a lower concentration of pertussis components (known as Tdap vaccines for their tetanus and diphtheria toxoids and acellular pertussis components) were recommended for the first time as a booster dose among adolescents and adults (*7*,*8*).

Researchers in several countries have used methods such as pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), and multilocus variable-number tandem-repeat analysis (MLVA) to study the evolution of molecular changes in *B. pertussis* populations over time (*9*–*25*). Recent studies have documented genetic changes in currently circulating *B. pertussis* strains, including shifts in virulence-associated protein phenotypes and predominant molecular types between prevaccine and postvaccine eras. These shifts may also be contributing to the resurgence of pertussis as a result of pathogen adaptation to current pertussis vaccines (*26*–*28*). In addition, recent changes in the epidemiology of pertussis have highlighted an increasing number of cases among older children and adolescents who have been fully vaccinated with ACVs; this increase further supports a role for vaccine pressure in the reemergence of pertussis in the United States (*1*,*3*–*5*,*7*).

PFGE, which can differentiate between individual isolates, has been used to characterize US *B. pertussis* isolates for >15 years (*19*,*29*). Because this method uses the entire genome, PFGE typing is more discriminatory for *B. pertussis* than PCR-based typing methods, such as MLST and MLVA, which analyze the sequences of a few select loci (*22*,*30*). MLST and MLVA typing must be used in tandem to obtain discriminatory power similar to that of PFGE typing (*13*,*30*).

Our objective was to increase understanding of the reemergence of pertussis and characteristics of circulating *B. pertussis* strains in the United States. To determine the current distribution of circulating PFGE profiles and identify changes in profile distributions over time, we analyzed PFGE profiles of *B. pertussis* isolates collected in the United States during 2000–2012.

Materials and Methods

A total of 5,262 isolates were available for testing, collected from 32 states during 2000–2012 (Table). Isolates were identified and collected through Enhanced Pertussis

Author affiliations: Centers for Disease Control and Prevention, Atlanta, Georgia, USA (P.K. Cassiday, T.H. Skoff, M.L. Tondella); Minnesota Department of Health, St. Paul, Minnesota, USA (S. Jawahir)

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Surveillance (EPS) conducted as part of the Emerging Infections Program Network, through routine state and local health department pertussis surveillance, or during localized outbreaks. Isolates from Massachusetts and Minnesota were characterized by PFGE in their respective state public health laboratories, and tagged image file format images of PFGE gels were sent to the Centers for Disease Control and Prevention (CDC) for analysis; isolates from all other states were sent directly to CDC for PFGE characterization (Table). Because running conditions differed from those of the PFGE method commonly used in Europe, images produced by each method could not be directly compared (*9*,*11*,*12*,*16*–*18*,*23*). An additional set of 5 *B. pertussis* isolates, representing European profiles BpSR3, BpSR5, BpSR10, BpSR11, and BpSR12 was also included in this study with which to compare US isolates with the most commonly circulating PFGE profiles in several European countries during 1998–2009 (*12*).

PFGE was performed by using restriction enzyme *Xba*I (*19*), based on the method developed by Gautom et al. (*31*) and similar to that currently used by US state health departments participating in CDC's PulseNet (http://www.cdc.gov/pulsenet/pathogens/index.html) for the typing of foodborne pathogens (*32*). PFGE patterns were compared with those in a database of *B. pertussis* isolate profiles maintained at CDC, and profiles were assigned on the basis of bands in the 125- to 450-kb range by using BioNumerics software version 5.01 (Applied Maths, Austin, TX, USA).

To identify predominantly circulating profiles, we analyzed overall circulating profiles for each individual year of the study period. Distributions were also compared during 2 shorter periods (2000–2009 and 2010–2012) to assess differences between recent peak years in disease and the remainder of the study period. To assess how states that submitted a large proportion of isolates affected the overall study findings, we performed 2 subanalyses. First, since Massachusetts and Minnesota together contributed >50% of isolates annually during 2000–2010 (Table), we conducted a subanalysis to check for profile local bias by comparing isolates collected from these states during 2000–2010 (Massachusetts, n = 1,758; Minnesota, n = 897), with isolates collected from all other states combined (n = 1,639). A second subanalysis was conducted in which isolates obtained during 2012 from 2 states that experienced

Table. *Bordetella pertussis* isolates by state and year of collection, United States, 2000–2012

State	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	Total no. (%)
Arizona	52	3	70	56	33	184	12	0	0	0	0	0	1	411 (8)
California	27	10	36	13	7	35	22	1	0	2	38	10	1	202 (4)
Colorado	0	0	0	0	0	0	0	1	2	0	2	0	9	14 (>1)
Connecticut	0	0	0	0	0	0	0	0	0	0	0	7	16	23 (>1)
Delaware	0	0	0	0	0	3	0	0	0	0	0	0	0	3 (>1)
Florida	0	0	0	0	0	0	0	0	0	1	0	6	9	16 (>1)
Georgia	34	13	21	14	13	22	20	3	12	56	4	1	5	218 (4)
Idaho	1	0	1	0	0	0	0	0	0	0	0	0	0	2 (>1)
Illinois	32	27	14	25	0	19	2	11	7	0	0	0	0	137 (3)
Indiana	3	0	0	0	0	0	0	0	0	0	0	0	0	3 (>1)
Kentucky	0	0	0	1	0	0	0	0	0	0	0	0	0	1 (>1)
Maryland	0	0	0	2	0	0	0	0	0	0	0	0	0	2 (>1)
Massachusetts*	227	66	84	178	296	204	194	216	133	79	81	19	0	1,777 (34)
Michigan	0	0	0	0	0	0	0	0	0	0	2	0	0	2 (>1)
Minnesota*	186	98	113	67	83	109	40	30	98	44	29	5	91	993 (19)
Missouri	0	2	1	0	8	0	0	0	0	0	0	1	0	12 (>1)
Nebraska	0	0	0	0	0	3	0	0	0	0	0	0	0	3 (>1)
Nevada	0	0	6	0	0	0	0	0	0	0	0	0	0	6 (>1)
New Jersey	1	1	1	0	0	0	0	0	0	0	0	0	0	3 (>1)
New Mexico	0	0	0	0	0	0	0	8	8	10	0	2	4	32 (1)
New York	29	21	29	73	169	41	0	0	0	0	2	9	53	426 (8)
North Carolina	0	0	0	0	0	0	0	0	11	0	0	0	0	11 (>1)
Ohio	74	72	0	1	1	0	0	0	0	0	0	0	0	148 (3)
Oregon	0	0	0	0	0	0	0	0	0	0	6	19	86	111 (2)
Pennsylvania	0	0	1	2	0	0	0	0	0	3	19	6	0	31 (1)
South Carolina	0	0	0	10	0	2	0	0	3	1	3	0	0	19 (>1)
Tennessee	0	0	0	0	0	0	0	0	0	0	2	0	0	2 (>1)
Texas	11	0	2	0	0	0	0	0	0	0	0	0	0	13 (>1)
Utah	0	1	0	0	0	0	0	0	0	0	0	0	0	1 (>1)
Vermont*	0	0	0	0	0	0	0	18	1	2	1	44	333	399 (8)
Virginia	0	0	0	0	0	0	0	0	1	0	0	0	0	1 (>1)
Washington*	0	0	0	9	0	0	0	0	0	0	0	9	222	240 (5)
Total no. (%)	677 (13)	314 (6)	379 (7)	451 (8)	610 (12)	622 (12)	290 (5)	288 (5)	276 (5)	198 (4)	189 (4)	138 (3)	830 (16)	5,262 (100)

*Isolates from these states were included in the subanalyses presented in Figure 3.

substantial statewide epidemics of pertussis, Vermont (n = 333) and Washington (n = 222), were compared with isolates obtained from the other 10 states (n = 275) in 2012 to assess the distribution of circulating PFGE profiles during a period of widespread disease. The χ^2 test was used for comparison of proportions; $p<0.005$ was considered significant.

A dendrogram of predominant profiles was created using BioNumerics software to visualize the degree of similarity between identified PFGE patterns. Dendrogram analysis also included the 5 predominant profiles found recently circulating in Europe. Clustering was determined by using unweighted pair group method with arithmetic mean with 1% band tolerance and optimization settings.

To assess changes among the study isolates over time, we calculated genetic diversity overall and by year of isolation using the Simpson Index of Diversity (*33*). CIs were calculated as described by Grundmann et al. (*34*).

Results

Overall, we identified 199 distinct PFGE profiles were the study isolates. CDC013, CDC010, CDC082, CDC002, and CDC046 were the predominant 5 profiles among our study population and accounted for 72% of all isolates tested (Figure 1). One additional profile, CDC237, accounted for 5% of isolates overall but was only observed in isolates collected in 2009 and subsequently (Figure 2). None of the remaining 193 profiles accounted for >5% of isolates overall.

When we assessed the most predominant profiles by year, we noted differences in the order of predominance beginning in 2010 (Figure 2). CDC013 was consistently predominant during 2000–2009, but CDC082 was predominant among isolates collected in 2010. CDC002 appeared to be fading from circulation during 2000–2009, but it emerged as the most common profile among 2011 isolates. CDC237 and CDC002 predominated in 2012; each accounted for ≈29% of circulating profiles.

When the 5 most predominant profiles were compared in each period, we found CDC013 in 41% of isolates collected during 2000–2009 (35%–46% annually) but only in 9% of isolates analyzed during 2010–2012 ($p<0.0001$). Prevalence also declined significantly between periods for CDC010 (from 15% to 4.5%, $p<0.0001$) and CDC082 (from 12% to 3%, $p<0.0001$). Conversely, profile CDC002, which comprised 4% of isolates in the earlier period, increased to 25% among isolates collected during 2010–2012 ($p<0.0001$). No changes were observed for profile CDC046, which accounted for a consistent 6% of isolates in each period. In addition, frequency of 3 profiles (CDC217, CDC237, CDC253), each accounting for <1% of isolates during 2000–2009, increased significantly to 4%, 24%, and 6%, respectively, of isolates collected during 2010–2012 ($p<0.0001$ for all).

When we compared profiles of Massachusetts and Minnesota isolates to isolates of all other states combined during 2000–2010, our subanalysis revealed that CDC013 was the most common PFGE profile among all 3 groups, ranging from 37% to 44% of isolates (Figure 3, panel A). In addition, CDC010 accounted for a similar proportion of isolates across all groups (12%–17%). Whereas a similar proportion of isolates were of profile CDC082 in Minnesota and in all other states combined (9% and 10%, respectively), this profile was found in significantly higher proportion in Massachusetts (17%, $p<0.0001$), where it was the second most predominant profile. CDC046 accounted for a consistent 5%–7% of isolates from all 3 groups, and CDC002 frequency ranged between from 2% to 5%.

Our subanalysis of the predominant profiles in 2012 isolates from 2 statewide epidemics (Vermont and Washington) and 10 other states combined revealed marked differences between the collections (Figure 3, panel B). CDC013 predominated among Washington isolates at 22%, which was significantly more than the 1% and 4% of isolates with this profile found among isolates from Vermont and combined states, respectively ($p<0.0001$). In Vermont, a higher percentage of isolates was CDC237 (41%), compared with 18% and 22% of isolates from Washington and the other states combined, respectively ($p<0.0001$). CDC046 was also found in significantly higher proportion among Vermont isolates (11%) than among those from Washington (1%; $p<0.0001$), but the proportion among isolates from the other states (5%, $p = 0.0098$) was not significantly higher. The most predominant profile from the other states combined group was CDC002 (35%), which accounted for

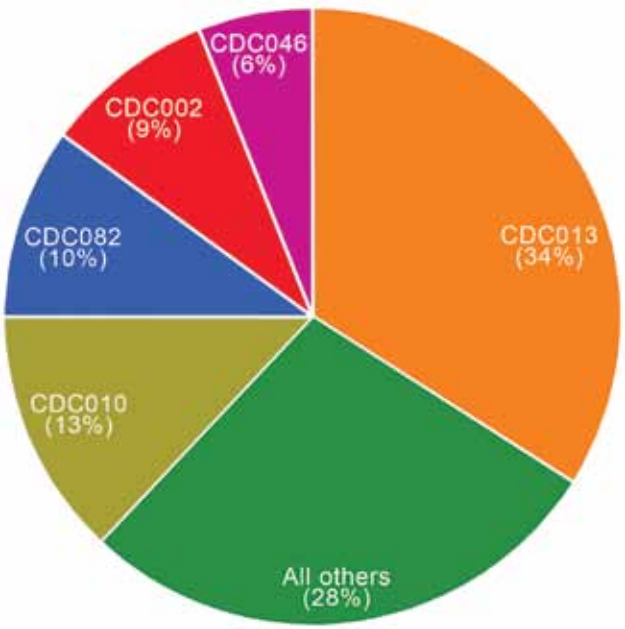

Figure 1. Predominant pulsed-field gel electrophoresis profiles of 5,262 *Bordetella pertussis* isolates, United States, 2000–2012.

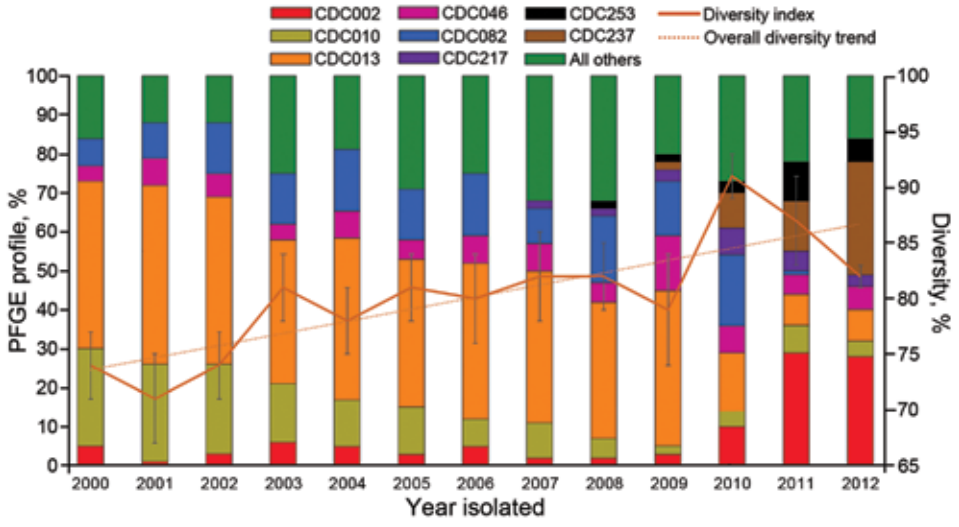

Figure 2. Predominant pulsed-field gel electrophoresis (PFGE) profiles and genetic diversity of 5,262 *Bordetella pertussis* isolates, by year of collection, United States, 2000–2012. Genetic diversity (D) was calculated overall and by year of isolation using the Simpson Index of Diversity (expressed as 1-D) (*33*). Confidence intervals were calculated as described by Grundmann et al.(*34*).

a similar proportion of isolates from Vermont (29%, p = 0.0966), but a significantly higher proportion of isolates from Washington (18%, p<0.0001). CDC010 comprised only 1% of isolates from Vermont, significantly fewer than those from Washington (7%, p = 0.0003) and the other states combined (6%, p = 0.0021). CDC082 accounted for <1% of isolates in each group.

A dendrogram analysis comparing the 5 most predominant profiles overall in the United States during the study period (CDC013, CDC010, CDC082, CDC002, CDC046) with 3 increasingly circulating profiles (CDC217, CDC237, CDC253) revealed 2 clusters with 75% similarity. Cluster 1 contains profiles CDC013, CDC046, and CDC082, first seen among US isolates collected during 1993–1997. Cluster 2 contains profiles CDC002, CDC010, CDC217, CDC237, and CDC253. CDC002 and CDC010 were first seen among US isolates collected in the 1980s, whereas the other 3 profiles were not seen until 2007. In the area of band analysis used in this study (125 kb–450 kb), the profiles of the 5 predominant strains from Europe, (BpSR3, BpSR10, BpSR11, BpSR5, BpSR12) were indistinguishable from the 5 predominantly circulating US profiles (Figure 4).

Overall genetic diversity among the study isolates was 84%. Genetic diversity ranged from 71% to 82% annually for isolates collected during 2000–2009 before a high of 91% was reached in 2010 (Figure 2). By 2012, diversity decreased slightly to 82%.

Discussion

Of the 199 *B. pertussis* PFGE profiles observed in this study, a single profile, CDC013, predominated between 2000 and 2009, and 5 profiles comprised most isolates tested from 2000 to 2012. These finding are similar to those previously reported by Hardwick et al., who also found 3 predominant profiles circulating in the United States during 1986–1999 (*19*). Hardwick et al. reported the 3 predominant profiles as CYXXI-010 (now CDC010) (37%), CYXXI-002 (now CDC002) (11%), and CYXXI-013 (now CDC013) (10%), which are still among the currently circulating predominant profiles in the United States. Additionally, 42% of the profiles observed during 1986–1999 were still circulating in the United States during our study. Other studies have also shown that a small number of PFGE profiles usually make up most of the circulating *B. pertussis* strains during outbreak and nonoutbreak periods (*12*,*29*).

In contrast to the isolates collected during 2000–2009, profile predominance changed rapidly among isolates collected during 2010–2012, and a different profile predominated each year. Rare profiles such as CDC237 quickly became more common than the previously predominant CDC013 profile. Two additional rarely seen profiles, CDC217 and CDC253, which were closely related to the CDC237 profile, also increased significantly in recent years. These findings prompted us to explore possible associations between PFGE profile and other molecular changes that have recently occurred in the organism. Specifically, in recent years, pertactin-deficient *B. pertussis* isolates have rapidly emerged. Pertactin is a key immunogen included in all ACVs currently used in the United States (*21*,*30*), and data have suggested a possible selective advantage of pertactin-deficient mutants among ACV-vaccinated populations (*27*). Using supplemental data, we observed that 87% of 2012 isolates included in our analysis were pertactin-deficient. Of interest, isolates with PFGE profiles CDC002 and CDC237, the most common profiles among 2012 isolates, were significantly more likely to be pertactin-deficient than isolates with profile CDC013, the most common profile during 2000–2009. That these 2 profiles started becoming more predominant in 2010, coinciding with the rapid increase in pertactin-deficient isolates in the United States (*21*), suggests a linkage between the 2 changes. To date, no associations have been reported between pertactin-deficiency and specific PCR-based MLVA

or MLST types, which reinforces the discrimatory power of PFGE typing methods and highlights the value of this method for elucidating *B. pertussis* evolution on a more granular level (*24*,*30*).

Although Massachusetts and Minnesota contributed a large proportion of isolates during 2000–2010, we observed similar distributions among 4 of the 5 predominant PFGE profiles when we compared isolates collected in these 2 states to isolates in all other states combined. These results suggest that location bias did not affect the findings. This observation contrasts with results of our subanalysis of 2012 isolates, in which significant differences were noted in the predominant strains found in Vermont, Washington, and all other states combined. This finding could be caused by many factors, including clonal expansion within epidemic areas and, as previously noted, selective advantage of pertactin-deficient *B. pertussis* among vaccinated populations. Exploration of epidemiologic data associated with these isolates may further explain the observed geographic differences in PFGE profiles.

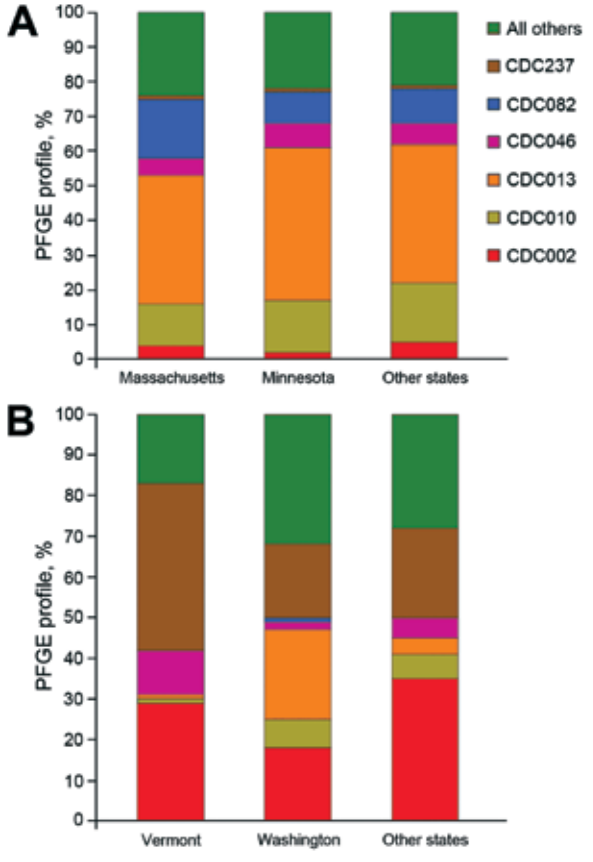

Figure 3. Geographic comparison of predominant pulsed-field gel electrophoresis (PFGE) profiles of *Bordetella pertussis* isolates, United States. A) Predominant profiles in Massachusetts and Minnesota compared with isolates from other states, 2000–2010. B) Predominant profiles in Vermont and Washington compared with isolates from other states, 2012.

Our analysis showed that the 5 most predominant profiles circulating in several countries in Europe (Denmark, Finland, France, Germany, the Netherlands, Norway, Sweden, United Kingdom) during 1998–2009 (*12*) were also the 5 most predominant profiles circulating in the United States during 2000–2012. The proportion of the most predominant profile in Europe, BpSR11, changed from 25%–30% of 1998–2005 isolates to 13% of 2007–2009 isolates, which was similar to changes observed in the United States equivalent CDC013 profile (41%, 2000–2009, to 9%, 2010–2012). Comparable increases were also noted for CDC002 (4%, 2000–2009, to 25%, 2010–2012) and its European equivalent, BpSR3 (0%, 1998–2005, to 21%, 2007–2009).

We did not detect a large increase in CDC010 during 2010–2012, such as was reported for BpSR10 during 2007–2009 in Europe, but instead detected a large increase in CDC237, first seen among US isolates in 2009. As shown, CDC010 and CDC0237 are extremely similar and cluster together in our dendrogram (Figure 4), which indicates that they are closely related. Possible explanations for differences in these profiles include a point mutation with the gain of a restriction site in CDC237 isolates or a rearrangement at the chromosomal level (*23*,*35*,*36*).

Similar to the findings for US isolates, the study in Europe also reported increased diversity (*12*). However, even though circulating PFGE profiles appear to be similar in the United States and Europe, percentages of pertactin-deficient isolates collected during 2010–2014 in European countries are much lower than in the United States, which suggests that even though PFGE changes and pertactin deficiency are occurring simultaneously in the United States, these changes are not necessarily linked and that virulence and transmission of pertussis depend on many factors (*37*,*38*).

A limitation of our study is that the differences in switch times and running time of our PFGE method prevents direct comparison with isolates tested by the European PFGE method (*23*). Our method results in low resolution of 6–8 small (45- to 125-kb) DNA bands, which are not included as part of our analysis, leading to fewer PFGE profiles overall when compared to the European method. However, by using the same PFGE method as in our previous study, we were able to directly compare 6,595 US isolates collected over 77 years. The profile changes observed in the course of the 2 studies have provided useful information for the development of current molecular typing systems and whole-genome sequencing strategies.

Our work reveals that *B. pertussis* PFGE profiles are changing rapidly in the United States, as they are in Europe (*12*). The previously held assumption that *B. pertussis* was a highly clonal organism has been challenged because recent isolates of *B. pertussis* have been shown

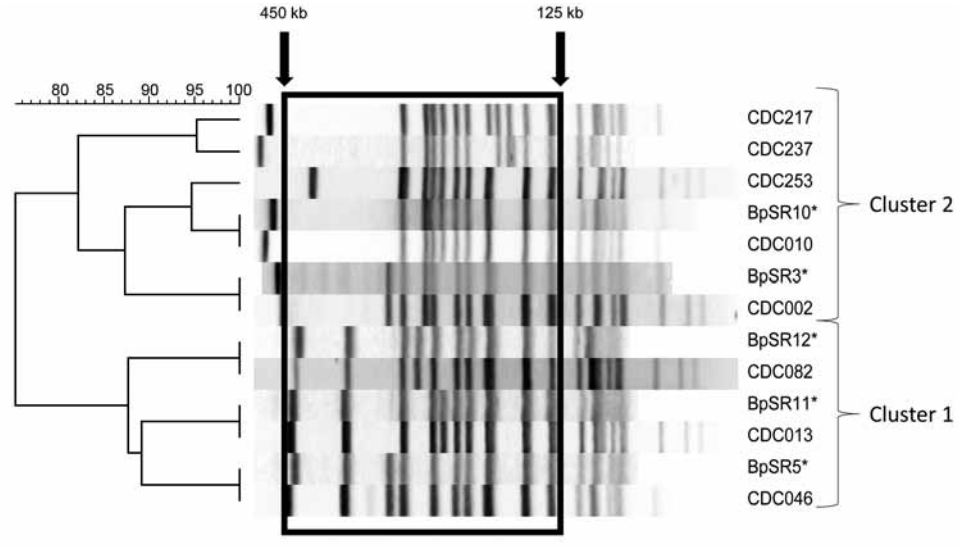

Figure 4. Dendrogram of the predominant *Bordetella pertussis* pulsed-field gell electrophoresis (PFGE) profiles currently circulating in the United States. Clusters were determined by using unweighted pair group method with arithmetic mean (UPGMA) with 1% band tolerance and optimization settings. Box indicates area of band analysis (125 kb–450 kb). *Indicates the predominant *B. pertussis* PFGE profiles currently circulating in Europe, as reported by Advani et al. (*12*). In the area of band analysis, these profiles (BpSR3, BpSR10, BpSR11, BpSR5, and BpSR12) were indistinguishable from US profiles CDC002, CDC010, CDC013, CDC046, and CDC082, respectively. Scale bar indicates percent similarity.

to be quite variable at the genome level, particularly in those genes that code for ACV components (*21*,*27*,*28*). Although more emphasis is now being placed on molecular typing methods that do not require the availability of isolates, PFGE, in tandem with other typing methods, continues to remain a valuable tool for understanding *B. pertussis* because it can detect a greater level of diversity among circulating strains.

As we move more toward whole-genome sequencing and rearrangement analysis, PFGE can be useful for strain selection when genetic diversity is a desired parameter for analysis. Having a more complete picture of the molecular evolution of *B. pertussis* through whole-genome methods such as PFGE and sequencing may provide key data to help guide development of the next generation of pertussis vaccines or, in the near term, inform us on ways to more effectively use existing vaccines. Continued monitoring of the molecular epidemiology of circulating *B. pertussis* continues to be critical for understanding the changing epidemiology of the disease both in the United States and abroad to optimize current prevention and control strategies.

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Ms. Cassiday is a research microbiologist in the Meningitis and Vaccine Preventable Diseases Branch, Division of Bacterial Diseases, National Center for Immunization and Respiratory Diseases, CDC. Her research focuses on *Bordetella pertussis* and *Corynebacterium diphtheriae*.

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Address for correspondence: Pamela K. Cassiday, Centers for Disease Control and Prevention, 1600 Clifton Rd NE, Mailstop D11, Atlanta, GA 30249-4027, USA; email: PCassiday@cdc.gov

Faster Detection of Poliomyelitis Outbreaks to Support Polio Eradication

Isobel M. Blake, Paul Chenoweth, Hiro Okayasu, Christl A. Donnelly, R. Bruce Aylward, Nicholas C. Grassly

As the global eradication of poliomyelitis approaches the final stages, prompt detection of new outbreaks is critical to enable a fast and effective outbreak response. Surveillance relies on reporting of acute flaccid paralysis (AFP) cases and laboratory confirmation through isolation of poliovirus from stool. However, delayed sample collection and testing can delay outbreak detection. We investigated whether weekly testing for clusters of AFP by location and time, using the Kulldorff scan statistic, could provide an early warning for outbreaks in 20 countries. A mixed-effects regression model was used to predict background rates of nonpolio AFP at the district level. In Tajikistan and Congo, testing for AFP clusters would have resulted in an outbreak warning 39 and 11 days, respectively, before official confirmation of large outbreaks. This method has relatively high specificity and could be integrated into the current polio information system to support rapid outbreak response activities.

The global eradication of polio is entering its final stages. The last case of poliomyelitis associated with serotype 2 wild poliovirus was reported in 1999 and of serotype 3 in 2012. In Africa, the last reported case of serotype 1 wild poliovirus was in Somalia in August 2014. Transmission of this serotype has yet to be interrupted in Afghanistan and Pakistan, and in 2014, 359 serotype 1–associated cases were reported worldwide, 81% of which occurred in Pakistan (*1*).

Transmission of wild poliovirus persists in countries where the disease is endemic, but outbreaks can also occur in previously polio-free populations in which population immunity is not sustained. For example, the 2013 polio outbreak in the Middle East was linked to importation of poliovirus from Pakistan (*2*). The live-attenuated oral poliovirus vaccine (OPV) has played a huge role in achieving >99% reduction in global annual incidence of poliomyelitis, but its continued use also means there is a risk for emergence and spread of circulating vaccine-derived poliovirus (cVDPV) (*3*). In 2015, cVDPV outbreaks were reported in at least 5 countries (*1*). The risk for serotype 2 cVDPV may be heightened during the planned global switch from trivalent to bivalent (containing Sabin virus types 1 and 3) OPV during routine vaccination in April 2016 (*4*). Poliomyelitis outbreaks substantially raise the cost of the eradication program and hinder progress toward eradication, particularly if they are not swiftly controlled (*5*). Early detection is therefore critical to the program to enable a fast outbreak response to quickly stop transmission.

Surveillance for poliomyelitis relies on the reporting of cases of acute flaccid paralysis (AFP) in children <15 years of age by healthcare providers (Figure 1 at http://dx.doi.org/10.5281/zenodo.44361) (*4*,*6*). In some areas this surveillance is supplemented by environmental surveillance, which involves the periodic collection and testing of sewage samples for the presence of polioviruses. Surveillance is challenging because of the large number of asymptomatic cases (100–1,000 infections/AFP case) and because there are multiple causes of AFP (e.g., trauma, toxins, enteroviruses), thus requiring laboratory testing of stool samples to confirm the presence of poliovirus (*7*–*9*).

In 2010, large outbreaks of poliomyelitis in Tajikistan and Republic of the Congo (Congo) were detected relatively late, partly due to delays in laboratory processing of stool samples; the delayed detection resulted in a limited effect from the outbreak response vaccination campaigns (*10*). The high transmissibility and pathogenicity of wild and vaccine-derived polioviruses means that poliomyelitis cases may be expected to cluster in space and time to a greater extent than do cases of AFP associated with other enteroviruses or noninfectious causes. We therefore decided to investigate whether clusters of AFP could herald poliomyelitis outbreaks and be identified as an early warning of outbreaks before laboratory confirmation.

Methods

Data

Cases of AFP are reported through a network of healthcare providers as part of routine surveillance for poliomyelitis

Author affiliations: Imperial College London, London, UK (I.M. Blake, C.A. Donnelly, N.C. Grassly); World Health Organization, Geneva, Switzerland (P. Chenoweth, H. Okayasu, R.B. Aylward)

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(*6*). We analyzed 67,218 AFP cases with clinical onset during 2003–2013; the patients resided in 3 countries that had >150 confirmed cases of polio annually since 2005 (Tajikistan, Congo, and Somalia) or in countries in Africa considered to be at high risk for an outbreak of wild poliovirus. For each AFP case, the following information was recorded: the first and second administrative levels (province and district, respectively) in which the patient resided; the dates of AFP onset, case notification, and stool sample collection; and the patient's age and sex.

AFP cases with stool samples adequate for testing were distinguished as virologically confirmed cases of poliomyelitis caused by wild poliovirus type 1 or 3 (3,089 cases), cVDPV (70 cases), or nonpolio AFP. Cases of AFP in persons without adequate stool samples were defined as polio-compatible cases (i.e., cases with clinical symptoms compatible with poliomyelitis, as determined by a panel of experts; 1,436 cases) or nonpolio AFP cases. The total number of nonpolio AFP cases was 62,623.

Institutional ethics approval for this study was not sought because the databases are free of personally identifiable information. National and subnational (first administrative and second administrative levels, respectively) boundaries were obtained from the World Health Organization.

For each of the 20 countries in the study, we obtained raster population size data for 2010 from the WorldPop project (http://www.worldpop.org.uk) (*11*,*12*). The data contained estimates of population distribution at ≈100-m^2 spatial resolution. Population size estimates at the district level were acquired by aggregating the raster data within each district by using the R package raster (*13*) (Figures 2 and 3 at http://dx.doi.org/10.5281/zenodo.44361) implemented in the R programming language (*14*).

Time from Paralysis Onset to Case Notification to Specimen Delivery for Laboratory Testing

Delays in reporting and testing were determined for all AFP cases reported from Africa during 2010–2013 with available date information. We computed the length of time between paralysis onset and case notification and between case notification and the date stool samples were sent to a global polio laboratory for testing.

Space–Time Analysis of Nonpolio AFP data

We fitted a mixed-effects spatiotemporal statistical model to the data for each country (http://dx.doi.org/10.5281/zenodo.44361). In brief, the number of nonpolio AFP cases reported in a district at a given time was assumed to follow a Poisson or negative binomial distribution. In accordance with the model of Besag, York, and Mollié (*15*), the linear predictor was based on spatially structured and spatially unstructured random effects, with an additional offset of population size, and a random walk over time to account for temporal trends in reporting. The models were fitted to the nonpolio AFP data for each country in a Bayesian framework by using INLA (integrated nested Laplace approximation) (*16*) implemented in the INLA package (*17*). We selected the most parsimonious model, according to the deviance information criterion (*18*), to determine whether the count data followed a Poisson or negative binomial distribution.

Creation of Real-Time AFP Databases

A record is not kept of when each AFP case enters the AFP database. To test whether clusters of AFP cases could be identified in advance of an outbreak, we created real-time AFP databases for each Monday during 2003–2013 by assuming cases entered the database on the date the case was notified by local healthcare providers. These real-time databases partly capture the delay between symptom onset and reporting of AFP cases (Figure 1 at http://dx.doi.org/10.5281/zenodo.44361) and are a best-case scenario of timely reporting. When the date of notification was missing, we used the date of investigation, first stool collection, or second stool collection (in that preferential order) as proxy for when the case entered the database.

Testing of Real-Time AFP Databases to Detect Polio Outbreaks

For each country, the prospective Kulldorff Poisson space–time scan statistic (*19*) was evaluated at weekly intervals from the real-time AFP database to identify clustering of AFP cases in space and time. In summary, for every district in a given country, space–time cylinders were created; the cylinders were centered on the centroid of the district, and each had a different radius (representing various distances from the centroid to other district centroids) and height (representing different time periods up to and including the current week of surveillance). Cases of AFP were included in a given sized cylinder if the onset date for the case was within the interval of the start and end dates of the cylinder and the radius passed through the centroid of their reporting district. The cylinder end date was always the date of the real-time database; the start date varied from 1 to 90 days before the end date (we assumed standard methods of poliomyelitis outbreak detection would have detected an outbreak >90 days after the date of paralysis onset of the first AFP case). The maximum radius of the cylinder was restricted to 500 km, a conservative distance given the observed spatial clustering of polio cases at the start of an outbreak (Figure 9 at http://dx.doi.org/10.5281/zenodo.44361). The radius did not extend outside a given country.

The number of AFP cases observed within each cylinder was summed, and the likelihood ratio function, defining how likely there is an elevated risk within the cylinder

compared with outside the cylinder, was maximized across cylinders of all locations and sizes. The expected rate of AFP reporting in the absence of a polio outbreak was obtained from the spatiotemporal regression model. The cylinder with the maximum likelihood ratio corresponded to the identified cluster (http://dx.doi.org/10.5281/zenodo.44361). The p value of the cluster was determined by Monte Carlo hypothesis testing by simulating cases under the null hypothesis and comparing the rank of the maximum likelihood ratio of the data with the simulations (http://dx.doi.org/10.5281/zenodo.44361). A cluster of AFP cases was defined to trigger an alarm of a potential outbreak when $p<0.05$. The space–time scan statistic was evaluated by using SaTScan version 9.3 (*20*), which was called using the R programming language (*14*), and the computation was parallelized over a 16-core, high performance cluster. We also tested the ability of the space–time permutation scan statistic (*21*) as an alternative to the space–time Poisson scan statistic (http://dx.doi.org/10.5281/zenodo.44361) because the space–time permutation scan statistic only relies on case data.

An outbreak was classified as detected by the algorithm if a warning alarm was raised within the outbreak period and if the location of the alarm occurred in at least 1 district containing reported outbreak-associated polio cases. The algorithm was assessed in its ability to detect confirmed serotype 1 and 3 wild poliovirus and cVDPV outbreaks. An outbreak period was defined as the length of time that consecutive, type-specific cases occurred with dates of paralysis onset <6 months apart. The percentage of outbreaks that were correctly identified was recorded (sensitivity of algorithm). The time of the alarm was compared with the date the outbreak was officially confirmed. The date of confirmation was not available for the smaller outbreaks; therefore, it was not possible to evaluate the timeliness of these alarms, apart from observing the time between the alarm and the date of onset of the first case. The specificity of the cluster detection algorithm was evaluated at the country level as the percentage of outbreak-free weeks without a false alarm. Sensitivity to this definition was examined (http://dx.doi.org/10.5281/zenodo.44361).

Results

Nonpolio AFP Reporting

The number of nonpolio AFP cases reported at the district level was spatially heterogeneous within each country investigated throughout 2003–2013 (Figure 1; Figure 4 at http://dx.doi.org/10.5281/zenodo.44361). Spatial heterogeneity remained across all the Africa countries when adjusting for the population size of each district (Figure 5 at http://dx.doi.org/10.5281/zenodo.44361). The number of nonpolio AFP cases reported during 2003–2013 increased over time in all countries except Equatorial Guinea, although the rate of increase differed by country (Figure 6 at http://dx.doi.org/10.5281/zenodo.44361).

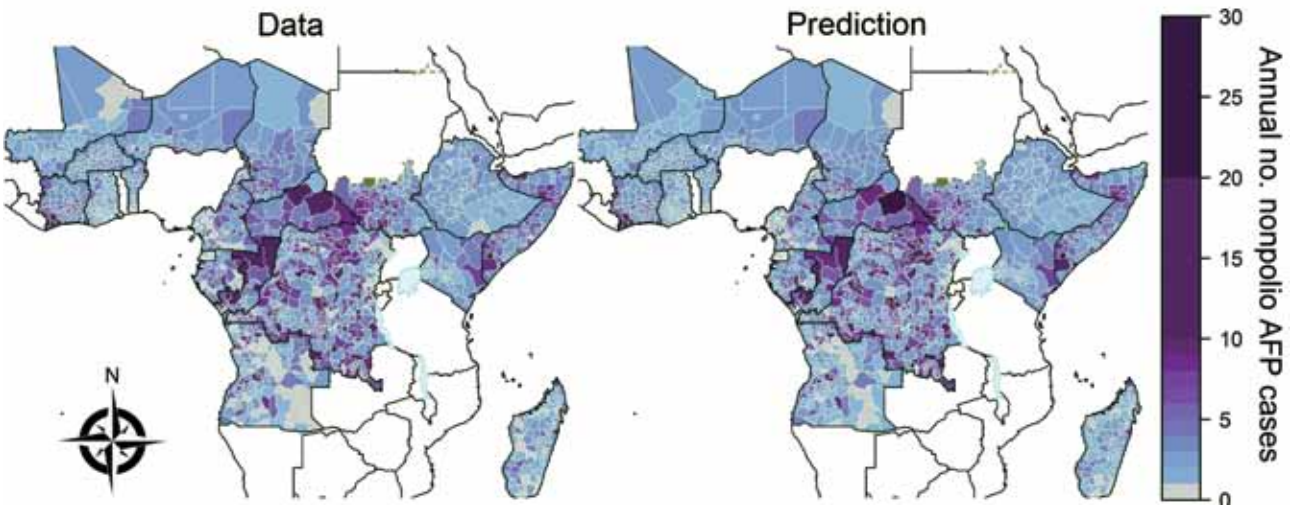

Figure 1. Nonpolio acute flaccid paralysis (AFP) cases in sub-Saharan Africa, 2003–2013. Left, mean annual number of cases reported at the second administrative unit (district) in countries in sub-Saharan Africa that have recently experienced a polio importation or outbreak or are considered to be at high risk for these events. Right, expected annual number of nonpolio acute flaccid paralysis cases reported at the district level; the number was obtained by fitting a spatiotemporal mixed-effects regression model to nonpolio AFP data from 2003–2013. Areas that report ≥25 annual cases are grouped into the 25–30 category (the maximum observed annual reported number was 128 in Tshopo, Democratic Republic of the Congo, in 2007). South Sudan gained independence in 2011, but reporting in this area before independence is shown for comparison. The publication of these maps does not imply the expression of any opinion whatsoever on the part of the World Health Organization (WHO) concerning the legal status of any territory, city, or area or of its authorities or concerning the delimitation of its frontiers or boundaries. WHO does not endorse or approve the use of subnational boundaries in this map. Disputed borders and areas are shown in green and lakes at borders are shown in pale blue.

Time from Paralysis Onset to Case Notification to Specimen Delivery for Laboratory Testing

The median delay between onset of paralysis and AFP case notification was <1 week in all countries across the time period analyzed, although the distribution was skewed such that 5.9% (range 2.0% [South Sudan] to 10.7% [Côte D'Ivoire]) of AFP cases were notified >3 weeks after onset of paralysis (Figure 2; Figure 7 at http://dx.doi.org/10.5281/zenodo.44361). The median delay between AFP case notification and stool sample delivery to a global polio laboratory was <1 week across 2010–2013 in Central African Republic, Kenya, Madagascar, Cote D'Ivoire, Ethiopia, and Benin, and 1–2 weeks for the remaining countries (Figure 2; Figure 8 at http://dx.doi.org/10.5281/zenodo.44361). However the distributions were also skewed such that 3.9% of stool samples were dispatched to the laboratory >3 weeks after notification.

Spatiotemporal Model of AFP Reporting

Spatiotemporal mixed-effects modeling enabled characterization of the temporal trend and variability at the district level for each country through estimating the precision of the spatial and temporal random effects (Table 1 at http://dx.doi.org/10.5281/zenodo.44361). In all countries, there was evidence for at least 1 type of spatial random effect, indicating that the estimated district population sizes alone were not sufficient to explain differences in reprting rates. Evidence indicated overdispersion in the nonpolio reporting rate in 9 countries where a negative binomial model of nonpolio AFP case reporting provided a lower deviance information criterion value than that provided by a Poisson model. The country-specific model fits over time corresponded with the country data (Figure 6 at http://dx.doi.org/10.5281/zenodo.44361). It was possible to obtain the expected number of nonpolio AFP cases independent of time from these fitted models (Figure 1; http://dx.doi.org/10.5281/zenodo.44361).

Distribution of Poliomyelitis Cases and Nonpolio AFP Cases in Space and Time

Overall, compared with nonpolio AFP cases, poliomyelitis cases during the beginning of a large outbreak occurred closer together in space and time (Figure 9 at http://dx.doi.org/10.5281/zenodo.44361). In all large outbreaks during 2003–2013, most cases occurred within 100 km and 0–6 days of each other.

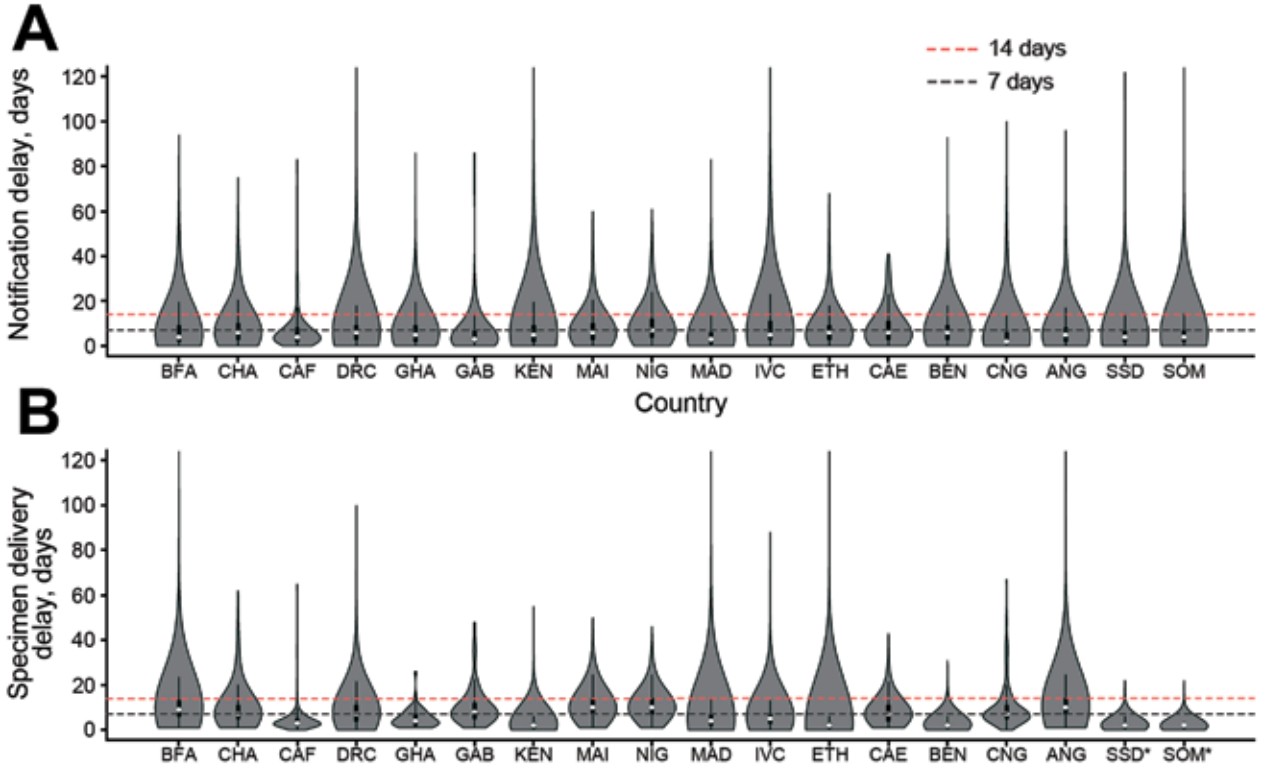

Figure 2. Distribution (violin plots) of time delays in notification of acute flaccid paralysis (AFP) cases and in sending samples for laboratory testing, by country, Africa, 2010–2013. A) Delay between onset of acute flaccid paralysis and notification of cases. B) Delay between notification of acute flaccid paralysis cases and the date collected stool samples were sent to a global polio laboratory. Asterisks (*) indicate that the date stool samples were sent to the laboratory was not available; in these instances, the date of the second stool collection was used instead. In the violin plots, white dots correspond to the median value, the rectangle indicates the interquartile range, and the vertical line corresponds to the range between upper and lower adjacent values. ANG, Angola; BEN, Benin; BFA, Burkina Faso; CAE, Cameroon; CAF, Central African Republic; CHA, Chad; CNG, Republic of the Congo; DRC, Democratic Republic of the Congo; ETH, Ethiopia; GAB, Gabon; GHA, Ghana; IVC, Côte D'Ivoire; KEN, Kenya; MAD, Madagascar; NIG, Niger; SOM, Somalia; SSD, South Sudan.

Testing of Real-Time AFP Databases to Detect Polio Outbreaks

Using the Poisson space–time scan statistic to test for the presence of AFP clusters at weekly intervals resulted in prompt warnings of a polio outbreak in the 4 recent large outbreaks (Figure 3). In Tajikistan, the detection of significant clustering would have occurred on March 15, 2010, which is 39 days before official confirmation of isolation of wild

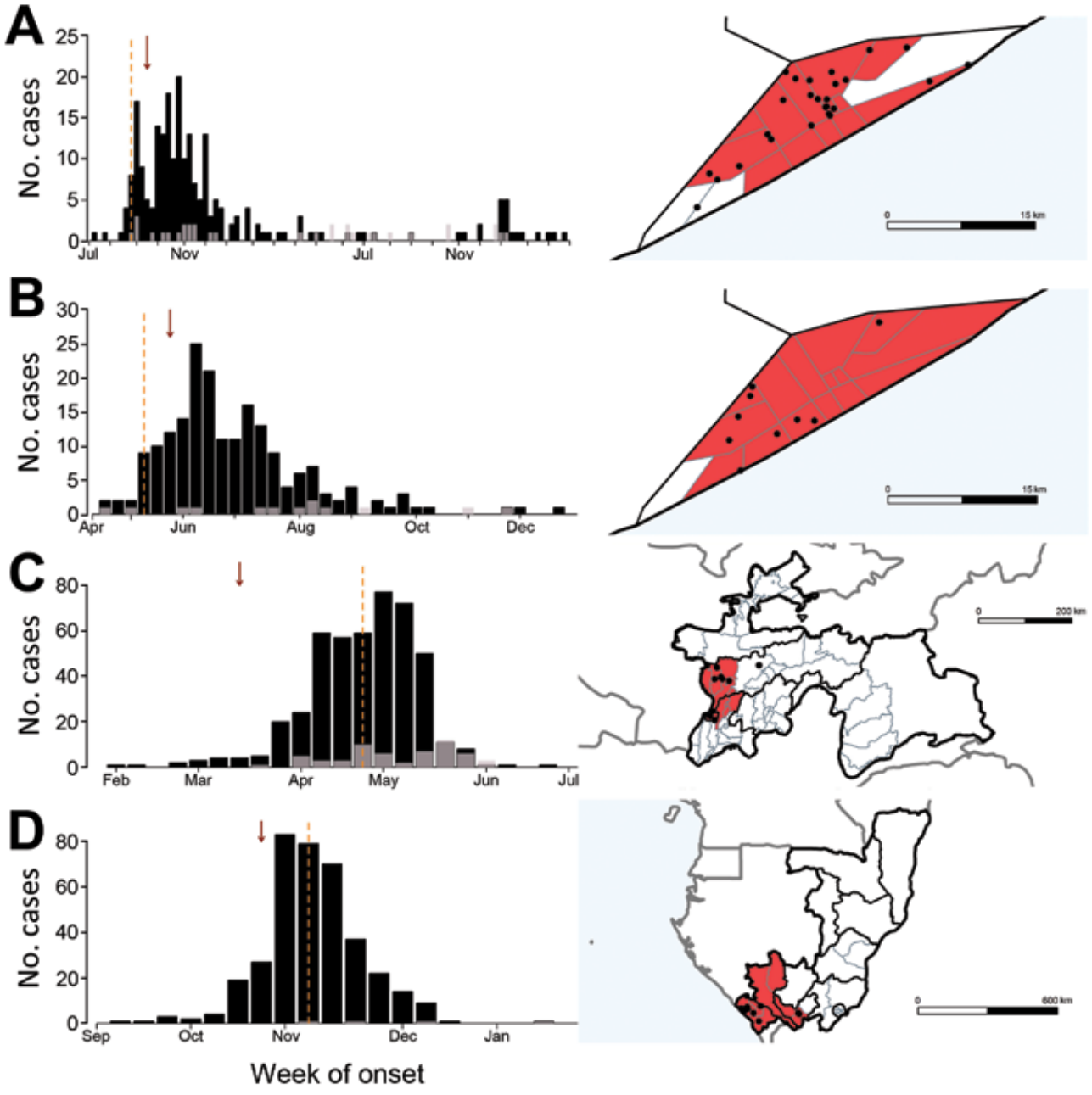

Figure 3. Incidence of serotype 1 poliomyelitis cases and time and location of 4 large outbreaks in Somalia, Tajikistan, and Congo: A) Somalia, 2005–2007; B) Somalia, 2013; C) Tajikistan, 2010; D) Congo, 2010. The charts on the left indicate weekly incidence of confirmed polio (black) and polio-compatible (gray) cases (by onset date of acute flaccid paralysis, AFP); vertical dashed lines indicate the date the outbreak was officially confirmed and arrows the date that an alarm would have been raised for detection of AFP clustering. Maps on the right show the second administrative divisions (districts) containing the significant cluster of acute flaccid paralysis cases; divisions are colored red if the alarm cylinder included its centroid. Each dot corresponds to a confirmed poliomyelitis case, plotted randomly within the corresponding district of occurrence. Maps in panels A and B show the administrative divisions of Banadir in Somalia. Gray lines in maps in panels C and D indicate neighboring countries. Blue shading indicates the sea. The publication of these maps does not imply the expression of any opinion whatsoever on the part of World Health Organization (WHO) concerning the legal status of any territory, city, or area or of its authorities or concerning the delimitation of its frontiers or boundaries. WHO does not endorse or approve the use of subnational boundaries in this map.

poliovirus on April 23, 2010. Clustering of AFP cases would also have been detected 11 days before official confirmation of the large outbreak in Congo (October 25, 2010, and November 4, 2010, respectively). However, in Somalia, 2 large outbreaks were detected by standard surveillance methods on August 23, 2005, and May 9, 2013, respectively, whereas the clustering algorithm would not have detected significant clusters of AFP cases until September 19, 2005, and May 20, 2013, respectively. The radius of each detected outbreak was 55.4 km (Tajikistan), 239.5 km (Congo), and 10.9 km and 21.1 km (Somalia); significance levels of the alarms were all $p<0.001$. In other settings, the algorithm detected some smaller outbreaks of polio (Table), although the time to detection was slow (Figure 10 at http://dx.doi.org/10.5281/zenodo.44361), and other outbreaks were not detected. In all countries, with the exception of the Democratic Republic of the Congo (DRC), relatively few false alarms were raised during outbreak-free periods (Table; Figure 10 and Table 4 at http://dx.doi.org/10.5281/zenodo.44361).

Overall, the space–time permutation scan statistic performed less well than the Poisson space–time scan statistic. The space–time permutation scan statistic would have resulted in a later detection of the 2010 Tajikistan and Congo outbreaks, and it detected fewer outbreaks in other countries (Tables 5 and 6 at http://dx.doi.org/10.5281/zenodo.44361).

Discussion

Maintaining high-quality surveillance for polio outbreaks is essential to achieve global eradication of poliomyelitis. The longer the delay between the start of a polio outbreak and its detection (and subsequent response), the higher the chance of wide-scale spread and reestablished transmission. The large outbreak in Tajikistan in 2010 was detected relatively late (*10*), and during 2009–2010, outbreaks in Angola, Chad, DRC, and Sudan have led to reestablished transmission (*5*).

The duration of time between the onset of symptoms in the first reported polio case and confirmation of an outbreak can be prolonged due to delays in sending stool samples for laboratory testing and the time taken to perform the test. In addition, many countries do not consistently perform adequate stool collection to test for the presence for poliovirus (*22*). Our findings show that, compared with nonpolio AFP cases, poliomyelitis cases cluster in time and space, and that, in some instances, detection of spatiotemporal clustering of all-cause AFP cases can provide an early warning of outbreaks. Such a method has been shown to be an effective early-warning system for outbreaks of other infectious diseases (*23–25*). The method could be run on a weekly basis, as new AFP cases enter the database, and detection of a significant cluster would warrant fast-track laboratory processing of the stool samples from the associated AFP patients and alert countries to prepare for a possible outbreak.

By creating a real-time database, in which AFP cases were assumed to enter the database on the date of notification (best-case scenario of reporting), and running the spatiotemporal scan statistic at weekly intervals, an early warning of the large 2010 Tajikistan outbreak could have been raised 39 days before the date that the outbreak was officially confirmed. If outbreak response immunization campaigns had commenced 2–4 weeks earlier, substantially more poliomyelitis cases would have been prevented (*10*). In addition, an early-warning alarm of the 2010 Congo outbreak could have been raised 11 days before official confirmation. Therefore, incorporation of this early-warning system into the polio information system would benefit the Global Polio Eradication Initiative (GPEI). Although we found that the scan statistic would not have raised an early warning regarding the large 2005 and 2013 outbreaks in Somalia, the dates of the alarm were not long after the dates of official outbreak confirmation.

The algorithm performed less well at detecting much smaller outbreaks that have occurred during the past decade in countries of sub-Saharan Africa. During these outbreaks, the initial growth rate was relatively low compared with that in the outbreaks in Tajikistan, Congo, and Somalia, meaning there was little temporal clustering of polio cases. However, even if the sensitivity of early outbreak detection is not high for small outbreaks, the large outbreaks for which it does provide an early warning and hence a faster response will be of public health benefit, enabling more

Table. Performance of cluster detection of acute flaccid paralysis cases as an early-warning system for detection of polio outbreaks, 2003–2013*

Country	No. confirmed polio outbreaks	% Identified outbreaks	Specificity, %
Somalia	5	60	97
Tajikistan	1	100	99
Congo	1	100	96
Chad	8	62	89
CAR	4	50	91
DRC	5	80	63
Gabon	0	NA	100
Kenya	4	75	91
Mali	4	50	95
Niger	6	67	87
South Sudan	3	67	96
Madagascar	0	NA	92
Côte D'Ivoire	3	100	81
Ethiopia	5	40	93
Equatorial Guinea	0	NA	100
Cameroon	5	20	93
Benin	2	100	94
Angola	4	100	96
Ghana	1	100	92
Burkina Faso	2	0	88

*Outbreak is defined as ≥2 reported cases of wild poliovirus type 1 or 3 or circulating vaccine-derived poliovirus poliomyelitis <6 mo apart in the given country. CAR, Central African Republic; DRC, Democratic Republic of the Congo; NA, not applicable.

rapid outbreak control and a reduction in the number of poliomyelitis cases. The algorithm can be automated and, after future work to test the algorithm in other settings, would complement the current surveillance system.

Part of the polio endgame strategy is the globally synchronized removal of serotype 2 OPV from routine immunization in April 2016 (*4*). After this transition, there is a risk that cVDPV2 outbreaks will arise as population immunity against serotype 2 declines. Therefore, surveillance for cVDPV2 outbreaks will be critical during the transition period. Our results show that the algorithm we used would have generated alarms during cVDPV outbreaks in DRC, Cameroon, Kenya, and Somalia (Figure 10 at http://dx.doi.org/10.5281/zenodo.44361) and, thus, could be of help during the vaccine transition period.

A critical feature of an early-warning system is the false-alarm rate. A system with infrequent false alarms could benefit countries by providing a means to check the level of outbreak preparedness. However, a system that results in frequent false alarms is likely to be ignored when a true alarm is raised. In general, the false-alarm rate in our study was relatively low; an exception occurred in DRC, where false alarms would have been raised in late 2012–2013.

To obtain the expected proportion of AFP cases reported at the district level in the absence of a polio outbreak over a given time period, we fitted a spatiotemporal regression model to the incidence of reported nonpolio AFP during 2003–2013. We found that the number of nonpolio AFP cases reported per district was not simply a function of population size and that reporting is heterogeneous within countries. Subnational heterogeneous AFP reporting has been demonstrated at the first administrative level (province) in many settings (*6*,*26*,*27*), but fewer studies have investigated differences at the district level. Possible errors in population estimates, which could arise, in part, due to infrequent censuses, are a potential explanation for some of the heterogeneous reporting. Heterogeneities in nonpolio AFP reporting may also occur at the subnational level, reflecting differential access of populations to healthcare facilities (*28*), differences in security across the country (*26*), and local transmission of other infectious causes of AFP, such as nonpolio enteroviruses (*29*).

The number of reported nonpolio AFP cases has increased over time. We did not account for population growth in the spatiotemporal regression model of nonpolio AFP reporting, but population growth, along with improved surveillance, is likely to be a contributing factor toward the observed increase in reporting in most countries. When testing for AFP clustering in the real-time AFP database, we based the expected incidence of AFP in the absence of an outbreak only upon reported AFP cases in the preceding 2 years from the current week of surveillance. Therefore, the general time trend toward increased reporting of AFP cases would not give lead to false identification of clusters unless there was a large shift in reporting practices during those years. We assume that geographic differences in the incidence of reported AFP cases do not change over time. The low false-alarm rate in the majority of countries suggests that this is a reasonable assumption. However, the spatial random effects in our model could also be updated annually to account for such changes.

During the years of our study, no record was kept of when AFP cases were recorded in a central database. Thus, a limitation of our work is that we assumed that AFP cases were reported to the polio information system on the date the case was notified by local healthcare providers. In practice there may be further delays in collation of local information into the global polio information system AFP database. We compared the date of notification of AFP cases by local healthcare providers with the date of entry into this database by downloading this database every week during 2015 and found a median delay of 25 days (interquartile range 21–32 days) (Figure 11 at http://dx.doi.org/10.5281/zenodo.44361). If this delay were to persist it would postpone the date of an early-warning alarm by the duration of the delay; thus, there is a strong case for faster collation of data into national and global databases.

Polio outbreaks that are detected late will threaten the progress of the GPEI, and consequently there is a need to strengthen ongoing surveillance. Although future work is required to test our algorithm in other settings, we have shown that integrating an automated early-warning system based on detection of AFP clusters into the polio information system could be of value to the GPEI, helping to identify large outbreaks earlier and stop transmission faster.

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Dr. Blake is a research fellow in the Department of Infectious Disease Epidemiology, Imperial College London. Her research is focused on using mathematical and statistical methods to understand and quantify the spread of infectious diseases.

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Address for correspondence: Isobel M. Blake, MRC Centre for Outbreak analysis and Modelling, Department of Infectious Disease Epidemiology, Imperial College London, Norfolk Pl, London W2 1PG, UK; email: isobel.blake@imperial.ac.uk

Identification of Novel Zoonotic Activity of *Bartonella* spp., France

Muriel Vayssier-Taussat, Sara Moutailler, Françoise Féménia, Philippe Raymond, Olivier Croce, Bernard La Scola, Pierre-Edouard Fournier, Didier Raoult

Certain *Bartonella* species are known to cause afebrile bacteremia in humans and other mammals, including *B. quintana*, the agent of trench fever, and *B. henselae*, the agent of cat scratch disease. Reports have indicated that animal-associated *Bartonella* species may cause paucisymptomatic bacteremia and endocarditis in humans. We identified potentially zoonotic strains from 6 *Bartonella* species in samples from patients who had chronic, subjective symptoms and who reported tick bites. Three strains were *B. henselae* and 3 were from other animal-associated *Bartonella* spp. (*B. doshiae, B. schoenbuchensis*, and *B. tribocorum*). Genomic analysis of the isolated strains revealed differences from previously sequenced *Bartonella* strains. Our investigation identifed 3 novel *Bartonella* spp. strains with human pathogenic potential and showed that *Bartonella* spp. may be the cause of undifferentiated chronic illness in humans who have been bitten by ticks.

Bartonella spp. cause varied and multifaceted human diseases, including cat scratch disease (*B. henselae*), Carrion's disease (*B. bacilliformis*), trench fever (*B. quintana*), endocarditis (*B. quintana* and *B. henselae*) (*1*,*2*), bacillary angiomatosis (*B. quintana* and *B. henselae*), and hepatic peliosis (*B. henselae*). *Bartonella* spp. can also cause prolonged intra-erythrocytic bacteremia in both humans and animals (*3*): in humans, *B. quintana*, *B. bacilliformis*, and *B. rochalimae* are known pathogens, and in animals, *B. henselae*, *B. clarridgeiae*, and *B. koehlerae* have been identified in felids; *B. grahamii, B. taylorii, B. doshiae, B. birtlesii*, and others in rodents; and *B. bovis, B. chomelii, B. schoenbuchensis,* in ruminants. In humans, chronic bacteremia caused by *B. quintana* causes few obvious symptoms apart from generalized fatigue and nonspecific leg pain (*1*,*4*).

It has been assumed that each *Bartonella* species infected 1 or a few closely related mammalian reservoir hosts, in which infection caused long-lasting bacteremia. Nonreservoir hosts were considered incidentally infected without bacteria being detected in blood. Recently, these assumptions has been contradicted by studies describing animal-associated *Bartonella* spp. indirectly associated with bacteremia and a spectrum of diverse symptoms in immune-competent persons who had contact with animals, arthropods, or both, which are natural routes of *Bartonella* transmission (*5*–*7*). In some cases, the source of infection remains unknown; ticks have been suggested as a possible source of animal-associated *Bartonella* infection in humans (*6*,*8*–*10*).

Related to a patient's history of tick bites, it is common for physicians to suspect Lyme disease, some rickettsial diseases, or tickborne encephalitis. However, in many cases, the diagnosis is not confirmed by serologic or DNA-based tests. In recent years, alternate interpretations of Lyme disease serology have flourished, leading to considerable discord between formal institutions for infectious disease and patient advocacy associations. Thus, unexplained symptoms after tick bites have become an issue of increasing importance for patients and their physicians (*11*,*12*).

In this context, we screened for the presence of *Bartonella* in the blood of patients reporting tick bites and with unexplained and aspecific symptoms. Here we report the isolation and genomic sequencing of 6 *Bartonella* strains obtained by blood culture from 66 patients. Three strains were identified as *B. henselae*, and 3 other strains were identified as different animal–associated species (*B. doshiae, B. tribocorum*, and *B. schoenbuchensis*).

Methods

Patients

During January–June 2013, we conducted a study of a cohort of 66 French patients who had consulted their doctors for chronic symptoms appearing after a tick bite. The entire study protocol was approved by the ethics committee of the Institut Federatif de Recherche 48 under reference 13–022–1.

All patients associated symptom onset with tick bites that occurred during 2008–2012 (Table 1). At symptom onset, local doctors were consulted, and serologic tests for Lyme borreliosis were performed. All patient samples

Author affiliations: Agence Nationale de Sécurité Sanitaire de l'Alimentation, de l'Environnement et du Travail, Maisons Alfort, France (M. Vayssier-Taussat, S. Moutailler, F. Féménia); Chronimed, Minnetonka, Minnesota, USA (P. Raymond); Aix-Marseille Université, Marseille, France (O. Croce, B. La Scola, P.-E. Fournier, D. Raoult)

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Table 1. Patients whose blood cultures were positive for *Bartonella* spp. that had no previously known zoonotic activity, France

Case-patient no./age, y/sex	*Bartonella* spp.	Tick bite date	Pets	Wild animal contact	Main complaints	Bacteremia, CFU/mL
1/49/F	*B. henselae*	Multiple since 2008	Cats, dogs, horses	Rats, fish	Fatigue, muscle pain, headache	50
2/58/M	*B. henselae*	2011	Birds, rabbits	No	Fatigue, muscle pain	70
3/47/F	*B. henselae*	2012	Dog, hamster	No	Fatigue, generalized pain, insomnia	80
4/45/F	*B. doshiae*	2009	No	No	Fatigue, blurred vision, arthralgia	50
5/64/M	*B. tribocorum*	2012	Dog	Game animals (hunter)	Fatigue, muscle pain, headache	60
6/40/F	*B. schoenbuchensis*	2011	No	No	Fatigue, muscle pain, fever	850

tested were seronegative for Lyme borreliosis bacteria; however, since that time, their symptoms had become chronic. The patients completed information forms giving informed consent for the use of their samples in the study. All of the patients lived in the countryside, where ticks were abundant and contact with wild animals was possible. The patients reported that they had not undergone antibacterial drug treatment for ≥3 months before the study.

We collected blood samples from each patient in EDTA-containing sample tubes. For a control population, we used anticoagulated blood samples from 70 anonymous healthy blood donors from Paris (France). All samples (control and patients) were tested simultaneously.

Bartonella Isolation from Blood

To specifically isolate *Bartonella* spp., 100 mL of blood samples from patients or healthy donors were directly plated onto sheep blood agar plates and incubated at 35°C in a humidified atmosphere with 5% CO_2 for 45 days. The plates were assessed daily from days 7–45 before the culture was deemed negative (i.e., absence of colony in the absence of contamination) (*1*). Colony-forming units (CFU) were counted and bacteremia (UFD/mL of blood) evaluated.

Genome Sequencing, Assembly, and Analysis

We extracted genomic DNA from each isolated strain by using the EZ1 automated extraction system (QIAGEN, Hilden, Germany), following the manufacturer's recommendations. Bacterial genomic DNA was sequenced by using the Nextera XT DNA sample prep kit (Illumina Inc., San Diego, CA, USA) and a 2×250 paired-end protocol with the MiSeq pyrosequencer (Illumina), according to the manufacturer's instructions. We aligned each genome by using Mira version 3.2 software in the mapping mode (*13*). The resulting contigs were combined by using Opera version 1.2 (*14*) and GapFiller (*15*) software. Finally, the genomic assemblies were improved with manual refinement by using the CLC Genomics version 4.7.2 software package (CLC Bio, Aarhus, Denmark). Noncoding genes and miscellaneous features were predicted by using RNAmmer (*16*) and ARAGORN (*17*). Coding DNA sequences were predicted by using Prodigal (*18*), and functional annotation was achieved by using BLAST+ (*19*) and HMMER3 (*20*) against the UniProtKB database (*21*). Coding DNA sequences were also annotated by using the Clusters of Orthologous Groups databases (*22*) with blastp (http://blast.ncbi.nlm.nih.gov/Blast.cgi) default parameters.

Single-nucleotide polymorphisms (SNPs) among genomes were identified by using SNIT software (*23*). SNPs were searched in regions exhibiting >95% nt sequence identity and the SNIT software was used with default parameters except for the Tandem Repeat Finder filter for avoiding ambiguous SNPs in repeat regions. We also performed in silico DNA–DNA hybridization (DDH) between *Bartonella* strains by using GGDC software (*24*).

Taxonomic Classification

To determine the taxonomic classification of the 6 isolates, we used previously proposed criteria (*25*) in which the *gltA* and *rpoB* gene sequences from each strain were compared to those of validated published *Bartonella* species. These criteria classify *Bartonella* isolates within a particular species if they share >96% and 95.4% nucleotide sequence similarity for the *gltA* and *rpoB* genes, respectively (*25*). In our study, *gltA* and *rpoB* sequences were retrieved from the genomes.

Results

Bartonella spp. Isolation

Bartonella spp. were isolated by prolonged culture from blood samples of 6 of the 66 patients who reported chronic symptoms following a tick bite. In contrast, samples from the 70 healthy blood donors remained negative after 45 days of incubation.

Bacteremia in the *Bartonella* infected patients increased from 50 to 850 CFU/mL. For 1 patient (case-patient 2), we had access to 2 blood samples that were taken at a 1-month interval. *B. henselae* was grown from the 2 samples, with similar bacteremia (50 and 60 CFU/mL, respectively), suggesting chronic bacteremia.

The case-patients who tested positive for *Bartonella* (Table 1) reported tick bites occurred 1–5 years before

blood samples were collected. All of them live in the countryside, in contrast to the healthy blood donors, who were all from Paris, France. The main complaint of the case-patients was chronic fatigue, but they also reported other subjective or nonspecific symptoms (or both), such as headaches and myalgia. A qualifying characteristic of the 70 healthy blood donors was absence of chronic fatigue. Even though potential exposure to ticks is difficult to evaluate, because the anonymous blood donors all lived in Paris, we assumed they were not likely to have frequent tick exposure or wild animal contact.

Taxonomic Classification

Of the 6 *Bartonella* isolates from this study, 3 (MVT01, MVT02, and MVT03) were classified within the *B. henselae* species on the basis of both their phylogenetic position and *gltA* and *rpoB* sequence similarities (Figure; Tables 1, 2). The isolates from samples from case-patient 2 at a 1-month interval shared 100% identity, based on *gltA* and *rpoB* gene comparison. Isolates MVT04, MVT05, and MVT07 were classified within the *B. tribocorum, B. doshiae,* and *B. schoenbuchensis* species, respectively.

The assembly data and main genomic characteristics of each isolated strain are summarized in Table 3; in silico DDH values and SNP numbers are described in Table 4 (http://wwwnc.cdc.gov/EID/article/22/3/15-0269-T4.htm). All studied strains displayed a similar genomic content when compared with reference genomes. The GGDC software we used proposes that DDH values >70% could classify isolates in the same species. Here, intraspecies values ranged from 80.3% to 100% (Table 4). Nevertheless, from 10 to 1,938 SNPs were identified among *B. henselae* isolates MVT01, MVT02, and MVT03 and from 693 to 2,093 SNPs when comparing these strains to *B. henselae* Houston-1 (Table 4), confirming that each strain was unique and did not result from cross-contamination or contamination from laboratory strains.

Of note, *B. tribocorum* isolate MVT04 and *B. schoenbuchensis* isolate MVT07 were the only 2 that exhibited plasmids. However, when compared with reference strain m07a, MVT04 and MVT07 carried a large plasmid and not the small plasmid homologous to the cryptic pBGR plasmid harbored by *B. grahamii* (*26*).

Discussion

In this study, animal-associated *Bartonella* isolates were individually cultured from the blood of patients who had been bitten by ticks and reported subjective symptoms, whereas no strains were isolated from healthy blood donors. This report describes the isolation of 3 different animal-associated *Bartonella* species from human samples, highlighting their potential novel zoonotic properties. Moreover, we found

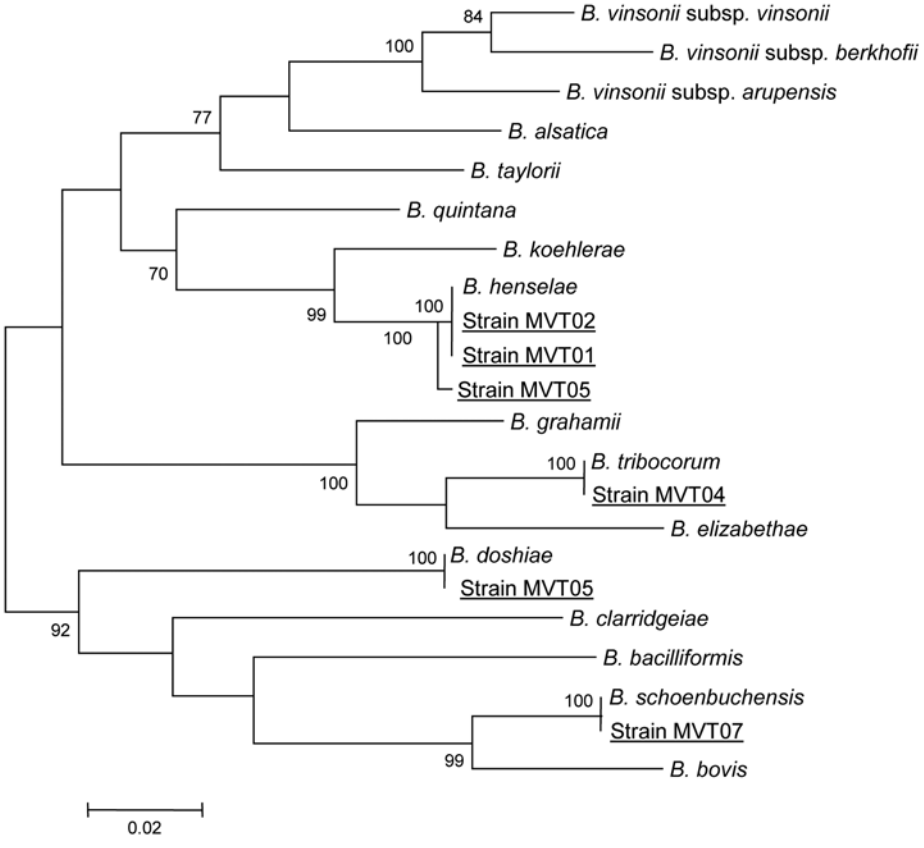

Figure. *rpoB* gene-based phylogenetic tree showing the relationships of 6 *Bartonella* isolates (underlined). Briefly, *rpoB* nucleotide sequences were aligned by using ClustalW software (http://www.clustal.org/clustal2/), and phylogenetic relationships were inferred by using the maximum-likelihood strategy and MEGA software (http://www.megasoftware.net). Bootstrap values above 70%, obtained from 500 analyses, are indicated at the nodes. Scale bar represents a 2% nucleotide sequence divergence.

Table 2. Nucleotide similarity of 6 *Bartonella* isolates from patients in France expressing novel zoonotic activity and their most phylogenetically similar published validated species*

Isolate	*gltA*, %	*rpoB*, %	Species
MVT01	100	100	*B. henselae*
MVT02	100	100	*B. henselae*
MVT03	99.7	99.6	*B. henselae*
MVT04	100	100	*B. tribocorum*
MVT05	98.7	100	*B. doshiae*
MVT07	100	99.9	*B. schoenbuchensis*

**gltA* and *rpoB* sequences were obtained from genomic sequences. Genome sequences of *B. henselae* strains MVT01, MVT02, and MVT03 were deposited in GenBank under accession numbers HG965802, NZ_LN879429, and HG969191, respectively; the genome sequence of *B. tribocorum* strain MVT04 was deposited in GenBank under accession numbers HG969192 and HG969193; the genome sequence of *B. doshiae* strain MVT05 was deposited in GenBank under accession numbers CCBL010000001–CCBL010000013; the genome sequence of *B. schoenbuchensis* strain MVT07 was deposited in GenBank under accession numbers HG977193–HG977197; the genome sequences of the reference strains *B. henselae* strain Houston-1, *B. tribocorum* strain CIP 105476, *B. doshiae* strain NCTC 12862, and *B. schoenbuchensis* strain m07a are available in genBank under accession numbers NC_005956, NC_010161, and NC_010160, NZ_JH725094 to NZ_JH725100 and NZ_KB915627-NZ_KB915629, NZ_CM001846-NZ_CM001845, respectively.

that zoonotic *Bartonella* spp. can be detected in the blood of afebrile patients, as has been shown for human-specific *B. quintana* and *B. bacilliformis* and as was recently reported for *Candidatus* Bartonella ancashi (*27*). Chronic bacteremia caused by infection by *Bartonella* spp. is well-described in many mammals, including humans (*4*,*28*). The *Bartonella*–mammalian host association is considered to be species-specific and attributable to co-evolution between host and pathogen (*28*). However, we show that animal-associated species can also chronically infect human blood, highlighting the possibility of host shift despite apparent host specificity (*28*,*29*).

This work is similar to that of E.B. Breitschwerdt et al. (*5*–*7*), who also recovered zoonotic *Bartonella* spp. from human samples using an in-house technique based on results of blood pre-enrichment followed by PCR detection of *Bartonella* spp.; members of the same team have investigated many cases of persons who had nonspecific symptoms, including arthralgia, muscle pain, fatigue, headaches, visual blurring, neurocognitive symptoms, and, in 2 case-patients, hemangioendothelioma (*30*). In total, *B. henselae* DNA was detected in 47 cases (*5*,*30*–*33*); *B. koehlerae* (another common agent of feline bacteremia) DNA in 96 cases, including 2 co-infected with *B. henselae* (*31*,*32*,*34*,*35*); and *B. vinsonii berkhoffii* (an agent of canine bacteremia and endocarditis) DNA in 24 cases (*31*–*34*), including 16 case-patients with *B. henselae* and 2 cases of *B. melophagi* (*36*). These results have been questioned because minute levels of contamination can result in false positives by PCR. Therefore, we deliberately avoided PCR to overcome this problem, and the resulting strain isolation was consequently straightforward and indisputable. These isolates (Table 2) have been archived in our collection (Collection de Souches de l'Unité des Rickettsies, World Data Center for Microorganisms no. 875, http://www.mediterranee-infection.com/article.php?laref=14&titre=collection-de-souches) and are available upon request under references B546, B547, B548, B549, B550, and B551 for isolates MVT01, MVT02, MVT03, MVT04, MVT05, and MVT07, respectively.

Our findings also confirm studies identifying zoonotic *Bartonella* in the blood of patients with nonspecific complaints. Among them, *B. henselae* is well known worldwide

Table 3. Assembly information and main characteristics of 6 sequenced *Bartonella* genomes from patients in France expressing novel zoonotic activity

	Species and isolate identification					
Genome characteristics	*B. henselae* MVT01	*B. henselae* MVT02	*B. henselae* MVT03	*B. tribocorum* MVT04	*B. doshiae* MVT05	*B. schoenbuchensis* MVT07
GenBank accession nos.	HG965802	NZ_LN879429	HG969191	HG969192–HG969193	CCBL010000001–CCBL010000013	HG977193–HG977197
Size, bp	1,902,535	1,905,383	1,975,503	2,609,404	1,919,109	1,734,324
No. contigs	1	1	1	2	13	5
Average read coverage	87	94	110	46	15	41
Average read length, trimmed	183	190	192	194	168	193
Total no. reads, trimmed	946,882	1,034,894	1,263,492	738,522	261,0852	666,371
Total no. predicted genes	1,659	1,658	1,726	2,335	1,720	1,574
Protein-coding genes	1,603	1,602	1,668	2,279	1,654	1,519
rRNA operons	2	2	2	2	2	2
tRNAs	43	43	45	43	53	41
Other RNAs	7	7	7	7	9	8
GC% content	38.18	38.18	38.09	38.84	37.82	35.58
Plasmid	0	0	0	1	0	1
Genome used as a reference for assembly (accession nos.)	*B. henselae* Houston-1 (NC_005956)	*B. henselae* Houston-1 (NC_005956)	*B. henselae* Houston-1 (NC_005956)	*B. tribocorum* CIP 105476 (NC_010161, NC_010160)	*B. doshiae* NCTC 12862 (NZ_JH725094–NZ_JH725100)	*B. schoenbuchensis* m07a (NZ_KB915627–NZ_KB915629, NZ_CM001846, NZ_CM001845)

as a zoonotic agent infecting both cats and their fleas and has also been found in ticks (*10*). *B. henselae* has been detected in the blood of a patient without apparent symptoms 4 months after recovering from cat scratch disease. For this particular case, the sequence of manifestation of cat scratch disease, then bacteremia, followed by endocarditis was proposed because it has been known to occur for *B. quintana* bacteremia. One of the case-patients in this study owns a cat and may have been infected by this pet.

The 3 other animal-associated species we detected should now be considered zoonotic *Bartonella* spp. *B. doshiae* and *B. tribocorum* are both rodent-associated species; in France and worldwide, these species have mainly been recovered from rats (*Microtus agentiis* for *B. doshiae* and *Rattus rattus* for *B. tribocorum*). *B. schoenbuchensis* is normally found in deer, elk, and cattle (*37*,*38*).

The zoonotic agents we isolated from patients from France have also been detected in animals in France. Similarly, in the United States and Thailand, *Bartonella* species known to be prevalent in animals have also been identified in humans: (*B. henselae, B. vinsonii berkhoffii,* and *B. koehlerae* in the United States (*33*,*35*) and *B. tribocorum* and *B. rattimassiliensis* in Thailand (*39*). Therefore, the zoonotic *Bartonella* species discovered in humans in this study generally appear to be related to the prevalence among animals.

The significance of these *Bartonella* spp. in the genesis of the clinical picture is difficult to determine. *Bartonella* spp. are present in ticks, and we have previously reported *Bartonella* infections following tick bites, such as SENLAT (scalp eschar and neck lymphadenopathy after tick bite [*40*]). However, the causal link between the conditions observed here, *Bartonella* and tick bite, cannot yet be concretely established, especially for persons with tick bites occurring up to 5 years previously, which introduces innumerable potential confounding exposures within the same period, including bites by other arthropods. For instance, 1 of the 3 patients with *B. henselae* bacteremia reported contact with cats; this contact was a more plausible source of infection than tick bites. Furthermore, it is crucial to determine whether *Bartonella* played a notable role in the observed pathologies, because treatment for chronic *Bartonella* bacteremia (as for *B. quintana*) is particularly arduous and may require 6 weeks of doxycycline treatment together with 3 weeks of gentamicin, as these are the only antimicrobial drugs known to be effective in eradication of *Bartonella* (*1*). Many *Bartonella* spp. can also cause endocarditis, including *B. quintana* and *B. henselae*; therefore, reports of rare cases of endocarditis attributed to zoonotic *Bartonella* such as *B. kohlerae*, *B. alsatica*, *Candidatus* B. mayotimonensis*, B. vinsonii*, or *B. elizabethae* may actually be the final manifestation of asymptomatic bacteremia, similar to that reported by our infected patients (*28*).

In summary, our major finding is the isolation of zoonotic *Bartonella* other than *B. quintana* in the blood of patients with poorly qualified syndromes. These results indicate that zoonotic *Bartonella* spp. infection may cause undifferentiated chronic illness in humans.

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Dr. Vayssier-Taussat is a senior scientist at the French National Institute of Agronomical Research, where she leads a research team involved in the study of Bartonella and other vectorborne pathogens.

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Address for correspondence: Didier Raoult, URMITE, UM63, CNRS 7278, IRD 198, INSERM 1095, Aix-Marseille Université, Marseille, France; email: didier.raoult@gmail.com

Improved Detection of Tuberculosis and Multidrug-Resistant Tuberculosis among Tibetan Refugees, India

Kerry L. Dierberg, Kunchok Dorjee, Fulvio Salvo, Wendy A. Cronin, J'Belle Boddy, Daniela Cirillo, Tsetan Sadutshang, Richard E. Chaisson

The incidence of tuberculosis (TB) among Tibetan refugees in India is 431 cases/100,000 persons, compared with 181 cases/100,000 persons overall in India in 2010. More than half of TB cases in these refugees occur among students, monks, and nuns in congregate settings. We sought to increase TB case detection rates for this population through active case finding and rapid molecular diagnostics. We screened 27,714 persons for symptoms of TB and tested 3,830 symptomatic persons by using an algorithm incorporating chest radiography, sputum smear microscopy, culture, and a rapid diagnostic test; 96 (2.5%) cases of TB were detected (prevalence 346 cases/100,000 persons). Of these cases, 5% were multidrug-resistant TB. Use of the rapid diagnostic test and active case finding enabled rapid detection of undiagnosed TB cases in congregate living settings, which would not have otherwise been identified. The burden of TB in the Tibetan exile population in India is extremely high and requires urgent attention.

Tuberculosis (TB) continues to be one of the leading causes of death worldwide, and the largest prevalence of this disease in Asia (59%) and Africa (26%) (*1*). In 2014, a total of 23% of all incident TB cases were in India and 10% were in China. Approximately 4% of all new TB cases and 20% of previously treated cases are multidrug-resistant TB (MDR TB, characterized by resistance to isoniazid and rifampin), and >50% of these cases are in India, China, and Russia (*1*).

Refugee populations are known to be at increased risk for TB. This finding is believed to be caused, in part, by increased risks for malnutrition and overcrowding, which lead to increased susceptibility to and transmission of TB (*1–3*). Limited data for the incidence of TB among Tibetan refugees has demonstrated that it is among the highest in the world. In the mid-1990s, the incidence of TB in Tibetans living in India was estimated to be 835–1,700 cases/100,000 persons (*2*,*4*). Studies performed among Tibetan refugees in Minnesota, USA, and Toronto, Ontario, Canada, showed positive rates of 98% and 97%, respectively, for tuberculin skin tests, which indicated high rates of latent TB infection (*5*,*6*). In 2009, the incidence of TB in Tibetan refugees in New York, NY, USA was 561 cases/100,000 persons (New York City Department of Health and Mental Hygiene, unpub. data), which was 10-fold higher than the incidence for natives of India and China (*7*).

In 2010, the reported incidence rate of TB among Tibetans living in India was 431 cases/100,000 persons (Central Tibetan Administration Department of Health TB program [CTA DOH], unpub. data). More than half of cases were in students, monks, and nuns who live in congregate settings, where the potential exists for high rates of TB and MDR TB transmission. An estimated 90% of all new cases were in persons <35 years of age and ≈10% of these persons had MDR TB. The prevalence of HIV among TB patients was <1%. In 2011, the rate was similarly high (412 cases/100,000 persons; CTA DOH, unpub. data), much higher than the overall TB incidence of 181 cases/100,000 persons in India (*1*).

TB REACH is an initiative of the Stop TB Partnership, supported by the Canadian International Development Agency, that seeks to find innovative approaches for improving TB case detection in populations at high risk for this disease and with limited access to TB services (*8*). Historically, TB is diagnosed by passive case detection, which relies on symptomatic persons to seek care and the healthcare system to detect the disease. In settings where the incidence of TB is high and health services are weak, these limitations results in prolonged illness in patients and ongoing transmission of undetected TB.

Active case finding (ACF) for TB in such settings can identify persons with disease earlier than would occur

Author affiliations: Johns Hopkins University School of Medicine, Baltimore, Maryland, USA (K.L. Dierberg, J.'B. Boddy, R.E. Chaisson); Tibetan Delek Hospital, Dharamsala, India (K. Dorjee, T. Sadutshang); Central Tibetan Administration Department of Health, Dharamsala (K. Dorjee, T. Sadutshang); San Raffaele Scientific Institute, Milan, Italy (F. Salvo, D. Cirillo); Maryland Department of Health and Mental Hygiene, Baltimore (W.A. Cronin)

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under routine services and reduce illness and transmission (*9*). Given previously documented high TB incidence rates in the Tibetan refugee population and increased risk for TB in persons living in congregate living settings, we sought to improve case finding in Tibetans in India. We received support from TB REACH. Our main objective was to increase TB and MDR TB case detection rates through ACF in Tibetan congregate living centers in India, where the risk for TB is high.

Methods

Study Sites and Population

The study was conducted from September 2011 through March 2013 and approved by the Institutional Review Board of Johns Hopkins Medicine (Baltimore, MD, USA) and the Ethics Committee at Tibetan Delek Hospital (Dharamsala, India). We performed ACF for TB in Tibetan residential schools, monasteries, and nunneries in Himachal Pradesh, Karnataka, and Uttarakhand states in India, and in the Tibetan Reception Center in Dharamsala, a dormitory-style facility for newly arrived refugees from Tibet. In Karnataka, ACF was conducted among residents of congregate facilities and Tibetan households in Tibetan settlements in Bylakuppe and Mundgod. On the basis of a census conducted in 2009, the total population of Tibetans living in India is estimated to be 94,203 (*10*). The target population for this study, those living in congregate facilities, is estimated to be 53,150. However, more detailed population numbers for each congregate living setting were not available.

Patient Screening and Enrollment

Before each ACF outreach activity, a member of the study team contacted the responsible administrator at every school, monastery, and nunnery to arrange dates and times for TB screening. Verbal consent was obtained from every participant before screening and enrollment. All children ≥12 years of age were screened at schools; children <12 years of age were screened if they had symptoms. The head nurse or teacher provided consent for screening. Each person was screened for symptoms of TB by using World Health organization (WHO) screening criteria (cough >2 weeks, fever, night sweats, or weight loss of any duration) through verbal or written questionnaires (*11*).

At the schools, a questionnaire with WHO symptom criteria was distributed to every student and staff member. At the smaller (<1,000 persons) monasteries and nunneries, each person was asked by study staff if they had 1 of these symptoms. Study staff interviewed any person who reported having >1 symptom. All persons previously given a diagnosis of TB and currently receiving treatment were excluded. At monasteries and nunneries that had >1,000 persons, an educational lecture was conducted for all residents. After being given information for signs and symptoms of TB, residents were asked to complete a questionnaire and notify project staff if they had ≥1 symptoms of TB. These persons were then interviewed.

The estimated total population screened was determined by using the most recent enrollment numbers at the Tibetan schools, monasteries, and nunneries. All newly arrived refugees at the reception center were screened and tested through interview by study staff.

All persons who were close contacts with someone known to have TB or MDR TB or who had been previously given treatment for TB were screened for symptoms of TB and received diagnostic testing (chest radiograph only versus chest radiograph and Xpert MTB/RIF test [Cepheid, Sunnyvale, CA, USA]). Contact information was obtained from school medical records, individual medical books (records), and patient interviews. A close contact was considered a person who lived in the same home, slept or studied in the same room, or spent time indoors with a person with TB.

Data were collected by use of a structured questionnaire for all persons with a history of TB, contact with a person with TB, MDR TB, or with symptoms of TB. Data collected included demographic information (age, sex, place of birth, date of arrival from Tibet), history of TB contact, smoking status, and history of TB. Persons with a history of TB were asked about the date of diagnosis, disease site, treatment duration, and treatment completion. TB contact history was further defined as recent (within the previous 6 months) or remote (>6 months) contact. Validation of data on previous TB was performed by personal medical records (individual medical information) carried by each person or TB treatment cards whenever possible. Symptoms were again reviewed and diagnostic testing was ordered as needed. When other members of the Tibetan community came to a site where ACF was being conducted, they were interviewed by using the same protocol described and enrolled in the database.

Diagnostic Testing

Diagnostic testing included chest radiography or sputum testing with routine acid-fast bacilli (AFB) microscopy or Xpert MTB/RIF on the basis of a diagnostic algorithm developed for this study (Figure). The Xpert MTB/RIF is a rapid molecular diagnostic assay that can detect *Mycobacterium tuberculosis* in sputum and other specimens in <2 hours, and that identifies mutations in the *rpoB* gene associated with rifampin resistance. This cartridge-based system has high sensitivity (70%–90%) for *M. tuberculosis* and detects rifampin resistance with an accuracy >95% (*12*,*13*). As part of this study, an Xpert MTB/RIF apparatus was installed in the main health centers in the Bylakuppe

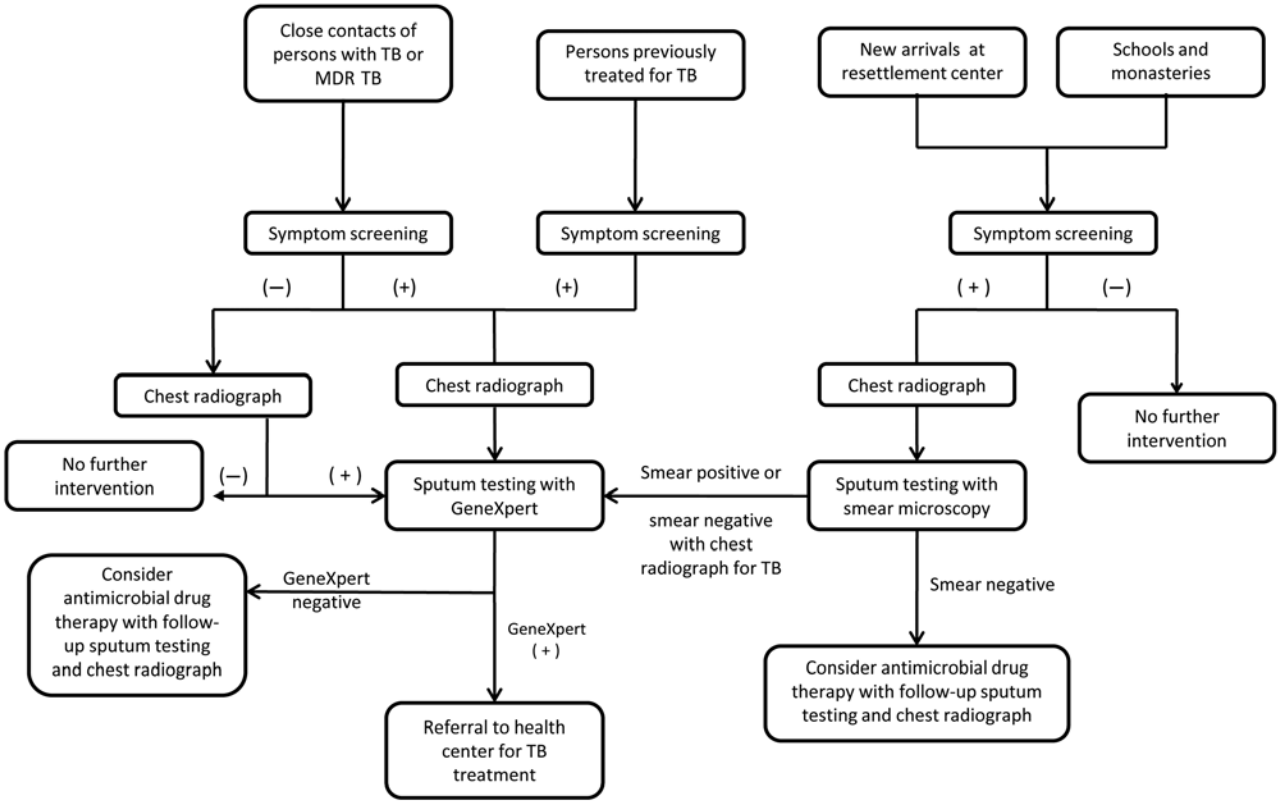

Figure. Diagnostic algorithm for improved detection of tuberculosis (TB) and multidrug-resistant TB (MDR TB) among Tibetan refugees, India. GeneXpert, Xpert MTB/RIF test (Cepheid, Sunnyvale, CA, USA). MTB, *Mycobacterium tuberculosis*; RIF, rifampin; –, negative; +, positive.

and Mundgod Tibetan settlements. One Xpert MTB/RIF apparatus was already in place at Tibetan Delek Hospital.

For all persons who were close contacts of a person with TB or MDR TB or had been previously given treatment for TB, the following diagnostic sequence was performed. First, screening was performed by using WHO symptom criteria and a written questionnaire. Second, persons who were symptomatic (≥1 symptoms) received a chest radiograph and sputum testing (if able to produce sputum) by Xpert MIB/RIF. On the basis of these test results, the following actions were taken. If a person had a positive result for Xpert MTB/RIF, the person was referred to the nearest treatment center for TB treatment. If a person had a chest radiograph suggestive of TB and a negative or unavailable result for Xpert MTB/RIF, the person was referred to a physician for further evaluation. If a person had a negative results for Xpert MTB/RIF and chest radiograph, the person was referred to a physician for appropriate treatment or further diagnostic testing. Finally, all persons who were asymptomatic contacts of a TB patient received a chest radiograph. If active disease was suspected, further diagnostic testing was performed as described.

For persons who were not given previous TB treatment and who had no contact with a person known to have TB, the same symptom criteria were used; symptomatic persons received a chest radiograph and sputum testing with AFB smear microscopy, if they were able to produce sputum. All chest radiographs and sputum tests were performed at the nearest Tibetan health center. If the chest radiograph suggested TB but the AFB microscopy result was negative, testing by using Xpert MTB/RIF was then performed.

Persons who met symptom criteria or had chest radiograph findings suggestive for TB but who had negative results for AFB smear and Xpert MTB/RIF were given antimicrobial drugs for treatment of pneumonia. Patients with persistent symptoms were then retested by using chest radiography and sputum testing with Xpert MTB/RIF. If sputum results were negative but chest radiograph and symptoms still suggested TB, patients were identified as having TB and given anti-TB therapy.

Regardless of the diagnostic methods used, all persons given a diagnosis of TB were recorded in the study database. These patients were given their test results and referred to the nearest TB treatment center to initiate anti-TB treatment. The study database was cross-referenced against the records of the health facility to which the patient was referred. If referred patients were not found in the health facility records, then the patient was contacted to investigate

the reasons why this omission occurred (e.g., failure of the patient to seek follow-up treatment, accessed a different health facility) and treatment was ensured for all patients given a new diagnosis.

Data Collection and Statistical Analysis

Data for study forms and laboratory reports were entered into a Microsoft (Redmond, WA, USA) Excel database by project staff. Data analysis was performed by using descriptive summaries, χ^2 or Fisher exact tests, and *t*-tests, as appropriate.

Results

During September 2011–March 2013, a total of 27,714 persons were screened for symptoms of TB at 21 Tibetan schools, 36 monasteries/nunneries, and the reception center for newly arrived refugees. These activities were conducted in Himachal Pradesh, Karnataka, and Uttarakhand states. Because data on age and sex for Tibetan school populations were not available, it was not possible to determine the age and sex distributions of the total population screened in this study. However, most (64%) of the persons screened were male. Of the total population screened, 55.0% (15,291) were residents of monasteries, and 1.6% (437) were refugees screened at the reception center.

A total of 3,830 (13.8%) persons with symptoms of TB or who were asymptomatic contacts of someone with TB were further evaluated and received chest radiography, sputum testing by AFB microscopy, Xpert MTB/RIF testing, or some combination (Table 1). Of 3,830 persons evaluated, 2,464 (64%) were male and 1,366 (36%) were female. Median age was 18 years (range 3–86 years) (Table 1); a total of 52.6% (2,016) were ≤18 years of age and 89% (3,413) were ≤30 years of age.

Table 1. Characteristics of 3,830 Tibetan refugees in India screened for tuberculosis*

Characteristic	No. (%)
Sex	
M	2,464 (64.3)
F	1,366 (35.7)
Age, y	
<18	1,633 (42.6)
18–24	1,363 (35.6)
25–34	529 (13.8)
35–44	194 (5.1)
45–54	53 (1.4)
≥55	58 (1.5)
Enrollment group	
Students	2,118 (55.3)
Monks/nuns	1,155 (30.2)
Reception center personnel	438 (11.4)
Other (community members, contacts)	73 (1.9)
Ethnicity	
Tibetan	3,585 (93.6)
Other (India, Nepal, Bhutan, Mongolia)	245 (6.4)
Risk factors	
History of TB	391 (10.2)
Any history of TB contact	2,221 (58.0)
Recent close TB contact (<6 mo)	1,883 (49.2)
No known TB contact	1,609 (42.0)

*TB, tuberculosis.

Most (93.6%) persons evaluated were Tibetan; there were smaller numbers of persons from India, Bhutan, Nepal, and Mongolia. Of the persons enrolled and tested, 47% were born in Tibet and had immigrated to India. Exposure to TB was common; 49.2% reporting having close contact with someone with TB or MDR TB in the previous 6 months, and 58.0% reporting having close contact with a TB patient at any time.

TB was diagnosed in 96 (2.5%) of 3,830 persons evaluated (346 cases/100,000 persons in the surveyed population). When community members not resident in schools, monasteries, and the reception center were excluded from this analysis, the prevalence of undiagnosed TB among persons tested was 2.3% (85/3,757) or 307 cases/100,000 persons in the screened population. Of the 96 cases, 77 (80.0%) were in male participants and 19 (20.0%) were in female participants. There were 47 TB cases in Tibetan schools (394 cases/100,000 persons) and 36 cases in monasteries (235 cases/100,000 persons). The rates of newly diagnosed TB in Mundgod (486 cases/100,000 persons) and Uttarakhand (721 cases/100,000 persons) monasteries were much higher than in Bylakuppe (53 cases/100,000 persons) and Himachal Pradesh (94 cases/100,000 persons) monasteries. Two cases were identified at the reception center, and 11 cases were identified through screening of community members who came to our ACF outreach activities. No cases of TB were identified in nunneries. Because additional laboratory testing to determine the strain of *M. tuberculosis* for each of these cases was not performed, clustering of similar strains could not be assessed.

Of the 96 TB patients identified, 81 had pulmonary TB; 65 (80.2%) of those patients had positive results by sputum smear or Xpert MTB/RIF. Fifteen patients had extrapulmonary TB, and 8 patients had both pulmonary and extrapulmonary TB. A total of 5 (5.2%) patients with MDR TB (5.2%) were identified by Xpert MTB/RIF; 3 of these patients reported a history of TB. One patient with extrapulmonary TB who did not respond to first-line anti-TB treatment was later identified as having MDR TB on the basis of an Xpert MTB/RIF test of ascitic fluid that showed a positive result for rifampin resistance.

A total of 31 (32%) cases detected were positive by sputum smear, and 34 (35%) were negative by sputum smear but positive by Xpert MTB/RIF (Table 2). Twenty (19%) patients with confirmed TB had no symptoms at the time of screening (TB contacts or history of TB), and 40 patients (42%) denied having a cough.

Patients with a known close contact (within the previous 6 months) accounted for 45% (59) of all cases identified.

Table 2. Results of microscopy for AFB and Xpert MTB/RIF test for detection of tuberculosis in 96 Tibetan refugees, India*

Results	No. (%) cases
Sputum AFB positive	31 (32.3)
Sputum AFB negative, Xpert MTB/RIF positive	34 (35.4)
Sputum AFB negative, Xpert MTB/RIF negative	16 (16.7)
Extrapulmonary TB (no sputum sample available)	15 (15.6)

*AFB, acid fast bacilli; MTB, *Mycobacterium tuberculosis*; RIF, rifampin; TB, tuberculosis.

A total of 56 (59%) patients reported contact with a person with TB at any time in the past. Nineteen (20%) patients had a previous history of TB, and only 1 person had HIV. All 96 patients were given anti-TB treatment, and 51% (49) had completed treatment by the end of the study; all other patients were still receiving treatment.

Discussion

This study confirms that the rate of TB remains high in the Tibetan refugee population in India; the overall prevalence of undiagnosed disease was 346 cases/100,000 persons in the population screened. Our results document that estimated TB prevalence rates are high in schools (394 cases/100,000 persons) and monasteries (235 cases/100,000 persons). We found unexpectedly large variation between case rates for TB in monasteries in different Tibetan settlements; this finding might be caused, in part, by differences in persons seeking care from local private physicians. Fewer monks than expected were screened in Bylakuppe, where the detected case rate was low, and many monks reported that a local private physician was providing TB testing and treatment to residents. Use of private physicians is less common in Himachal Pradesh, where most Tibetan residents use the Tibetan services, including the Delek Hospital, for treatment. Further exploration into the causes of this variation would potentially be useful to improve TB control.

Another factor that might have contributed to lower than expected screening numbers in some locations was high mobility of the Tibetan population. Persons move throughout India for work, for religious pilgrimage, and to visit family. This finding had a substantial effect on population size in different locations throughout the year and was a challenge during ACF screening at the monasteries in Bylakuppe and Mundgod. Patient mobility was also a major challenge for ensuring continuity of care for receiving TB treatment. During ACF activities in Bylakuppe, many monks were on pilgrimage for the annual Kalachakra celebration.

Implementation of ACF with Xpert MTB/RIF enabled early and rapid detection of undiagnosed TB cases in Tibetan congregate living settings and resulted in diagnosis of additional cases of pulmonary TB not identified by routine sputum smear microscopy. Through ACF outreach activities conducted during this study, we identified an additional 96 cases of TB. Although it was likely that many of these patients would have eventually come to a Tibetan health center or other health facility because of signs and symptoms of TB, many persons had minimal or no symptoms, and nearly half reported no cough at the time of screening. These results suggest that ACF played a major role in identifying cases earlier in the course of disease, which might have resulted in lower subsequent transmission. The 11 case-patients identified in the community were all symptomatic at the time of evaluation and came later for treatment in the course of their disease than other patients identified in congregate settings.

One limitation of this study was that different recruitment methods were used for identifying symptomatic persons in large (>1,000 persons) and small (<1,000 persons) congregate settings, which might have had an effect on our ability to detect differences in prevalence between different congregate settings. However, because the proportion of small versus large congregate settings in settlements in Bylakuppe and Mundgod were similar, this factor probably does not affect differences in prevalence between these settlements. A second limitation was that asymptomatic persons not identified as a TB contact or with known history of TB were not evaluated in this study.

ACF was time-intensive and required major human resource capacity for screening and testing with Xpert MTB/RIF. To accommodate ACF activities, limit effects on routine medical services, and improve testing efficiency, we recruited an additional laboratory technician at each testing site. Lack of a laboratory technician has been a well-documented obstacle to implementing Xpert MTB/RIF in many settings and will be an ongoing issue for the CTA DOH if scale-up of ACF or Xpert MTB/RIF is planned. We also recruited and trained healthcare staff to provide TB education, assist with TB screening, and perform contact tracing. These workers continue to perform these activities at the health centers for which they work, demonstrating that this project also helped to build capacity of the TB program itself.

A formal cost analysis was beyond the scope of this study, but the estimated direct costs of the ACF activities was US $1,000–$1,200/case identified. However, benefits and disability-adjusted life years could not be calculated, and true cost-effectiveness is not known.

This study targeted school children, monks, and nuns who live in congregate settings and are known to be at higher risk for acquisition of TB. High rates of TB in these settings were confirmed in this study, but we also identified many TB cases in the general Tibetan community. In 2011, 42% of all TB cases registered at the Tibetan Delek Hospital were persons living in the community. During this study, 11 of 96 TB patients identified lived in the

settlement community and not in a congregate living setting. Because only a small number (73) of community members participated in the screening, this finding indicates a high prevalence of TB in this subgroup and suggests that symptomatic persons took advantage of the campaign to obtain a diagnosis.

Although local staff reported increasing awareness of TB among Tibetans in India, the disease still carries a major negative stigma. For many Tibetans, particularly those with MDR TB, having TB means being isolated from daily activities (i.e., school, work, religious practices). Educational campaigns continue to be a large priority for the CTA DOH and will be essential for ongoing TB control in the Tibetan community and congregate living settings. Furthermore, efforts to provide TB screening for the general Tibetan community will be essential for TB control in this population.

We identified only 5 case-patients with MDR TB (5% of all case-patients identified); 3 of these case-patients had a history of TB. These rates are lower than expected on the basis of rates previously documented in the Tibetan refugee population, including a recent drug resistance survey among Tibetans in India, for reasons that are unclear (*14*).

In summary, the burden of TB remains high among Tibetan refugees in India. ACF and implementation of Xpert MTB/RIF has been a successful strategy for increased case detection in congregate settings in which TB diagnosis would otherwise likely have been delayed or not given. Ongoing efforts with periodic ACF by using Xpert MTB/RIF or similar rapid molecular tests, along with contact tracing and infection control measures, are warranted and will be essential for control of TB in this population.

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Dr. Dierberg is an infectious diseases physician working in Sierra Leone with Partners in Health and an associate scientist at the Brigham and Women's Hospital in Boston, MA. Her research interests are TB, MDR TB, TB/HIV co-infection, and global infectious diseases.

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Address for correspondence: Richard E. Chaisson, Center for Tuberculosis Research, Johns Hopkins University, 1550 Orleans St, 1M.08, Baltimore, MD 21287, USA; email: rchaiss@jhmi.edu

Underestimation of Invasive Meningococcal Disease in Italy

Chiara Azzari,[1] Francesco Nieddu,[1] Maria Moriondo,[1] Giuseppe Indolfi, Clementina Canessa, Silvia Ricci, Leila Bianchi, Daniele Serranti, Giovanni Maria Poggi, Massimo Resti

Knowing the incidence of invasive meningococcal disease (IMD) is essential for planning appropriate vaccination policies. However, IMD may be underestimated because of misdiagnosis or insufficiently sensitive laboratory methods. Using a national molecular surveillance register, we assessed the number of cases misdiagnosed and diagnoses obtained postmortem with real-time PCR (rPCR), and we compared sensitivity of rPCR versus culture-based testing. A total of 222 IMD cases were identified: 11 (42%) of 26 fatal cases had been misdiagnosed or undiagnosed and were reclassified as IMD after rPCR showed meningococcal DNA in all available specimens taken postmortem. Of the samples tested with both rPCR and culture, 58% were diagnosed by using rPCR alone. The underestimation factor associated with the use of culture alone was 3.28. In countries such as Italy, where rPCR is in limited use, IMD incidence may be largely underestimated; thus, assessments of benefits of meningococcal vaccination may be prone to error.

Neisseria meningitidis is the major etiologic agent of bacterial meningitis and one of the most important causes of invasive bacterial disease worldwide (*1*,*2*). The annual number of invasive meningococcal disease (IMD) cases is estimated to be at least 1.2 million, resulting in ≈135,000 deaths (*3*). Meningococcal meningitis is the most common form of meningococcal disease, accounting for 80%–85% of all reported cases of this illness. In nearly half of these cases, sepsis is also present. The remaining 15%–20% of cases are sepsis only (*1*–*3*); however, in the elderly, *N. meningitidis* can also cause pneumonia (*4*).

In Italy, recent data show that IMD in children results in a death for ≈13% (7% 8% for meningitis and 20% for sepsis) of case-patients (*5*). Among survivors, 10%–30% have disabling, long-term sequelae such as seizures, motor impairments, hydrocephalus, sensorineural hearing loss, mental retardation, and cognitive and behavioral problems (*2*,*6*).

Author affiliations: Anna Meyer Children's University Hospital, Florence (C. Azzari, F. Nieddu, M. Moriondo, G. Indolfi, C. Canessa, S. Ricci, L. Bianchi, D. Serranti, G.M. Poggi, M. Resti); University of Florence Department of Health Sciences, Florence, Italy (C. Azzari, F. Nieddu, M. Moriondo, S. Ricci, D. Serranti, G.M. Poggi)

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IMD has a high economic and social impact, and a vaccination program could be useful in reducing incidence of disease. However, to gauge the value of vaccination through the use of health technology assessment (*7*), precise data on IMD incidence are needed. Furthermore, meningococcal infection has a rapid and severe clinical progression and clinical signs and symptoms that are similar to severe invasive infections caused by other pathogens. Consequently, a fast and sensitive method of diagnosis is needed to ensure that contacts of meningococcal disease patients receive appropriate prophylaxis to prevent secondary cases. Standard diagnostic microbiology using culture-based methods is critical, enabling molecular characterization of isolates and providing information on antimicrobial drug resistance. However, culture-based methods are strictly dependent on viability of microorganisms. That characteristic may be a serious limiting factor, especially in patients who have a rapid fatal outcome or who have already undergone antimicrobial therapy (*8*).

N. meningitidis is a fastidious pathogen that frequently undergoes autolysis, and its growth can be inhibited by a single dose of antimicrobial drug therapy, even in cases when the patient dies from the infection (*9*). Therefore, molecular tests such as real-time PCR (rPCR) are used alone or in combination with culture to diagnose IMD and determine the serogroup of the implicated pathogen (*5*,*10*). However, in countries where use of rPCR is limited, IMD may go undiagnosed. Failure to diagnose IMD is undermining prevention efforts and evaluation of IMD incidence and leads to underestimation of IMD and imprecise assessments of the relative risks and benefits of vaccination. By using data from Italy's national register for molecular surveillance of invasive bacterial disease, we attempted to identify factors contributing to the underestimation of IMD, including suddenly fatal cases and the use of different diagnostic procedures.

Methods

Patients

Our study evaluated retrospectively all patients included in the molecular surveillance register during 2007–2014. The

[1]These authors contributed equally to this article.

register was started in 2006 and has been expanded since 2007 with dedicated funds from Italy's Center for Disease Control through a project titled "Improving Diagnosis of Invasive Bacterial Infection by Molecular Methods." The project and, consequently, the register were initially focused on pediatric hospitals. All pediatric hospitals or pediatric wards in general hospitals in Italy were invited to participate. Upon request by clinicians, samples obtained from adults were also accepted, tested, and included in the register, and the number of adults tested has increased over the years. Molecular surveillance was organized and is still active as a voluntary, nonmandatory surveillance. To be included in the register, at least 1 sample from each patient had to be analyzed by using rPCR, whereas use of a culture-based test was not an inclusion criterion. All clinical and laboratory data were recorded.

Sample Collection and Testing

Samples of blood, cerebrospinal fluid (CSF), or other normally sterile fluids were obtained as soon as possible (in most cases, before start of treatment) from patients in whom, on the basis of clinical signs and symptoms, invasive bacterial disease was suspected upon hospital admission. Samples were then sent for molecular testing to the reference center (Immunology and Infectious Disease Laboratory, Anna Meyer Children's Hospital, Florence, Italy) by using a freepost parcel carrier service; samples were delivered by the following day and tested within 2 hours after arrival. A report was produced and immediately sent back (by fax or email) to the sending hospital so that clinicians had the report within 24 hours after shipment of the sample. Samples for cultures were collected and sent to local laboratories in accordance with the hospitals' own procedures. Sepsis was clinically suspected in the presence of previously described signs and confirmed by blood tests (*11*). Meningitis was clinically suspected in the presence of a compatible clinical syndrome and abnormal chemical test results (*12*). A case was considered to be confirmed in the presence of positive microbiologic tests (culture or molecular tests). Our study evaluated all patients included in the molecular surveillance register and was approved by the Institutional Review Board at Anna Meyer Children's University Hospital.

Diagnostic Criteria

A diagnosis of laboratory-confirmed IMD was made if a patient's samples were culture positive for *N. meningitidis,* rPCR positive for the *ctrA* gene, or both, as described previously (*5*). If no increase in the fluorescent signal occurred before the 40th cycle of amplification, the sample was assumed to be negative. All samples in which the *ctrA* gene was detected by rPCR were included in a serogrouping analysis. The serogroups A, B, C, W, and Y (*13*) were identified by rPCR or endpoint PCR (for serogroups W and Y) by using appropriate primers and probes (Table 1).

Results

Samples Received and Diagnosis of Meningococcal Infection

Patients were selected from among 85 hospitals in 19 of Italy's 20 regions. The only region that did not include any patients represents 0.2% of Italy's population. Of 222 patients evaluated, 211 (95.0%) were tested during hospitalization and 11 (5.0%) were tested postmortem (Figure 1). At least 1 sample from each of the 222 patients included in the study was tested by rPCR. Because the reporting of a culture-based test (or lack of one) was welcome but not required for a case to be included in the register, samples for culture-based tests were not available for all patients, but at least 1 sample for culture-based tests was available for 187 of the 211 hospitalized patients. No culture-based test was performed for the 11 patients whose IMD diagnosis was postmortem; instead, diagnosis was performed by using rPCR on autoptic specimens, including blood, CSF, and formalin-fixed tissue samples (e.g., kidney, adrenal gland, brain, and lung tissue).

Among the 222 patients with confirmed IMD, we found 171 (77.0%) meningitis cases (11 of which were associated with sepsis) and 51 (23.0%) sepsis cases. A total of 158 (71.2%) cases were found in the pediatric age group (0–18 years of age), and 64 (28.8%) cases were found in adults (>18 years of age) (Figure 2). Children <1 year of age had the highest number of cases (46/222; 20.7%). The male-to-female ratio was 121:101 (1.2).

The rPCR tests performed directly on normally sterile fluids (blood or CSF) were positive for all 222 patients, and

Table 1. Primers and probes used for *Neisseria meningitidis* serogrouping of isolates from samples from a national register for molecular surveillance of invasive bacterial disease, Italy, 2007–2014

Target	Gene	Forward primer	Reverse primer	Probe
N. meningitidis	*ctrA*	gctgcggtaggtggttcaa	ttgtcgcggatttgcaacta	FAM_cattgccacgtgtcagctgcacat_TAMRA
Serogroup A	*sacB*	cccccagcatggctagattt	agggcactttgtggcataattt	FAM_accctaaaattcaatgggtatatcacga_TAMRA
Serogroup B	*siaD* B	ttggacttggttaagctgacctaa	gttgacaacatctccattttatcttacc	FAM_ttagatatgacaaataaattgttacgtggg_TAMRA
Serogroup C	*siaD* C	agggaaccgcaacctatgc	cacaaaacgttgctcaaattttg	FAM_ccactcttagaatcatttacatacaaaccc_TAMRA
Serogroup W/Y	*siaD* W/Y	gctgataaattgttcttatggtctgaa	cggcaccagaaccaatctct	FAM_ttggaatcatgagcttttaccaaatccaaca_TAMRA
Serogroup W*	*siaD*	cagaagtgagggatttccata	cacaaccattttcattatagttactgt	
Serogroup Y*	*siaD*	ctcaaagcgaaggctttggtta	ctgaagcgttttcattataattgctaa	

*Identified by using endpoint PCR.

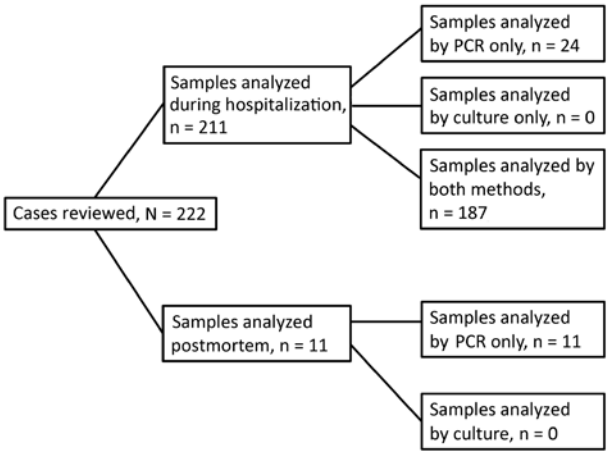

Figure 1. Distribution of patients diagnosed with invasive meningococcal disease during hospitalization or postmortem evaluation, by test performed for *Neisseria meningitidis* (real-time PCR [rPCR] or rPCR and culture), from a national register for molecular surveillance of invasive bacterial disease, Italy, 2007–2014.

rPCR enabled serogrouping in 218 (98.2%) cases (4 samples were not serogrouped because of insufficient sample material). Of the 218 samples that were serogrouped, 172 (78.9%) were serogroup B, 29 (13.3%) were serogroup C, 8 (3.7%) were serogroup W, and 8 (3.7%) were serogroup Y. No organisms from serogroup A were found.

During the study period (2007–2014), a total of 26 deaths occurred among the 222 patients, resulting in a case-fatality rate of 11.7%. Five (19.2%) deaths occurred in patients <1 year of age, 7 (26.9%) in patients 1–5 years of age, 8 (30.8%) in patients 6–18 years of age, and 4 (15.4%) in adult patients. Case-fatality rates were 22.6% (14/62 cases) in patients admitted with a diagnosis of sepsis or meningitis and sepsis and 7.5% (12/160 cases) in patients admitted for meningitis but with no mention of sepsis at admission.

Misdiagnosis and Postmortem Diagnosis of IMD

Postmortem diagnosis of IMD was obtained in 11 (5.0%) of the 222 cases. In all 11 cases, culture-based tests were either negative or impossible to perform because the patient died before being admitted to the hospital. Eight of these cases had been diagnosed as sepsis of unknown origin (Table 2). Here we describe the clinical progression of the other 3 cases.

Case 1

A 20-year-old, previously healthy woman had sudden onset of high fever with chills and general malaise. The next day, her general condition rapidly deteriorated. She was then referred to the emergency department but died on the way to the hospital. A diagnosis of sudden death was made; no blood test was performed. A few years later, autoptic specimens (formalin-fixed lung, kidney, and adrenal gland tissue) were tested (for legal reasons) at the Immunology and Infectious Disease Laboratory of the Anna Meyer Children's Hospital by using rPCR; all specimens were found to be positive for *N. meningitidis* serogroup B.

Case 2

A 5-month-old male infant was found dead in his crib. In the preceding days, he had shown poor feeding and irritability. He was born from healthy, nonconsanguineous parents at the end of a normal pregnancy. In accordance with the national diagnostic protocol for sudden infant death syndrome (SIDS), an autopsy was performed. Autoptic samples (i.e., formalin-fixed lung, kidney, brain, and adrenal gland tissue) for diagnosis of infectious diseases were immediately transferred to the Immunology and Infectious Disease Laboratory, where rPCR showed the presence of *N. meningitidis* serogroup C in all the specimens.

Case 3

A 17-year-old male adolescent was admitted to the hospital with fever, diarrhea, vomiting, purpuric rash, and lethargy, symptoms that had manifested suddenly during the preceding 6 hours. He had a normal clinical history and a normal history of school attendance, and he had participated in sports. Blood tests performed on his arrival showed a high leukocyte count (>70,000 cells/μL) and a low platelet count (<38,000/mL). He died in the hospital 1 hour after his arrival. During the following days, all culture-based test results were negative, and a diagnosis of acute myeloid leukemia resulting in death was made. Three days after his death, a family member was admitted to the hospital with a similar clinical signs and symptoms. The pathologist in charge of postmortem examination for the first patient was immediately alerted so that an infectious disease diagnosis could be considered. The pathologist decided to send formalin-fixed tissue samples to the Immunology and Infectious Disease Laboratory. Blood samples from the second patient were also sent, and rPCR results led to a diagnosis of *N. meningitidis* group C infection in both patients.

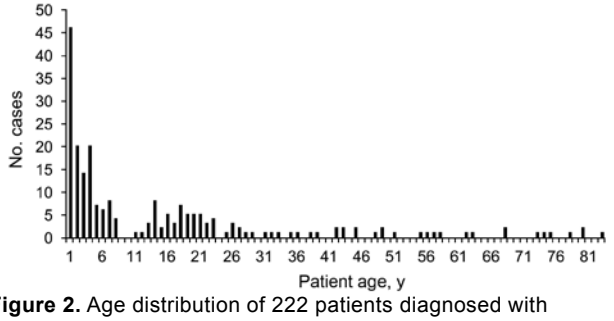

Figure 2. Age distribution of 222 patients diagnosed with invasive meningococcal disease from a national register for molecular surveillance of invasive bacterial disease, Italy, 2007–2014.

Table 2. Description of 11 case-patients with postmortem diagnosis of invasive meningococcal disease included in a national register for molecular surveillance of invasive bacterial disease, Italy, 2007–2014*

Patient no.	Sex	Age at death	Preexisting disease	Cause of missing or incorrect diagnosis	First diagnosis of cause of death	Culture result	rPCR result†	Serogroup
1	F	20 y	None	Died before being admitted to hospital	Sudden death	Not performed	Positive	B
2	M	5 mo	None	Died before being admitted to hospital	SIDS	Not performed	Positive	C
3	M	17 y	None	Misdiagnosis: acute myeloid leukemia	Acute myeloid leukemia	Negative	Positive	C
4	F	5 mo	None	Died before being admitted to hospital	Sepsis	Not performed	Positive	B
5	M	6 y	None	Died <1 h after hospital admission	Sepsis	Not performed	Positive	Y
6	F	11 mo	None	Died before being admitted to hospital	Sepsis	Not performed	Positive	C
7	M	4 y	None	Died before being admitted to hospital	Sepsis	Not performed	Positive	B
8	M	15 y	Previous meningitis at age 5 y	Negative culture-based tests	Sepsis	Negative	Positive	Y
9	M	20 y	Diabetes type I	Negative culture-based tests	Sepsis	Negative	Positive	C
10	M	13 y	None	Died at hospital admission	Sepsis	Not performed	Positive	C
11	M	6 y	None	Died at hospital admission	Sepsis	Not performed	Positive	B

*SIDSs, sudden infant death syndrome; rPCR, real-time PCR.
†Of *ctrA* gene of *Neisseria meningitidis*.

Standard Culture-Based Tests versus rPCR

A total of 116 blood samples were tested with rPCR, and 107 blood samples were tested with culture (Table 3). Blood was positive for *N. meningitidis* in 104 (89.7%) of 116 samples tested with rPCR and in 26 (24.3%) of 107 samples tested with blood culture (odds ratio [OR] 27.0, 95% CI 12.1–61.2; p<0.0001). One culture sample was reported as contaminated with *Streptococcus viridans*.

A total of 162 CSF samples were tested with rPCR, and 90 CSF samples were tested with culture (Table 3). CSF was positive in 160 (98.8%) of 162 samples tested with rPCR and in 33 (36.7%) of 90 samples tested with CSF culture (OR 138.1, 95% CI 30.7–862.6; p<0.0001). One culture sample was reported as contaminated with *S. epidermidis*. Overall, by considering both kinds of samples, rPCR was shown to be 3.28 times more sensitive than culture.

All 12 patients whose blood samples were negative by rPCR had CSF samples that tested positive by rPCR. Among the 81 patients whose samples tested negative by blood culture, CSF culture was not performed for 22 (27.2%); 18 (22.2%) had samples that tested positive by CSF culture and 41 (50.6%) had samples that tested negative by CSF culture.

The 2 patients whose CSF samples tested negative by rPCR had blood samples that tested positive by rPCR. Among the 57 patients whose samples tested negative by CSF culture, a blood culture was not performed for 14 (24.6%); a blood culture tested positive for *N. meningitidis* for 4 (7.0%) and negative for 39 (68.4%).

Overall (including CSF and blood samples), rPCR enabled a correct diagnosis of IMD in all (100%) patients. On the other hand, culture enabled a correct diagnosis in only 29 (42.0%) of 69 patients for whom blood and CSF cultures were performed at admission.

To better compare the sensitivity of rPCR versus culture, we evaluated samples collected at the same time and tested by using both methods. Of the 63 patients who had samples that were simultaneously tested with blood culture and rPCR on blood, 53 (84.1%) had samples that tested positive by rPCR, whereas 17 (26.9%) had samples that tested positive by culture (OR 14.3, 95% CI 5.5–38.2; p<0.0001); 45 (71.4%) had samples that tested negative by culture. One of the 17 samples that tested positive by culture was reported as contaminated. No sample found negative by rPCR was found positive by culture. Use of rPCR on blood was 3.12 times more sensitive than blood culture.

Eighty-eight patients had samples that were simultaneously tested with CSF culture and rPCR on CSF: 86 (97.7%) had samples that tested positive by rPCR, whereas 338 (37.5%) had samples that tested positive by culture (OR 71.6, 95% CI 15.7–451.1; p<0.0001); 54 (61.4%) had samples that tested negative by culture. One of the 33 samples that tested positive by culture was reported as contaminated. No sample found negative by rPCR was found positive by CSF culture, and rPCR on CSF was 2.61 times more sensitive than CSF culture.

Overall, *N. meningitidis* was identified only by rPCR in 36 of 63 blood samples and in 53 of 88 CSF samples. For enabling a laboratory diagnosis of IMD, rPCR was

Table 3. Distribution of rPCR and culture-based test results for *Neisseria meningitidis* for CSF and blood samples from a national register for molecular surveillance of invasive bacterial disease, Italy, 2007–2014*

Type of sample	No. samples/no. tested (%)			
	Positive by rPCR	Negative by rPCR	Not tested by rPCR	Total†
CSF				
Culture positive	33	0	0	33/90 (36.7)
Culture negative	55	2	0	57/90 (63.3)
Not tested with culture	72	0	0	0
Total	160/162 (98.8)	2/162 (1.2)	0	0
Blood				
Culture positive	16	0	10	26/107 (24.3)
Culture negative	37	10	34	81/107 (75.7)
Not tested with culture	51	2	0	0
Total	104/116 (89.7)	12/116 (10.3)	0	0
Total, CSF or blood				
Culture positive	49	0	10	59/197 (29.9)
Culture negative	92	12	34	138/197 (70.1)
Not tested with culture	123	2	0	0
Total	264/278 (95.0)	14/278 (5.0)	0	0

*A total of 162 CSF samples were tested with rPCR, and 90 were tested with culture-based methods. A total of 116 blood samples were tested with rPCR, and 107 were tested with culture-based methods. CSF, cerebrospinal fluid; rPCR, real-time PCR.
†Proportion of samples that were positive, negative, or not tested with culture-based methods.

significantly more sensitive than culture (Cohen's Kappa 0.59, OR 23.4, 95% CI 11.3–49.1; p<0.001).

Discussion

Our analysis of the national register for molecular surveillance of bacterial disease in Italy showed that at least 2 main factors cause underestimation of IMD: misdiagnosis and insufficiently sensitive laboratory methods. In the register, 3 deceased patients had previously had a different disease diagnosis (i.e., SIDS, acute myeloid leukemia, sudden death); later, when biological samples were tested for *N. meningitidis* for other reasons (e.g., a legal trial or a secondary case), samples from the patients were found to be positive for the pathogen. The extent of misdiagnosis is difficult to quantify. Although misdiagnoses account for 1.4% in the national register, the actual percentage is probably much higher because only cases for which a clinical doubt occurred and samples were tested posthumously had a chance of being found positive for *N. meningitidis*. In the 3 cases described in this article, samples were retrieved and tested posthumously. However, in absence of those incidental situations, all 3 cases would have been misdiagnosed, thus contributing to the underestimation of IMD.

Among the 26 fatal cases, >40% were undiagnosed by standard culture-based methods, thus substantially contributing to the underestimation of IMD. In all undiagnosed cases, culture-based test results were either negative or not performed because sudden death attributable to *N. meningitidis* infection occurred before the patients were admitted to the hospital or upon their arrival at the emergency department. Whereas rPCR can be used for postmortem analysis of samples and enables diagnosis and serogrouping, culture-based methods are not useful in those situations; rPCR can be used with formalin-fixed tissue (*14*,*15*), as occurred with 2 of our patients, and even with bodies in advanced decomposition (*16*). Diagnoses of IMD is important for timely administration of prophylaxis to contacts and for limiting underestimation of cases. Therefore, rPCR should be considered as a fundamental tool. Moreover, molecular techniques offer the opportunity to identify the serogroup in culture-negative and fulminant cases. The ability to identify serogroups has important implications for vaccination programs. In fact, if fatalities were more often associated with a specific serogroup, a dedicated vaccination program could be planned. Moreover, molecular techniques enable the meningococcus to be molecularly characterized, which is important for planning and monitoring vaccination with subcapsular meningococcal vaccines.

We found that all tissues tested postmortem were positive for *N. meningitidis* by using rPCR. No specific kind of tissue seems to be better suited for diagnostic testing.

As for laboratory confirmation of IMD in nonfatal cases, current data confirm what has been shown previously about meningococcal (*5*,*17*) and pneumococcal (*18*,*19*) infections: rPCR is approximately 3 times more sensitive than culture in identifying meningococcal infection, regardless of the type of biologic sample used or the patients' clinical signs and symptoms. Consequently, in countries (as in Italy) where most hospitals use only standard culture-based methods for diagnosis of invasive bacterial infections, incidence of IMD may be largely underestimated.

Testing with rPCR can enable etiologic diagnosis and serogrouping in culture-negative samples (*19–21*). Therefore, most countries have included rPCR techniques in addition to culture-based tests in surveillance programs. The results are encouraging: in developed areas, such as England or Wales, the number of diagnoses made has more than doubled with the use of rPCR because 58% of cases were confirmed by rPCR alone (*22*). Our study shows that

in Italy, as in England and Wales, >50% of cases are confirmed by rPCR alone. The advantage is even greater in countries with fewer health resources, where laboratory results might be negatively influenced by inadequate transport and storage of samples (*23*). Testing with rPCR has the additional advantage of providing results rapidly, enabling speedy initiation of prophylaxis of contacts, thus preventing secondary cases.

Other underestimation factors undoubtedly exist, and underreporting is surely one of the most important (*24*). Clinicians must be made aware that, besides curing patients, identifying and reporting the bacterial etiology are important for enabling a better understanding of the epidemiology of meningococcal disease and implementation of appropriate public health interventions, such as vaccination programs or prophylaxis for contacts. Hospitals unable to offer local rPCR should be encouraged to duly and promptly collect samples for offsite testing.

In summary, IMD is largely underestimated in Italy because of misdiagnosis, limited use of molecularly based laboratory methods, and undernotification. Using molecular methods for diagnosis of IMD in all patients with clinical evidence that results in a suspicion of *N. meningitidis* infection and for postmortem diagnoses can help reduce underestimation of IMD.

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Dr. Azzari specializes in pediatrics as well as allergy and immunology. She is director of the Immunology and Infectious Disease Laboratory of the Anna Meyer Children's Hospital (University of Florence) and the Regional Reference Center for Meningococcal Infections. Her main fields of interests are invasive bacterial disease, immunodeficiencies, and vaccines.

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Address for correspondence: Chiara Azzari, Pediatric Immunology Unit, Department of Health Sciences, University of Florence, Viale Pieraccini 24, Florence 50139, Italy; email: chiara.azzari@unifi.it

Whole-Genome Sequencing to Determine Origin of Multinational Outbreak of *Sarocladium kiliense* Bloodstream Infections

Kizee A. Etienne, Chandler C. Roe, Rachel M. Smith, Snigdha Vallabhaneni, Carolina Duarte, Patricia Escandón, Elizabeth Castañeda, Beatriz L. Gómez, Catalina de Bedout, Luisa F. López, Valentina Salas, Luz Maria Hederra, Jorge Fernández, Paola Pidal, Juan Carlos Hormazabel, Fernando Otaíza-O'Ryan, Fredrik O. Vannberg, John Gillece, Darrin Lemmer, Elizabeth M. Driebe, David M. Engelthaler, Anastasia P. Litvintseva

We used whole-genome sequence typing (WGST) to investigate an outbreak of *Sarocladium kiliense* bloodstream infections (BSI) associated with receipt of contaminated antinausea medication among oncology patients in Colombia and Chile during 2013–2014. Twenty-five outbreak isolates (18 from patients and 7 from medication vials) and 11 control isolates unrelated to this outbreak were subjected to WGST to elucidate a source of infection. All outbreak isolates were nearly indistinguishable (≤5 single-nucleotide polymorphisms), and >21,000 single-nucleotide polymorphisms were identified from unrelated control isolates, suggesting a point source for this outbreak. *S. kiliense* has been previously implicated in healthcare-related infections; however, the lack of available typing methods has precluded the ability to substantiate point sources. WGST for outbreak investigation caused by eukaryotic pathogens without reference genomes or existing genotyping methods enables accurate source identification to guide implementation of appropriate control and prevention measures.

Despite modern advances in technology to control fungal contamination in clinical settings, fungi are continuously implicated in clusters or outbreaks of infections, particularly among immunosuppressed patients (*1*). The sources of fungal nosocomial outbreaks often are difficult to assess because of the widespread prevalence of fungi in the environment. Specifically, differentiating between fungal infections originating from a single contaminated point source and those independently acquired from the environment frequently is difficult. Molecular typing methods for discriminating strains have been an essential tool to identify potential source(s) of fungal infections in outbreaks. Small-scale DNA-based typing methods, such as variable-number tandem-repeat typing and multilocus sequence typing (MLST), use genomic similarity to assist in determining epidemiologic relatedness of fungal isolates in an outbreak investigation (*2*). However, the robustness and accuracy of such genotyping tools depend largely on the discriminatory power of the genotyping method and availability of reference data, which often are inconsistent or incomplete for many fungi.

The advent of whole-genome sequence typing (WGST) has made fungal genotyping feasible for outbreak investigations (*3*–*7*), especially for fungi for which conventional genotyping methods do not exist. With WGST, genetic relationships among isolates are determined by the phylogenetic analysis of single-nucleotide polymorphism (SNP) differences among analyzed genomes a population: typically, the fewer the number of SNPs observed between the strains, the more closely the strains are related, and the more likely they are to have a point source, provided supporting epidemiologic evidence exists. Point source outbreaks are typically clonal, and the resulting isolate genomes display few to no SNP differences. Environment-linked outbreaks might have 1 or multiple source populations that also are identifiable and distinct from background and control strains (*5*).

In January 2014, the Chilean Ministry of Health contacted the Mycotic Diseases Branch (in the Division of Foodborne, Waterborne, and Environmental Disease,

Author affiliations: Centers for Disease Control and Prevention, Atlanta, Georgia, USA (K.A. Etienne, R.M. Smith, S. Vallabhaneni, A.P. Litvintseva); Georgia Institute of Technology, Atlanta (K.A. Etienne, F.O. Vannberg); Translational Genomics Research Institute, Flagstaff, Arizona, USA (C.C. Roe, J. Gillece, D. Lemmer, E.M. Driebe, D.M. Engelthaler); Instituto Nacional de Salud, Bogota, Colombia (C. Duarte, P. Escandón, E. Castañeda); Universidad del Rosario, Bogota (B.L. Gómez); Corporación para Investigaciones Biológicas, Medellín, Colombia (B.L. Gómez, C. de Bedout, L.F. López); Instituto de Salud Publica de Chile, Santiago, Chile (V. Salas, L.M. Hederra, J. Fernádez, P. Pidal, J.C. Hormazabel); Ministry of Health, Santiago (F. Otaíza-O'Ryan)

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National Center for Emerging and Zoonotic Infectious Diseases), US Centers for Disease Control and Prevention (CDC), about a cluster of 67 cases of *Sarocladium kiliense* (formerly *Acremonium kiliense*) bloodstream infections (BSI). This cluster was identified at 8 different hospitals in Santiago, Chile, by the National Infection Control Program. The infections occurred during June 2013–January 2014; 39 infections were in children and 2 in adults, all of whom were undergoing chemotherapy. The Chilean Ministry of Health initiated an epidemiologic investigation with technical assistance from CDC and the Pan American Health Organization. An environmental source was considered unlikely because of the spread of these infections among multiple locales in Chile; however, the possibility of environmental contamination could not be excluded.

A detailed review of medication administration records revealed that all patients received 4 intravenous medications: ondansetron, heparin, saline, and potassium. Heparin, saline, and potassium were products used in many different patient populations within the hospital. However, ondansetron, an antinausea medication, was used as standard protocol among oncology patients, and all patients with *S. kiliense* BSIs received ondansetron from a single source, pharmaceutical company A, in Colombia. Three lots of ondansetron, manufactured by pharmaceutical company A, were investigated in Chile Drug National Agency (ANAMED, ISP). In accordance with Mycotic Diseases Branch recommendations (S. Vallabhaneni, pers. comm.) the Chilean Ministry of Health laboratory cultured 10% of unopened ondansetron vials of these 3 lots. Vials from 2 of the 3 available lots yielded *S. kiliense* on February 15, 2014. All isolates were identified by traditional methods and confirmed by DNA sequencing by ISP. After this finding, all ondansetron products made by this manufacturer were recalled in Chile; the Pan American Health Organization issued an international health alert on February 17, 2014.

Concurrently, the Corporación para Investigaciones Biológicas (Medellin, Colombia) and the Instituto Nacional de Salud (Bogota, Colombia) contacted CDC about an isolate identified as *S. kiliense* by conventional DNA sequencing methods and 16 isolates originally identified as a *Fusarium* spp. by phenotypic methods in Colombia dating to November 2013. Because of the findings in Chile, these isolates were reevaluated and confirmed as *S. kiliense* by conventional DNA sequencing methods (*8*). Further investigation by officials in Colombia showed that at least 14 of the 16 patients also received ondansetron manufactured by pharmaceutical company A in Colombia; culturing and conventional DNA sequence identification methods also confirmed that ondansetron was contaminated with *S. kiliense*.

A subset of isolates from patients and medication vials in this investigation were sent to CDC's Mycotic Diseases Branch for further identification and molecular typing. To determine whether the contaminated lots of ondansetron harbored the same fungal strains as infected patients, we used WGST.

Methods

Isolates

On the basis of the availability of epidemiologic and patient information, we subjected a subset of patient isolates from each country to molecular analysis: 7 isolates from Chile and 11 isolates from Colombia, 1 isolate per patient. Additionally, 7 isolates from contaminated ondansetron vials were collected from both countries (Table). Eleven unrelated *S. kiliense* control isolates from the CDC culture collection, American Type Culture Collection, Centraalbureau voor Schimmelcultures, and Universitat Rovira I Virgili also were included for analysis. No background isolates of *S. kiliense* from the affected countries were available for analysis. Genomic DNA was extracted from cells grown on Sabouraud dextrose agar by using the DNeasy Blood and Tissue kit (QIAGEN, Hilden, Germany) as referenced in Litvintseva et al. (*3*).

Library Preparation and Illumina Sequencing

The 36 DNA samples were prepared for Illumina sequencing (Illumina, San Diego, CA, USA) by using the KAPA Biosystems Library Preparation with Standard PCR kit (KAPA Biosystems, Wilmington, MA, USA) protocol with a modified 8-bp index. Approximately 1 μg of double-stranded DNA was sheared by using a Sonicman sonicator (Brooks Automation, Spokane, WA, USA) to an average insert size of 650 bp, and DNA libraries were prepared for Illumina paired-end sequencing as described by the manufacturer. All 36 libraries were sequenced to a read length of 100 bp on the Illumina HiSeq 2500 system. Whole-genome sequence read files were deposited in the National Center for Biotechnology Information Sequence Read Archive under BioProject PRJNA291140.

Genome Assembly

We assembled raw sequencing reads from isolate CDC-B10657 using ABySS as a reference genome for *S. kiliense* (*9*). The assembly and its corresponding read data were subject to self-alignment to determine coverage. Contigs were filtered for length and coverage; contigs <200 bp were removed from samples assembled from HiSeq data. Contigs with coverage <20% of the average coverage of the 20 largest contigs per assembly were manually removed. We assessed the final assembly for erroneous sites using Pilon (*10*).

SNP Variant Detection and Phylogenetic Analysis

We applied a reference-based analysis method to determine genetic relatedness among all isolates. Read data of

Table. Whole-genome sequenced strains of *Sarocladium kiliense*, Chile and Colombia, 2013–2014*

Laboratory identification	Type of isolate	Source of isolate	Origin of isolate	Depth of coverage, ×
B10646	Patient	Chile	Blood	228
B10648	Patient	Chile	Blood	218
B10650	Patient	Chile	Blood	72
B10651	Patient	Chile	Blood	193
B10652	Patient	Chile	Blood	119
B10653	Patient	Chile	Blood	67
B10657	Patient	Chile	Blood	354
B10660	Ondansetron	Chile	Vial	78
B10661	Ondansetron	Chile	Vial	29
B10731	Patient	Colombia	Blood	75
B10732	Patient	Colombia	Blood	34
B10734	Patient	Colombia	Blood	67
B10743	Patient	Colombia	Blood	37
B10748	Ondansetron	Colombia	Vial	98
B10749	Ondansetron	Colombia	Vial	152
B10762	Patient	Germany	Skin lesion	54
B10763	Patient	Utah, USA	Eye	41
B10764	Patient	Wisconsin, USA	Skin lesion	43
B10765	Patient	Pennsylvania, USA	Blood	27
B10766	Patient	Florida, USA	CSF	74
B10767	Patient	Texas, USA	BAL	51
B10971	Patient	Colombia	Blood	101
B10972	Patient	Colombia	Blood	95
B10973	Patient	Colombia	Blood	62
B10974	Patient	Colombia	Blood	103
B10975	Patient	Colombia	Blood	113
B10976	Patient	Colombia	Blood	191
B10977	Patient	Colombia	Blood	261
B10978	Ondansetron	Colombia	Vial	150
B10979	Ondansetron	Colombia	Vial	126
B10980	Ondansetron	Colombia	Vial	152
B5504	Patient	Pennsylvania, USA	Eye	138
B5505	Patient	Pennsylvania, USA	Eye	38
ATCC64672	Dog	Costa Rica	Eye	26
CBS155	Environment	India	Soil	100
CBS157	Environment	India	Soil	82

*ATCC, American Type Culture Collection; CBS, Centraalbureau voor Schimmelcultures.

all samples were aligned to a reference by using Novoalign 3.00.03 (Novocraft Technologies, Selangor, Malaysia), and SNPs were identified by using the Genome Analysis Toolkit version 2.4 (*11*) from a custom pipeline, NASP (Northern Arizona SNP Pipeline, http://tgennorth.github.io/NASP/). The SNP calls were filtered in the final step of the pipeline and were included in the final matrix if they were not identified in repetitive regions, found in <90% of the base calls, and had a minimum read depth coverage of 10×. We excluded reads that mapped to multiple locations within the genome, as well as insertions and deletions. Only SNP loci present in all 36 isolates were included. This final matrix was created from NASP output and converted to FASTA format by using an in-house script NASP. We conducted genomewide SNP-based phylogenetics analyses using the simple parsimony algorithm in PAUP version 4.0b10 (*12*).

Results

The total length of the de novo assembled reference contigs showed that the genome size of *S. kiliense* was ≈38 MB. The average sequencing depth for the reference strain was 354×, which resulted in 98% coverage across the genome. The assembly contained 1,092 contigs ranging in length from 500 bp to 1.7 million bp (average contig length 35,528 bp; N50 591,374 bp) (*13*).

Reference-based phylogenetic analysis identified ≈117,000 shared SNPs, of which ≈60% were parsimoniously informative (Figure). The cladogram showed 1 distinct clade that comprised all outbreak isolates and included isolates from the patients and the contaminated drugs. No more than 5 SNPs were detected between any patient and drug isolates from Chile and Colombia, demonstrating that these isolates had nearly indistinguishable genomes. Conversely, all control isolates from different sources clustered separately from the outbreak clade.

The genomewide SNP analysis showed greater diversity among the control isolates. Although the closest control isolate, B10764, differed from the outbreak clade by 501 SNPs, the remaining majority of the control isolates diverged from the outbreak clade by ≈21,000 SNPs. Two control isolates, B5504 and B5505, isolated from a 1996 cluster of *S. kiliense* infections in a US hospital, were genetically indistinguishable from each other.

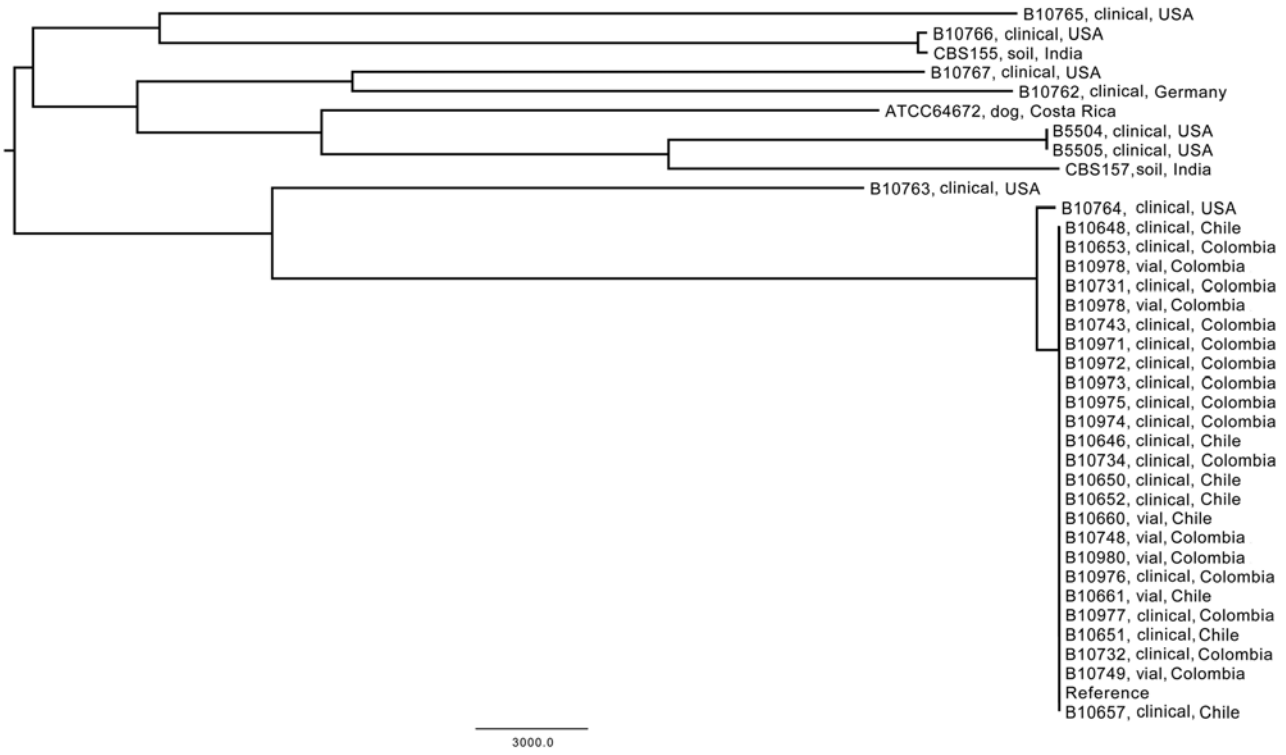

Figure. Whole-genome single-nucleotide polymorphism (SNP) typing of *Sarocladium kiliense* strains, Chile and Colombia, 2013–2014. All patient (clinical) and drug (vial) isolates from these 2 countries differed by ≤5 SNPs, and >21,000 SNPs were identified for the control isolates (≈117,000 total SNPs, ≈73,000 parsimoniously informative SNPs). Scale bar indicates nucleotide substitutions per site.

Discussion

S. kiliense is primarily a saprobic soil organism; it can cause opportunistic infections in humans that typically occur after inoculation of the fungus (*14*). Here we report the results of molecular epidemiologic investigation of an outbreak of *S. kiliense* BSI that affected >50 patients in 2 South American countries. The epidemiologic investigation suggested contaminated antinausea medication as a possible source of this infection. With no existing genotyping methods for this uncommon pathogen, we used WGST to understand the genetic relationships among the isolates and identify a potential source of this outbreak.

The use of WGST to investigate fungal outbreaks has become integral to epidemiologic investigations (*3*–*5*,*15*). During nosocomial outbreak investigations, ascertaining potential source(s) of infection based solely on the descriptive epidemiologic findings often is difficult. Molecular genotyping frequently is needed to test hypotheses generated by epidemiologic investigation: the presence of a single strain or dominant clone usually suggests a point source, whereas the presence of multiple strains is usually consistent with an environmental exposure or exposure to mixed populations from a single source. For example, in a recent outbreak of *Curvularia* spp. (formerly *Bipolaris* spp.) among cardiac surgery patients, initial epidemiologic investigation suggested a point source; however, a molecular epidemiology analysis demonstrated multiple strains, consistent with an environmental source (*16*,*17*). Similarly, in an outbreak of *Fusarium* spp. associated with the use of contact lenses, initial epidemiologic investigation linked the infections with a particular lens cleaning solution, suggesting a point source; however, molecular analysis indicated multiple sources (*18*). In our current investigation, the WGST analysis demonstrated that the patient isolates from Chile and Colombia were nearly genetically indistinguishable (≤5 SNPs) from those recovered from the medication vials, indicating the likely presence of a single-source infection. Conversely, the control isolates clustered differently from the outbreak clade by ≈21,000 SNPs, and except for 2 strains from the same cluster, thousands of SNPs separated any 2 control strains (Figure).

Although BSIs with this organism are rare, case reports/clusters of *S. kiliense* fungal infections have been reported in the literature (*19*–*22*). In a cluster of *S. kiliense* infections of endophthalmitis and catheter-related BSIs, an environmental source was strongly suggested but could not be confirmed because of the lack of available typing methods (*21*). Isolates from this cluster of infections served as controls in our study: specifically, isolates B5504 and B5505 from 2 patients who underwent

cataract extraction with intraocular lens implantation differed from each other by 1 SNP, indicating that the 2 genomes were nearly indistinguishable and suggesting a common source of infection.

In this investigation, the use of WGST identified a likely source of *S. kiliense* BSI in oncology patients in both countries by linking these infections to the receipt of contaminated medication. Results from this outbreak are consistent with those from other outbreaks of fungal pathogens in which WGST was used and a common source was hypothesized. For example, Litvintseva et al. investigated *Exerohilium rostratum* infections associated with the injection of contaminated methylprednisolone acetate by using whole-genome SNP typing; they found that all outbreak isolates were genotypically indistinguishable: no more than 2 SNPs separated the strains in the outbreak clade (*3*).

The genetic diversity among the *S. kiliense* control strains in our study was congruent to the level of diversity among control isolates found in other studies. We identified comparable levels of genetic similarities to those found among isolates of *Saprochaete clavata* from patients from a multicenter outbreak in France (*15*). Conversely, >28,000 and up to 1.2 million SNPs were observed among the control isolates of *Apophysomyces trapeziformis* infections associated with a tornado in Joplin, Missouri, USA (*5*). Although we were able to include a variety of controls from different sources, a major limitation in this study is the lack of background isolates of *S. kiliense* from the affected countries to assess genomic similarity among the unrelated isolates from South America.

Traditional molecular strain typing methods, such as MLST, are limited to analysis of specific genomic regions, typically protein-coding regions. However, the conservation in these regions might be insufficient to discriminate strains of certain fungi or provide the resolution needed to identify a likely source in a fungal outbreak. In the absence of established methods for fungi, the rapid development of traditional typing methods is often necessary. In this investigation, before deploying WGST, we evaluated MLST using 7 protein-coding regions to determine the relatedness among all isolates (*17*). Across 3,651 nt amplified, only a 1-nt polymorphism was identified in the β-tubulin gene of one of the control isolates, which did not provide sufficient resolution between outbreak and control isolates, indicating that the use of MLST was not informative in this outbreak investigation. Although conventional typing methods are rapid, the conclusions drawn from them might be confounded by either their discriminatory power or their character state conflict (i.e., homoplasy). Typically, genotyping methods with high resolution (e.g., variable-number tandem-repeat typing) have higher degrees of homoplasy, whereas methods with lower resolution (e.g., MLST) have less homoplasy; both might be at risk of misidentifying a common source. Conversely, WGST enables accurate deduction of genetic relationships among strains in fungal outbreaks for which current typing methods are ineffective or nonexistent. Furthermore, with the improvement of next-generation sequencing technologies, investigating fungal outbreaks in real time will soon be possible.

Contamination of medical products, particularly with rare fungi, poses growing concern and a public health threat, especially in vulnerable populations. Fungal clusters/outbreaks of common environmental species (rather than classical clinical pathogens) have been associated with medical products. For example, the 2002 outbreak of fungal meningitis was caused by injected steroids contaminated with *Exophiala dermatitidis*, and the 2012–2015 outbreak of fungal meningitis and other infections resulted from methylprednisolone contaminated by *Exserohilum rostratum* (*3*,*23*). Similarly, in 2009, intestinal zygomycosis resulted from ingestion of allopurinol tablets contaminated with *Rhizopus microsporus* (*24*). In 2012, an endophthalmitis outbreak was associated with use of an ophthalmologic dye contaminated with *Fusarium* sp. in the *Fusarium incarnatum-equiseti* species complex and triamcinolone contaminated with *Curvularia hawaiiensis* (*Bipolaris hawaiiensis*) (*16*,*25*,*26*). Increased vigilance and the use of advanced technologies are needed to rapidly identify the likely source(s) of infection to efficiently guide epidemiologic investigations and initiate appropriate control measures.

In summary, our study highlights the utility of advanced molecular methods to investigate outbreaks involving rare fungi. Next-generation sequencing and bioinformatics analyses will remain critical molecular epidemiology tools in such epidemiologic investigations.

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Ms. Etienne is a public health microbiologist at CDC's Mycotic Diseases Branch, Division of Foodborne, Waterborne, and Environmental Disease, National Center for Emerging and Zoonotic Infectious Diseases, in Atlanta and a Ph.D. student in the Bioinformatics program at Georgia Institute of Technology. Her research interests include integrating advanced laboratory methods, epidemiology and public health strategies to the study of fungal infections.

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Address for correspondence: Kizee A. Etienne, Centers for Disease Control and Prevention, 1600 Clifton Rd NE, Mailstop G11, Atlanta, GA 30329-4027, USA; email: guf1@cdc.gov

Decreased Time to Treatment Initiation for Multidrug-Resistant Tuberculosis Patients after Use of Xpert MTB/RIF Test, Latvia

Helen R. Stagg, Peter J. White, Vija Riekstiņa, Andra Cīrule, Ģirts Šķenders, Vaira Leimane,[1] Liga Kuksa,[1] Gunta Dravniece, James Brown, Charlotte Jackson

Few studies have examined whether the Xpert MTB/RIF test improves time to treatment initiation for persons with multidrug-resistant tuberculosis (MDR TB). We determined the impact of this test in Latvia, where it was introduced in 2010. After descriptive analyses of pulmonary MDR TB patients in Latvia during 2009–2012, time to treatment initiation was calculated, and univariate and multivariable accelerated failure time models were constructed. Univariate results showed strong evidence of an association between having rifampin-resistant TB detected by Xpert MTB/RIF and reduced time to treatment initiation versus the test not being used. A multivariable model stratifying by previous TB showed similar results. Our finding that in Latvia, time to treatment initiation was decreased for MDR TB cases that were rifampin-resistant TB by XpertMTB/RIF has implications for the use of this test in other settings with a high burden of MDR TB in which rifampin resistance is highly predictive of MDR TB.

Timely diagnosis and treatment of multidrug-resistant tuberculosis (MDR TB), of which there were an estimated 480,000 cases in 2014, have been identified as a critical challenge for TB control (*1,2*). In 2010, the World Health Organization endorsed the Xpert MTB/RIF (*Mycobacterium tuberculosis*/rifampin test, hereafter referred to as Xpert; Cepheid, Sunnyvale, CA, USA) as a rapid test for the diagnosis of TB, including rifampin-resistant cases, citing a strong recommendation in their 2013 policy update for its use "as an initial diagnostic test in individuals suspected of having MDR TB or HIV-associated TB" (*3,4*).

Implications of the implementation of Xpert for a country are closely linked to where in the clinical pathway it is placed and how clinicians view the technology. Although many studies (and reviews) have assessed sensitivity, specificity, and predictive values of Xpert (*5*), few studies have examined the impact of this technology on the rapidity with which appropriate treatment is given to TB patients (*6–11*). Even fewer studies have assessed the effects of Xpert on time to MDR TB treatment initiation for MDR TB patients. In South Africa, a reduction in time from first diagnostic sputum collection to treatment commencement from 43 days to 17 days was found when an algorithm based on Xpert was compared with an algorithm based on the line probe assay (LPA) (*12*).

Despite successes in TB and MDR TB control in recent years, Latvia is classified as having a high burden of MDR TB; in 2014, 8.2% of new TB cases and 30% of re-treatment TB cases were estimated to be MDR (*2,13*). Absolute case numbers (48 new and 37 re-treatment in 2014) are relatively low, which is a reflection of TB incidence and population size (*13*). A total of 99% of new cases and 86% of re-treatment cases were reported by the World Health Organization to have been tested phenotypically or genotypically for rifampin-resistant or MDR TB in 2014; the 86% reflects clinical use of prior drug-susceptibility results for re-treatment cases. All MDR TB cases were tested for resistance to second-line drugs in 2013 (*14*). Access to MDR TB treatment is universal in Latvia. Most MDR TB patients are hospitalized, at least for the initial treatment period, in Riga, the capital of Latvia.

Latvia has 2 Xpert systems, both in Riga, where Xpert has been used since 2010. Xpert was initially targeted toward groups at high risk for MDR TB (e.g., contacts of persons with MDR TB). Since 2012, wider use of Xpert was promoted (e.g., for re-treatment cases). Two diagnostic pathways for MDR TB patients were used (Figure 1).

Author affiliations: University College London, London, UK (H.R. Stagg, J. Brown, C. Jackson); Medical Research Council Centre for Outbreak Analysis and Modelling, London (P.J. White); National Institute for Health Research Health Protection Unit in Modelling Methodology, London (P.J. White); Imperial College School of Public Health, London (P.J. White); Public Health England, London (P.J. White); University of Latvia, Riga, Latvia (V. Riekstiņa); Riga East University Hospital, Riga (V. Riekstiņa, A. Cīrule, Ģ. Šķenders, V. Leimane, L. Kuksa); KNCV Tuberculosis Foundation, The Hague, the Netherlands (G. Dravniece); Royal Free London National Health Service Trust, London (J. Brown)

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[1]These authors contributed equally to this article.

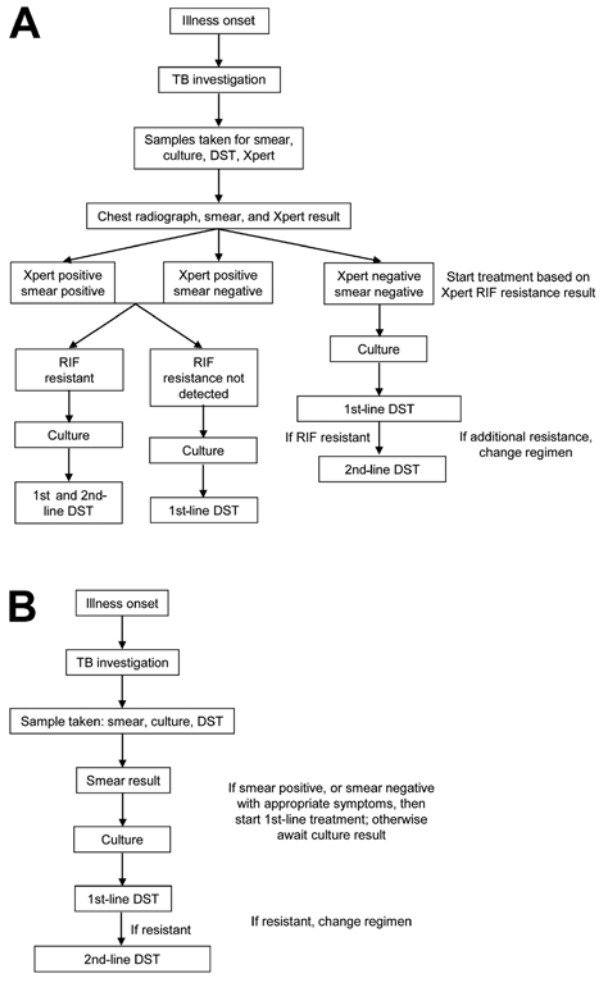

Figure 1. Diagnostic pathways for patients with multidrug-resistant tuberculosis, Latvia, 2012. A) With use of Xpert MTB/RIF; B) without use of Xpert MTB/RIF. A line probe assay was used if Xpert and DST showed discordant results. MTB, *Mycobacterium tuberculosis*; RIF, rifampin; TB, tuberculosis; DST, drug sensitivity testing; Xpert, Xpert MTB/RIF.

When Xpert was available, its results determined whether a patient was initially given treatment for MDR TB. In the absence of Xpert, patients were initially given first-line drugs. In Latvia, rifampin resistance is a good predictor of MDR TB (*15*). The positive predictive value (PPV) of rifampin resistance for MDR is partly determined by MDR TB prevalence (at an MDR TB prevalence ≥14.2% for new TB cases and ≥20% for re-treatment cases, the PPV is 90%) (*16*).

We used surveillance data to examine the relationship between use of Xpert and time to treatment initiation for MDR TB patients in Latvia. We aimed to provide useful data for clinicians and policy makers evaluating implementation of Xpert in settings with a high burden of MDR TB in which rifampin resistance is a good indicator of MDR TB.

Methods

The treatment cohorts for MDR TB patients in Latvia during 2009–2012, excluding cases in prisoners, were obtained from the national MDR TB surveillance system, together with their demographic and clinical data (supplemented from paper records where necessary). Persons with only extrapulmonary MDR TB were excluded, which is consistent with primary use of Xpert. Ethical approval for the study was provided by the ethics committee of Riga Stradins University. Informed consent was not required because this study used an anonymous surveillance dataset.

Age was grouped into 20-year categories (<20, 20–39, 40–59, and ≥60 years); nationality as Latvian or other; residency region in Latvia as Riga or elsewhere (consistent with diagnostic methods available in Riga); and social risk factors (history of imprisonment, history of or current drug abuse, current homelessness, current dependence on alcohol) into a single dichotomous variable of ≥1 risks versus none or unknown. Site of disease was classified as pulmonary or pulmonary and extrapulmonary and HIV status as positive, negative, or unknown. Use of Xpert was categorized as not conducted versus conducted for descriptive analysis; the conducted category was subdivided into conducted and rifampin-resistant TB and conducted and negative result for rifampin-resistant TB. History of TB and sex were treated as binary variables. Reporting date was grouped into year of reporting.

Time between date patient samples were obtained and date of MDR TB treatment initiation (start of MDR TB treatment was defined as start of use of second-line drugs) was calculated and used as the scale (time since entry) for regression analysis. Persons for whom either date was missing were excluded. When persons started MDR TB treatment on the day that their samples were obtained, time to treatment initiation was set to 0.25 days for regression analysis.

Demographic and clinical characteristics of patients were described, and time-to-event (MDR TB treatment initiation) data plotted, including using Kaplan-Meier plots to examine the proportion of persons who had completed treatment at given time points. Before regression modeling, we created a conceptual framework of the relationship between main exposure (Xpert use) and outcome (time to initiation of MDR TB treatment) (Figure 2) and used this framework to identify a priori and potential confounders, as well as effect modifiers (*17*).

We initially assessed use of linear regression to measure the effect of Xpert use on time to treatment initiation. However, the highly skewed distribution of time to treatment initiation (even after log or reciprocal transformation) showed that this technique was not appropriate. Accelerated failure time (AFT) models were chosen as an alternative technique. These models assume that the

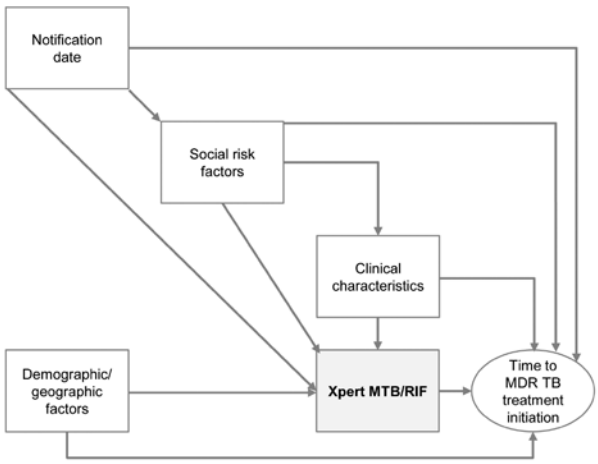

Figure 2. Conceptual framework of relationship between use of Xpert MTB/RIF and time to treatment initiation among patients with multidrug-resistant tuberculosis (MDR TB), Latvia, 2009–2012. Demographic and geographic variables were sex, age, country of birth, and region of Latvia. Clinical variables were previously having had tuberculosis, site of disease, and HIV status. Social risk factor variables were history of imprisonment, history of or current drug abuse, current homelessness, current dependence on alcohol. MTB, *Mycobacterium tuberculosis*; RIF, rifampin.

fraction of persons surviving (i.e., not receiving appropriate treatment) in 1 group at any given time point is proportional to the equivalent fraction of the other group.

We tested this assumption by using quantile–quantile plots, which plot quantiles of survival distribution for the exposed group against those for the unexposed group. AFT models are considered appropriate if these plots approximate a straight line. We then used a univariate model to calculate the time ratio, 95% CI, and p value for the association between Xpert use and time to MDR TB treatment initiation. The time ratio represents the relative time to MDR TB treatment initiation between groups (i.e., a ratio of 0.9 indicates that persons in the exposed [tested] group are 10% more likely to have initiated treatment than those in the unexposed [not tested] group). In a scenario in which everyone initiates treatment, a ratio of 0.9 indicates a 10% reduction in time to initiation among exposed persons. An exponential distribution of survival times was assumed; a Weibull distribution was also investigated in preliminary analyses, but was found to fit the data poorly.

Exposures classified as a priori confounders (sex, age, previous TB) and potential confounders associated with outcome and Xpert use, but not on the causal pathway between these 2 factors, were included in the initial multivariable model. We used a backward deletion strategy in which potential confounders were individually removed and the full (all potential confounders present) and reduced (1 potential confounder removed) models were compared by using square roots of estimated mean squared errors (*18,19*). Thus, a final model was derived while simultaneously enabling assessment of confounding, multicollinearity and maintenance of a priori confounders.

Likelihood ratio tests were conducted to test for linearity (age, year) and effect modification. Region and previous TB were pre-identified as potential effect modifiers. Year of reporting was excluded from initial modeling so that data for 2009 could be used, but was planned to be included as a confounder during sensitivity analysis. Analysis was conducted by using Stata 13 (StataCorp LP, College Station, TX, USA) and Excel (Microsoft, Redmond, WA, USA) software.

Results

Of 398 persons in treatment cohorts in Latvia during 2009–2012, five persons were excluded because of missing dates and 6 more were excluded because they had only extrapulmonary disease. This exclusion resulted in 387 pulmonary TB patients, of whom 262 did not have Xpert testing (100% for 2009, 84% for 2010, 45% for 2011, and 39% for 2012), 110 had rifampin-resistant TB by Xpert, and 15 had negative results for rifampin-resistant TB by Xpert (11 in which TB was not detected, 3 in which rifampin resistance was not detected, and 1 in which rifampin sensitivity was not determined). These 15 patients were subsequently found to have MDR TB after culture and phenotypic testing. Of 387 patients, 295 (76%) were male, 355 (92%) were Latvian, 239 (62%) lived outside Riga, 294 (76%) were HIV negative, and 348 (90%) had only pulmonary TB (Table 1). Data for current homelessness was missing for 1 person. Factors associated with use of Xpert appeared to be notification year, region, having previously had TB, site of disease, and HIV status (Table 1).

For the overall cohort, median time from the date samples were obtained to MDR TB treatment initiation was 27 (95% CI 22–30, range 0–385) days. Sixteen (4%) patients started treatment on the day samples were obtained. When Xpert was not used, median delay was 40 (95% CI 33–45) days. When Xpert was used, median delay was 7 (95% CI 6–8) days. Among persons for whom Xpert was used, median delay was 6 (95% CI 5–7) days when Xpert detected persons to have rifampin-resistant TB and 57 (95% CI 28–99) days when Xpert showed negative results. Overall, median time to treatment initiation in Latvia decreased over time (Figure 3, panel A) and was relatively consistent for persons for whom Xpert was not used, which indicated that additional persons selected for Xpert testing in 2012 were not different in terms of their risk for a longer time to MDR TB treatment initiation than the initial population selected (Figure 3, panel B). These unadjusted figures suggest that time to MDR TB treatment initiation was shorter in persons who underwent Xpert testing (Figure 3, panel B).

A quantile-quantile plot comparing persons for whom Xpert was not used with those who had rifampin-resistant TB by Xpert showed a linear relationship, which supported use of the AFT model (Figure 4, panel A). Linearity could only be tenuously assessed when persons who had negative results by Xpert were compared with either of the other groups. The final data quantile in both instances was highly influential and outlying in each plot, but could not be ignored because of the small number of data points (Figure 4, panels B, C). Given the small number of persons in this third group, their unexpected test results, and that they were likely to have had a different mechanism for a change in the time frame to initiating treatment, they were excluded from the main analysis, which resulted in 372 patients.

A Kaplan-Meier plot of time to MDR TB treatment initiation (Figure 5) indicated a pattern similar to that shown in Figure 3, panel B. This plot shows that persons with rifampin-resistant TB by Xpert began treatment sooner than those who did not have Xpert testing.

For the 372 patients, overall median time to diagnosis was 24 (95% CI 21–29) days (Table 2). A univariate AFT model showed strong evidence for an association between having rifampin-resistant TB by Xpert (baseline: Xpert not done) and reduced time to MDR TB treatment initiation (time ratio 0.19, 95% CI 0.15–0.23, p<0.001; baseline median in model 38 days) (Table 2). This finding corresponded to an absolute reduction of ≈31 days. HIV status also showed strong evidence for an association with time to treatment initiation. There was strong evidence that persons having extrapulmonary and pulmonary TB, having ≥1 social risk factor, and living in Riga led to quicker treatment initiation than for having only pulmonary TB, being without such risk factors, and living outside Riga, retrospectively.

Region, site of disease, and HIV status were associated with the outcome and main exposure and were included with a priori confounders (sex, age, and previous TB) in an initial multivariable model. The backwards deletion strategy removed HIV status. Age was included as a linear

Table 1. Descriptive analysis of 387 patients in MDR TB treatment cohorts, Latvia, 2009–2012*

Variable	Total, no. (%)	Xpert MTB/RIF, no. (%)		
		Not conducted	Conducted, rifampin-resistant TB	Conducted, negative result
Overall exposure	387 (100.0)	262 (67.7)	110 (28.4)	15 (3.9)
Xpert MTB/RIF				
Not conducted	262 (67.7)	NA	NA	NA
Conducted, rifampin-resistant TB	110 (28.4)	NA	NA	NA
Conducted, negative result	15 (3.9)	NA	NA	NA
Year reported				
2009	114 (29.5)	114 (100.0)	0	0
2010	80 (20.7)	67 (83.8)	10 (12.5)	3 (3.8)
2011	91 (23.5)	41 (45.1)	45 (49.5)	5 (5.5)
2012	102 (26.4)	40 (39.2)	55 (53.9)	7 (6.9)
Sex				
M	295 (76.2)	200 (67.8)	88 (29.8)	7 (2.4)
F	92 (23.8)	62 (67.4)	22 (23.9)	8 (8.7)
Age, y				
<20	5 (1.3)	4 (80.0)	1 (20.0)	0
20–39	151 (39.0)	92 (60.9)	53 (35.1)	6 (4.0)
40–59	190 (49.1)	135 (71.1)	47 (24.7)	8 (4.2)
≥60	41 (10.6)	31(75.6)	9 (22.0)	1 (2.4)
Country of birth				
Latvia	355 (91.7)	241 (67.9)	100 (28.2)	14 (3.9)
Other	32 (8.3)	21 (65.6)	10 (31.3)	1 (3.1)
Region of Latvia				
Riga	148 (38.2)	83 (56.1)	56 (37.8)	9 (6.1)
Other	239 (61.8)	179 (74.9)	54 (22.6)	6 (2.5)
Social risk factors				
None or unknown	201 (51.9)	137 (68.2)	58 (28.9)	6 (3.0)
≥1	186 (48.1)	125 (67.2)	52 (28.0)	9 (4.8)
Previous TB				
No	255 (65.9)	154 (60.4)	90 (35.3)	11 (4.3)
Yes	132 (34.1)	108 (81.8)	20 (15.2)	4 (3.0)
Site of disease				
Pulmonary	348 (89.9)	245 (70.4)	91 (26.1)	12 (3.4)
Pulmonary and extrapulmonary	39 (10.1)	17 (43.6)	19 (48.7)	3 (7.7)
HIV status				
Negative	294 (76.0)	210 (71.4)	74 (25.2)	10 (3.4)
Positive	56 (14.5)	24 (42.9)	29 (51.8)	3 (5.4)
Unknown	37 (9.6)	28 (75.7)	7 (18.9)	2 (5.4)

*Prisoners with MDR TB and persons with only extrapulmonary MDR TB were excluded. MDR TB, multidrug-resistant tuberculosis; MTB, *Mycobacterium tuberculosis*; RIF, rifampin; NA, not applicable.

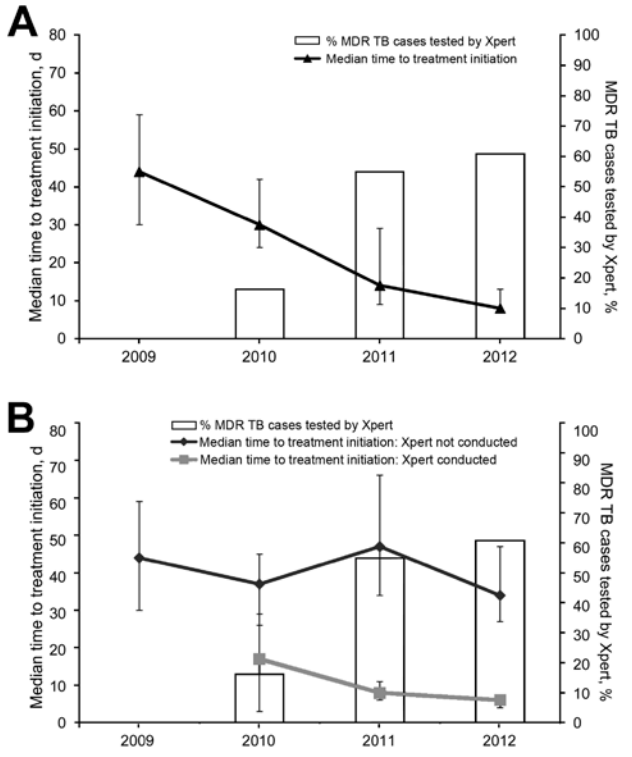

Figure 3. Relationship between use of Xpert MTB/RIF (Xpert) and time to treatment initiation among patients with multidrug-resistant tuberculosis (MDR TB) Latvia, 2009–2012. Shown are percentages of MDR-TB patients that underwent Xpert MTB/RIF testing (bars) and median time to treatment initiation (lines) with binomial distribution–derived CIs (error bars) for A) all patients and B) patients with and without testing by Xpert. MTB, *Mycobacterium tuberculosis*; RIF, rifampin.

variable (p = 0.42, by test for linearity). Region was not an effect modifier (p = 0.44, by test for interaction), unlike having previously had TB (p = 0.01, by test for interaction). Effect estimates for the effect of Xpert are therefore presented stratified by previous TB status (Table 3). Having extrapulmonary and pulmonary TB, and being a female patient were weakly associated with the outcome in the multivariable model, with a reduction in the time to treatment initiation (Table 2).

The effect of Xpert on time to MDR TB treatment initiation for persons who had not previously had TB differed little from the effect found by univariate analysis in the fully adjusted model (time ratio 0.16, 95% CI 0.12–0.21, Wald p<0.001; median 41 days in baseline strata of Xpert) (Table 3). A smaller time reduction was seen for persons who had previously had TB (time ratio 0.34, 95% CI 0.21–0.55, Wald p<0.001; median 34 days in baseline strata of Xpert). Thus, in this fully adjusted and stratified model, time to treatment initiation among persons found to have rifampin-resistant TB by Xpert was reduced by 35 days among patients who had previously had TB and by 22 days among those who had not previously had TB.

We found weak evidence for an association between previously having had TB and time to MDR TB treatment initiation in persons who had not had Xpert testing (time ratio 0.80, 95% CI 0.62–1.04, Wald p = 0.09). Evidence was stronger among persons found to have rifampin-resistant TB by Xpert (effect estimate 1.69, 95% CI 1.03–2.76, Wald p = 0.04).

A sensitivity analysis was conducted to determine the effect of including year as an additional confounder in the model (Figure 2), although time to treatment initiation in the absence of Xpert testing did not appear to have changed during 2010–2012 (Figure 3, panel B). This inclusion reduced overall power because Xpert was not used in 2009 (258 patients). Including year (as a categorical variable; p for linearity <0.001) had little impact on the effect estimate for the effect of Xpert (no previous TB 0.15, 95% CI 0.11–0.21, Wald p<0.001; previous TB 0.45, 95% CI 0.27–0.77, Wald p = 0.003; baseline median 41 days) (Table 4). Effect estimates per year were 1.43 (95% CI 1.00–2.03) for 2011 and 0.74 (95% CI 0.53–1.03) for 2012 (overall p<0.001; baseline 2010).

Inclusion of the 15 patients with MDR TB identified by phenotypic drug sensitivity testing, but not identified as having rifampin-resistant TB by Xpert, enabled Xpert results to be modeled as a 3-tiered variable. This inclusion had little influence on the effect estimate compared with analysis of Xpert use in 2 strata (Xpert not used vs. Xpert used and a positive result for rifampin-resistant TB) (Table 4). Having a negative result by Xpert was weakly associated with longer time to treatment initiation in persons who had previously had TB (effect estimate 2.97, 95% CI 1.07–8.28, Wald p = 0.04; baseline median 41 days), but this association was not seen for persons who had not previously had TB. Running such a model with year included as a confounder (273 patients) yielded similar results, and the previous association appeared stronger (effect estimate 3.63, 95% CI 1.27–10.37, Wald p = 0.02; baseline median 41 days) (Table 4).

Discussion

An unadjusted AFT model showed strong evidence for an association between having rifampin-resistant TB detected by Xpert and a reduction of ≈1 month in time to treatment initiation (time ratio 0.19, 95% CI 0.15 0.23, p<0.001; baseline median in model 38 days) for patients with MDR TB in Latvia during 2009–2012. In a fully adjusted model, this relationship was supported, although the effect of Xpert was smaller for persons who had previously had TB than for those with had not had TB. This finding corresponded to a reduction in median time to treatment initiation of 35 days for persons who had not previously

had TB and 22 days for those who had TB. This reduction is paralleled by a potential lengthening of the timeframe in persons with negative Xpert results who had previously had TB, although our estimates for these persons are highly uncertain. Inclusion of year as a confounder had little effect on the results obtained, which justifies our use of data for 2009 (when Xpert was not available in Latvia).

Data completeness in this cohort was high; thus, bias caused by missing data was not a major concern. Poor recording of dates causing measurement error, where present, was more likely to have been non-differential than differential, reducing precision around the effect estimate. Five patients had no recorded sampling date, but they represented a small fraction of the cohort. Patients with pulmonary TB for whom Xpert was used were likely to have been those producing sputum (i.e., potentially quicker to give a diagnosis of drug-resistant TB even without Xpert testing), although absence of an increase in time to diagnosis among persons for whom Xpert was not used seemed to negate this likelihood. We examined the impact of time to initiation of any MDR TB treatment regimen rather than a regimen tailored to second-line drug sensitivity testing. Given known high levels of additional drug resistance in MDR TB patients in Latvia, it is probable that many patients included in this study had their treatment regimen subsequently altered, which is not captured here and could be the subject of future research (*14*).

In South Africa, Naidoo et al. compared use of Xpert-based and LPA-based algorithms and found a decrease of 25 days in time to MDR TB treatment commencement when Xpert-based algorithms were used (*12*). Although the absolute size of such a decrease is highly context specific to the diagnostic pathway and available resources in each setting, this finding is consistent with our results.

The smaller improvement in time to treatment in persons who had previously had TB and who showed rifampin resistance by Xpert is assumed to be because these persons

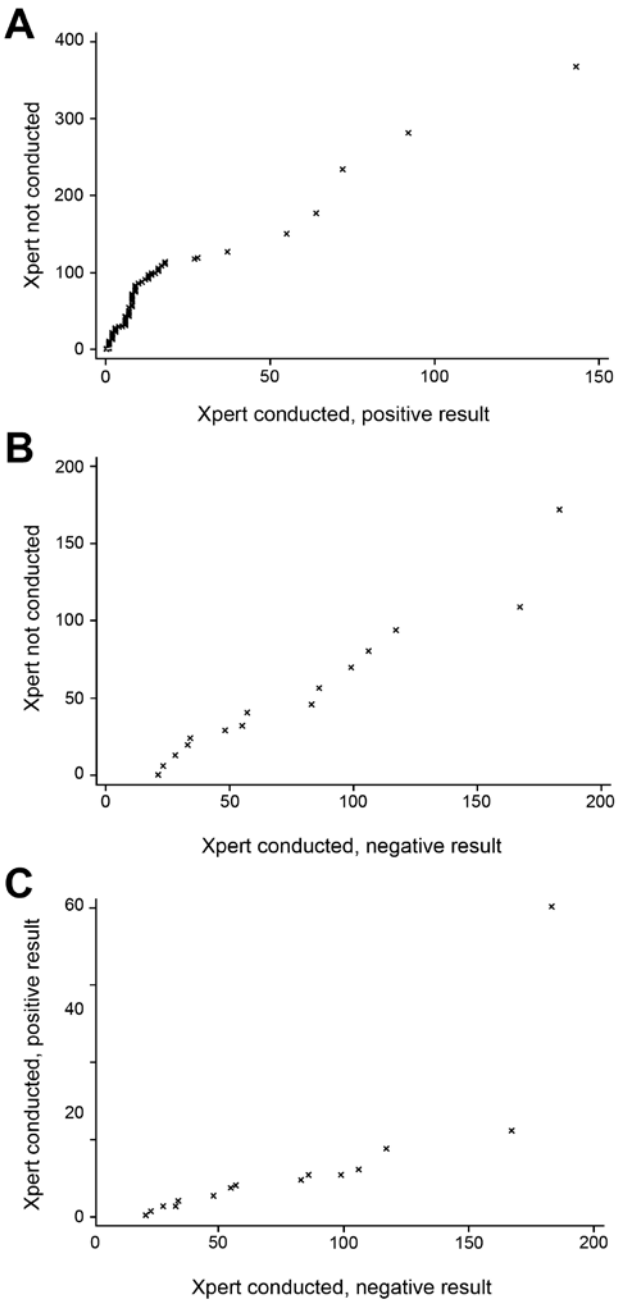

Figure 4. Quantile–quantile plots of time to multidrug-resistant tuberculosis (MDR TB) treatment initiation by use and results of Xpert MTB/RIF (Xpert) for patients with MDR TB, Latvia, 2009–2012. Shown are time to MDR TB treatment initiation (days) for patients A) who were not tested by Xpert vs. those who had rifampin-resistant TB by Xpert, B) those who were not tested vs. those who had a negative result for rifampin-resistant TB, and C) those who were tested by Xpert and had positive vs. negative results for rifampin-resistant TB. MTB, *Mycobacterium tuberculosis*; RIF, rifampin.

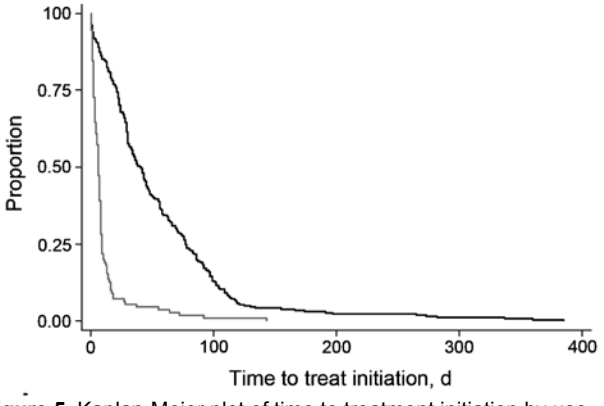

Figure 5. Kaplan-Meier plot of time to treatment initiation by use and results of Xpert MTB/RIF in patients with multidrug-resistant tuberculosis (MDR TB), Latvia, 2009–2012. Shown is time to MDR TB treatment initiation (days) for patients who were not tested by Xpert MTB/RIF (dark gray line) and those who had rifampin-resistant TB by Xpert MTB/RIF (light gray line). MTB, *Mycobacterium tuberculosis*; RIF, rifampin.

Table 2. Univariate and multivariable accelerated failure time models of association between Xpert MTB/RIF use and time to MDR TB treatment initiation for patients in MDR TB treatment cohorts, Latvia, 2009–2012*

Variable	Crude analysis, median days to diagnosis (95% CI)	Univariate regression		Multivariable regression	
		Median days to diagnosis	p value, time ratio (95% CI)	Median days to diagnosis	p value, time ratio (95% CI)
Overall	24 (21–29)	NA	NA	NA	NA
Xpert MTB/RIF					
Not conducted	40 (33–45)	38	<0.001	†	†
Conducted, rifampin-resistant TB	6 (5–7)	NA	0.19 (0.15–0.23)	†	†
Sex					
M	26 (21–30)	30	0.08	30	0.08
F	21 (11–30)	NA	0.80 (0.63–1.02)	NA	0.79 (0.62–1.02)
Age, y					
<20	64 (2–‡)	29	0.98	33	0.78
20–39	21 (9–29)	NA	1.00 (0.87–1.16)	NA	0.98 (0.84–1.14)
40–59	28 (22–30)	NA	NA	NA	NA
≥60	30 (14–48)	NA	NA	NA	NA
Country of birth					
Latvia	26 (21–30)	29	0.63	NA	NA
Other	14 (8–29)	NA	0.91 (0.63–1.32)	NA	NA
Region of Latvia					
Riga	16 (11–24)	NA	0.78 (0.63–0.96)	NA	0.93 (0.75–1.16)
Other	30 (24–36)	32	0.02	32	0.51
Social risk factors					
None or unknown	30 (23–35)	32	0.03	NA	NA
≥1	22 (11–28)	NA	0.79 (0.65–0.97)	NA	NA
Previous TB					
No	24 (18–29)	28	0.39	†	†
Yes	27 (16–35)	NA	1.10 (0.89–1.36)	†	†
Site of disease					
Pulmonary	28 (23–31)	30	0.01	30	0.05
Pulmonary and extrapulmonary	7 (4–11)	NA	0.61 (0.43–0.86)	NA	0.69 (0.49–0.98)
HIV status					
Negative	29 (24–33)	31	0.003	NA	NA
Positive	7 (4–11)	NA	0.59 (0.44–0.79)	NA	NA
Unknown	29 (14–48)	NA	1.00 (0.71–1.43)	NA	NA

*Crude median time to MDR TB treatment initiation was determined (with a binomially derived CI), followed by association between use of Xpert MTB/RIF and time to treatment initiation in univariate and multivariable (372 patients) accelerated failure time models and median time to treatment in such models. Prisoners with MDR TB and persons with only extrapulmonary MDR TB were excluded; 15 patients with negative results by Xpert MTB/RIF were also excluded. Age was treated as a linear variable in regression analyses. The final regression model adjusted for all variables except country of birth, social risk factors, and HIV status. MDR TB, multidrug-resistant tuberculosis; MTB, *Mycobacterium tuberculosis*; RIF, rifampin; NA, not applicable.
†Stratified results are shown in Table 3.
‡No binomial prediction as small stratum.

would be more likely to receive expedited MDR treatment even without use of Xpert. Few patients with MDR TB were not given a diagnosis of rifampin-resistant TB by Xpert (their mechanism for a differential timeframe to start treatment for MDR TB is probably different than that for other patient categories regardless, which made interpretations across strata of Xpert more difficult). However, the suggested increased time to MDR TB treatment initiation in this group when persons had previously had TB appears to indicate that clinicians in Latvia trust Xpert results to

Table 3. Stratified results for multivariable accelerated failure time model of association between Xpert MTB/RIF use and time to MDR TB treatment initiation for patients in MDR TB treatment cohorts, Latvia, 2009–2012*

Exposure	Stratifier	Multivariable regression, time ratio (95% CI)	p value
Xpert MTB/RIF	Previous TB		
Not conducted	No	Baseline	
Conducted, rifampin-resistant TB	No	0.16 (0.12–0.21)	<0.001
Not conducted	Yes	Baseline	
Conducted, rifampin-resistant TB	Yes	0.34 (0.21–0.55)	<0.001
Previous TB	Xpert MTB/RIF		
No	Not conducted	Baseline	
Yes	Not conducted	0.80 (0.62–1.04)	0.09
No	Conducted, rifampin-resistant TB	Baseline	
Yes	Conducted, rifampin-resistant TB	1.69 (1.03–2.76)	0.04

*Association between use of Xpert MTB/RIF and time to MDR TB treatment initiation was stratified by the effect modifier previous tuberculosis. Association between previous tuberculosis and time to MDR TB treatment initiation was stratified by Xpert use. There were 372 patients in the model. All results derived from the final model shown in Table 2. Median in baseline strata of Xpert MTB/RIF and previous tuberculosis was 41 days. MTB,

Table 4. Sensitivity analyses for findings of multivariable accelerated failure time model for association between Xpert MTB/RIF use and time to MDR TB treatment initiation for patients in MDR TB treatment cohorts, Latvia, 2009–2012*

		Time ratio (95% CI), p value		
Exposure: Xpert MTB/RIF	Stratifier: previous TB	Model 1: 2-Strata Xpert, year as confounder	Model 2: 3-Strata Xpert	Model 3: 3-Strata Xpert, year as confounder
Not conducted	No	Baseline	Baseline	Baseline
Conducted, rifampin-resistant TB	No	0.15 (0.11–0.21), <0.001	0.16 (0.12–0.21), <0.001	0.15 (0.11–0.20), <0.001
Conducted, negative result	No	NA	1.17 (0.62–2.22), 0.62	1.09 (0.57–2.09), 0.80
Not conducted	Yes	Baseline	Baseline	Baseline
Conducted, rifampin-resistant TB	Yes	0.45 (0.27–0.77), 0.003	0.33 (0.20–0.54), <0.001	0.45 (0.27–0.76), 0.003
Conducted, negative result	Yes	NA	2.97 (1.07–8.28), 0.04	3.63 (1.27–10.37), 0.02

*Three models of sensitivity of the association between Xpert MTB/RIF use and the time to MDR TB treatment initiation stratified by the effect modifier previous TB were used. Model 1 included year as a confounder (restricting the dataset to 2010–2012; 258 patients). Model 2 included persons who had negative results for rifampin resistance by Xpert MTB/RIF (387 patients). Model 3: models 1 and 2 assessed simultaneously (273 patients). A final model was adjusted for all variables except country of birth, social risk factors, and HIV status (plus year where stated). Median in baseline strata of Xpert MTB/RIF and previous tuberculosis was 41 days for all analyses. A positive result was rifampin-resistant TB, and a negative result was rifampin sensitivity or no TB detected. MTB, *Mycobacterium tuberculosis*; RIF, rifampin. MDR TB, multidrug-resistant tuberculosis; NA, not applicable.

the extent that clinical suspicion of MDR TB, which might expedite starting treatment for MDR TB in the absence of Xpert results, is overridden. Such delays could have negative consequences for patients in terms of having a successful treatment outcome and highlights the need for accurate clinical judgement in diagnostic algorithms.

Latvia also has a few TB cases each year that have discordant results in the opposite direction. A total of 7 patients in 2013 had rifampin-resistant TB by Xpert, but 5 (3 of which showed rifampin resistance by LPA) had rifampin-sensitive results by BACTEC (Becton, Dickinson and Company, Franklin Lakes, NJ, USA), and 2 were not culture positive by BACTEC or on solid media.

Sensitivity of Xpert for detection of rifampin resistance has been documented in many settings, and specific mutations and mixed infections are believed to play a role (*5,20,21*). No interaction was seen in analysis by residential region, which is noteworthy given the time lag incurred by transporting samples to Riga for patients living elsewhere in Latvia, but a positive sign for the over-arching functionality of the TB program.

We made adjustments for temporal changes in our sensitivity analyses. It was reassuring to see a relatively steady time to treatment initiation among patients for whom Xpert was not used, particularly given the change in target populations. This study was restricted to MDR TB treatment cohorts, whereby inclusion in a particular year cohort is determined by the date on which treatment started. Use of Xpert could have also reduced the proportion of patients who did not start treatment because earlier use of treatment might have reduced the likelihood of death and pretreatment default.

Our study in Latvia suggests that implementing Xpert as an early-stage diagnostic tool from which treatment decisions are rapidly made reduces the time to MDR TB treatment initiation. This implementation not only probably benefits patients with MDR TB but might also reduce nosocomial and community transmission (*1*). Other countries should undertake similar research to evaluate the effect of Xpert on time to treatment initiation and treatment outcomes. Careful monitoring of time to treatment initiation could provide valuable performance data for national TB programs, around which targets could be set.

Although cost implications of introducing Xpert are variable in different settings, cost and affordability analyses suggest its viability as a diagnostic tool for detecting MDR TB, particularly in settings that have a high burden of MDR TB in which rifampin resistance has a good PPV for detecting MDR TB (*16,22*). Nevertheless, implementation of Xpert needs to be considered carefully in terms of how it is introduced into diagnostic algorithms, where machines are located, how clinicians interpret test results, and the common sites of TB in a particular population.

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Dr. Stagg is a senior research fellow at University College London, London, UK. Her primary research interests are infectious disease epidemiology for underserved population groups and at the interface with laboratory sciences.

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Address for correspondence: Helen R. Stagg, Research Department of Infection and Population Health, University College London, 4th Fl Mortimer Market Centre, off Capper St, London WC1E 6JB, UK; email: h.stagg@ucl.ac.uk

Factors Associated with Loss to Follow-up during Treatment for Multidrug-Resistant Tuberculosis, the Philippines, 2012–2014

Thelma E. Tupasi, Anna Marie Celina G. Garfin, Ekaterina V. Kurbatova, Joan M. Mangan, Ruth Orillaza-Chi, Leilani C. Naval, Glenn I. Balane, Ramon Basilio, Alexander Golubkov, Evelyn S. Joson, Woo-jin Lew, Vivian Lofranco, Mariquita Mantala, Stuart Pancho, Jesus N. Sarol Jr.

To identify factors associated with loss to follow-up during treatment for multidrug-resistant (MDR) tuberculosis (TB) in the Philippines, we conducted a case–control study of adult patients who began receiving treatment for rifampin-resistant TB during July 1–December 31, 2012. Among 91 case-patients (those lost to follow-up) and 182 control-patients (those who adhered to treatment), independent factors associated with loss to follow-up included patients' higher self-rating of the severity of vomiting as an adverse drug reaction and alcohol abuse. Protective factors included receiving any type of assistance from the TB program, better TB knowledge, and higher levels of trust in and support from physicians and nurses. These results provide insights for designing interventions aimed at reducing patient loss to follow-up during treatment for MDR TB.

The Philippines is 1 of 22 countries considered to have a high burden of tuberculosis (TB) (*1*), including multidrug-resistant (MDR) TB (resistant to isoniazid and rifampin) (*1*). Compared with treatment for drug-susceptible TB, treatment for MDR TB is longer, more expensive, and less effective, and it causes more medication side effects (*2–5*). Resistance to anti-TB drugs has been detected in all regions of the Philippines; an estimated 8,500 MDR TB cases occurred in 2013 (*6*).

Programmatic Management of Drug-resistant Tuberculosis (PMDT) was jointly initiated in the Philippines in October 2000 by the private Makati Medical Center in Metro Manila and the Tropical Disease Foundation, Inc., in collaboration with the National TB Control Program and the local government unit, as the first directly observed therapy (DOTS)–plus pilot project for the management of MDR TB approved by the Green Light Committee (*7*). In 2003, a grant proposal from the Philippines for Round 2 of the Global Fund to Fight AIDS, Tuberculosis and Malaria included treatment for 500 patients with MDR TB (National Tuberculosis Control Program, the Philippines, 2013 Aug 25–Sep 6. Report of the Joint Program Review; 2013 Sep 30, unpub. data). This funding was approved and subsequently expanded to 2,500 MDR TB patients approved to receive treatment according to a Round 5 proposal. In 2010, a new coordination team for PMDT was established by the National TB Control Program/Department of Health; the Lung Center of the Philippines was the implementing arm for PMDT. As of September 2014, a total of 44 PMDT health facilities were located in 16 of 17 regions. The annual number of patients with drug-resistant TB who began receiving treatment under PMDT increased from 191 in the 2005 cohort to 2,056 in the 2012 cohort. Despite substantial progress made by PMDT in the Philippines, the proportion of patients for whom treatment was successful decreased from 73% in the 2005 cohort to 46% in the 2010 cohort, while the proportion of loss to follow-up increased from 13% to 38%, respectively (*8*). Even with recent efforts to improve retention of patients receiving treatment for TB (e.g., providing transportation allowance, financial incentives at treatment milestones, food baskets, and halfway houses for patients from remote areas), the proportion of patients lost to follow-up remains substantial.

Author affiliations: Tropical Disease Foundation, Inc., Makati City, the Philippines (T.E. Tupasi, L.C. Naval, G.I. Balane, E.S. Joson, J.N. Sarol Jr.); Department of Health, Manila, the Philippines (A.M.C.G. Garfin, R. Basilio); Centers for Disease Control and Prevention, Atlanta, Georgia, USA (E.V. Kurbatova, J.M. Mangan); Philippine Business for Social Progress–Innovations and Multisectoral Partnership to Achieve Control of Tuberculosis Project, Manila (R. Orillaza-Chi); US Agency for International Development (USAID), Washington, DC, USA (A. Golubkov); World Health Organization Philippines, Manila (W.-j. Lew); The Lung Center of the Philippines, Manila (V. Lofranco, S. Pancho); Technical Assistance to the Countries–USAID-funded activity, Manila (M. Mantala)

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An effective approach to reducing loss to follow-up during treatment for MDR TB is needed (National Tuberculosis Control Program, the Philippines, 2013 Aug 25–Sep 6. Report of the Joint Program Review; 2013 Sep 30, unpub. data), especially in light of data from a prospective multinational study demonstrating that among MDR TB patients lost to follow-up, almost a third had extensively drug-resistant or pre–extensively drug-resistant TB when treatment was started; drug resistance was acquired during treatment by an additional 12% (*9,10*). In addition, a third of those lost to follow-up remained culture-positive at last contact, enabling community transmission of strains with more extensive resistance (*10*). However, most studies of loss to follow-up were done retrospectively, through medical record reviews (*11–17*), and lacked a theoretical framework. Specific reasons why patients in the Philippines are lost to follow-up during MDR TB treatment are limited and based primarily on the views of healthcare providers. We aimed to determine which individual, diagnosis and treatment, interpersonal, healthcare setting, and social factors were significantly associated with patient loss to follow-up during MDR TB treatment in the Philippines.

Methods

Study Design and Patient Population

We conducted a case–control study among adult patients (≥18 years of age) with confirmed MDR or rifampin-resistant TB for whom treatment was initiated during July 1–December 31, 2012, at PMDT treatment facilities in the Philippines. We excluded from study inmates, children <18 years of age, patients enrolled in pharmaceutical clinical trials, and patients who had a major psychiatric disorder or were physically incapacitated.

The study was approved by the institutional review board of the Tropical Disease Foundation, Inc., the Lung Center of the Philippines–Ethics Review Committee, and the Ethics Research Committee of the Philippine Tuberculosis Society, Inc. The US Centers for Disease Control and Prevention (CDC) determined that CDC staff involvement did not constitute engagement in human subject research and that submission for CDC institutional review board review was not required.

In the Philippines, the standardized treatment regimen for MDR TB is pyrazinamide, kanamycin, levofloxacin, prothionamide, and cycloserine; the intensive phase lasts ≥6 months and the continuation phase an additional ≥12 months. For this study, case-patients were defined as patients who were lost to follow-up from MDR TB treatment (i.e., patients whose treatment was interrupted for ≥2 consecutive months) (*18*). Those who later returned (after being considered lost to follow-up) at the time of interview were eligible for inclusion in the study as case-patients. Control-patients were defined as patients who had continued treatment for MDR TB for ≥12 months or who had a documented treatment outcome of cured, completed, or failed (*18*). Data collection and interviews were conducted from April 14 through July 31, 2014; thus, all control-patients were receiving MDR TB treatment for ≥15 months.

Case-patients were identified by review of MDR TB registers. The number of patients who were lost to follow-up per treatment facility was assessed; centers with ≥3 patients with drug-resistant TB who had been lost to follow-up by January 1, 2014, and who were not known to have died, were selected for logistical reasons. Field study staff attempted to find all eligible patients who were lost to follow-up and invite them to participate in the study. Two control-patients were randomly selected for each enrolled case-patient from the same PMDT treatment facilities at which treatment was initiated for case-patients. Of 986 eligible patients, a total of 273 were enrolled: 91 case-patients and 182 control-patients (Figure 1).

To characterize factors associated with loss to follow-up during MDR TB treatment, we followed a 5-level social ecologic model (*19,20*) that focuses on individual and environmental factors that affect health outcomes: 1) individual factors; 2) interpersonal factors; 3) healthcare setting factors, such as individual experiences with services and relationships within the setting; 4) diagnosis and treatment factors; and 5) social factors (Figure 2). To operationalize each category of factors and develop data collection forms, investigators reviewed TB literature and a 2013 Joint Program Review of the National Tuberculosis Control Program in the Philippines, which was led by the World Health Organization (WHO), and solicited input from experts within the country.

Data Collection

Clinical and laboratory data were abstracted from participants' medical records by using standard data collection forms. In-depth interviews with a series of closed- and open-ended questions were conducted to collect information about the following: demographics, social history, adverse drug reaction experiences, TB knowledge, perceived barriers to treatment completion, self-efficacy to adhere to treatment (*21*), values and expectancies associated with treatment; psychosocial factors (e.g., stigma, sources of emotional support, and family dynamics), financial support, perceptions of the healthcare setting, their diagnosis, their prescribed treatment, impressions of and feedback for the PMDT program regarding the current TB enabler package and patient-centered activities, and interventions under consideration. Cumulative scores were calculated from items focused on 1) patient perceptions of disease severity and TB knowledge; 2) expected outcomes, treatment self-efficacy; 3) patient-reported social

support from family and friends; 4) trust in, rapport with, and support from health center staff; and 5) stigma (Table 1). Case-patients were asked to report their primary reason for stopping treatment. (Interview and scoring instruments are available from T.E.T.)

Data Analysis

Data were entered in a Microsoft (Redmond, WA, USA) Access electronic database. Statistical analyses were performed by using SAS software version 9.3 (SAS Institute Inc., Cary, NC, USA). Thematic qualitative analysis was conducted by using Microsoft Excel software.

We assessed associations between individual, interpersonal, healthcare setting, diagnosis and treatment, and social factor data and an outcome of lost to follow-up. For continuous variables, we compared means (SDs) or medians (percentiles) or both, depending on the underlying distributions. The proportions of patients with characteristics of interest were compared between case-patients and control-patients by χ^2 or Fisher exact tests, as appropriate. We calculated odds ratios with corresponding 95% CIs. To identify independent factors associated with loss to follow-up, we performed multivariable logistic regression analyses. The initial multivariable model included covariates with epidemiologic, biological, or statistical associations with the dependent variable. We evaluated effect modification and confounding in the full model, and then we performed backward elimination to improve precision of the estimates (*22*). All tests were 2-sided, and a p value of ≤ 0.05 was considered statistically significant.

Results

Mean ± SD age of the 273 study participants at start of MDR TB treatment was 39 ± 13 (median 40, range 16–68)

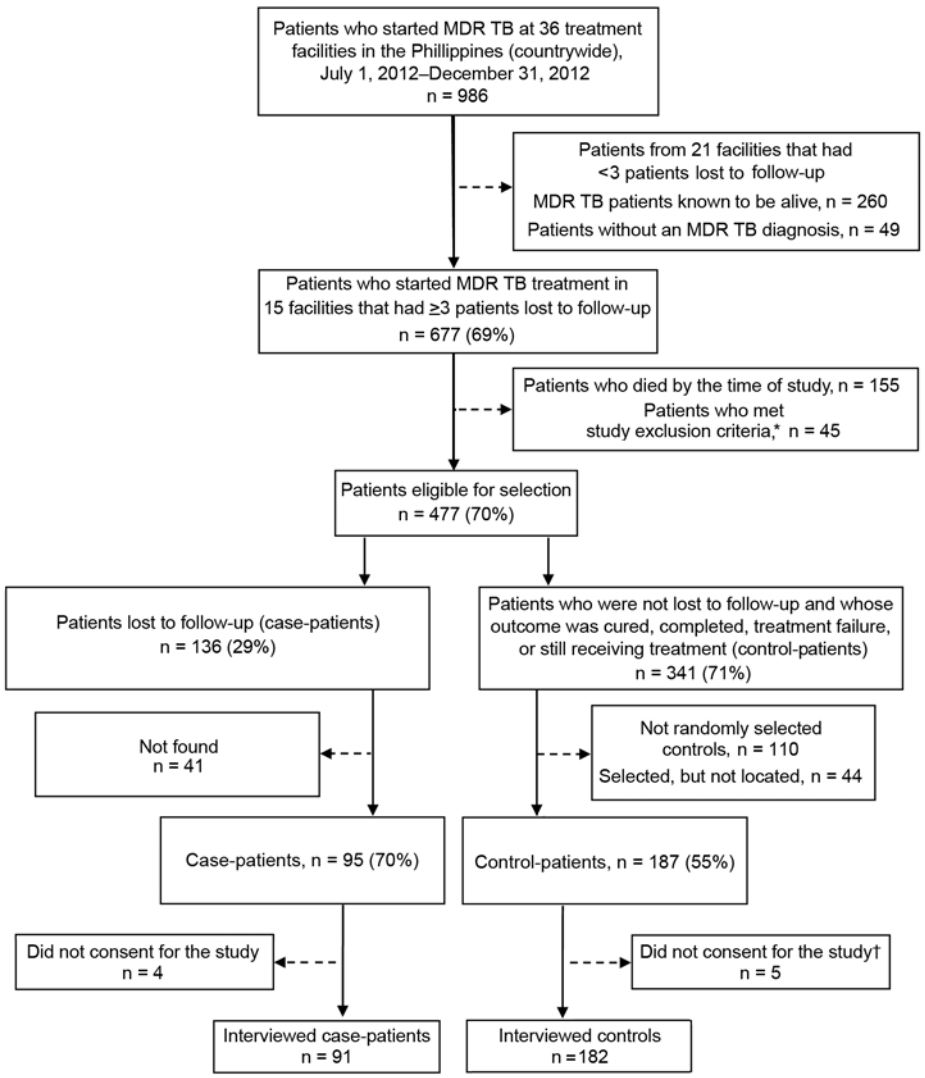

Figure 1. Selection of participants for study of loss to follow-up during treatment for multidrug resistant tuberculosis (MDR TB) in the Philippines, 2012–2014. *Study exclusion criteria were incarceration, age <18 years, enrollment in pharmaceutical clinical trials, and major psychiatric disorder or physical incapacitation. †Control-patients who did not give consent for the study were replaced by other randomly selected eligible patients.

years; 164 (60.1%) were male. An HIV test result was recorded for 56 (20.5%) of the 273 patients; 2 (3.5%) were HIV positive. All patients had pulmonary TB, and 35% had cavitary TB.

Most (70 [77.8%]) of the 90 case-patients for whom information on length of treatment was available were lost to follow-up during the intensive phase of treatment. Mean ± SD time receiving MDR TB treatment for case-patients was 7.8 ± 3.4 months (median 7 months; 25th percentile 4 months; 75th percentile 11 months) and for control-patients was 19.8 ± 1.7 months (median 20 months; 25th percentile 19 months; 75th percentile 21 months). Most (121 [66.5%]) of the 182 control-patients were still receiving treatment at the time of interview. Among 61 control-patients for whom treatment outcome was available, 35 (57.4%) experienced cure, 24 (39.3%) completed treatment, and 2 (3.3%) experienced treatment failure.

Univariate analysis indicated that individual factors significantly associated with loss to follow-up included older age (mean ± SD age 42 ± 13 years for case-patients vs. 38 ± 12 years for control-patients; p = 0.028); tobacco smoking (odds ratio [OR] 2.86, 95% CI 1.65–4.97); alcohol abuse (OR 1.93, 95% CI 1.09–3.40); and residence in an urban slum (OR 0.52, 95% CI 0.29–0.91) (Table 2). General knowledge of TB was significantly lower among case-patients than among control-patients; knowledge included understanding of the severity of and susceptibility to the disease (mean ± SD score 67.8 ± 16.3 vs. 74.3 ± 13.8, respectively; p<0.001), but recall for self-confidence for adhering to treatment at the time of treatment start was significantly higher among case-patients (4.9 ± 5.3 vs. 2.44 ± 3.8, respectively, p<0.001) (*20*).

Univariate analysis also indicated that the only interpersonal factor significantly associated with loss to follow-up was social support from family and friends. Scores were lower among case-patients than among control-patients (mean ± SD score 12.1 ± 3.4 vs. 12.9 ± 3.0, respectively, p = 0.047) (Table 3).

Among healthcare setting factors, case-patients reported having received any type of assistance from the TB program significantly less often than control-patients: overall (OR 0.11, 95% CI 0.04–0.29), food (OR 0.46, 95% CI 0.27–0.79), free medications for treatment of adverse drug reactions (OR 0.28, 95% CI 0.16–0.48), or money for transportation (OR 0.19, 95% CI 0.1–0.37) (Table 4). Scores for trust in, rapport with, and support from physicians and nursing staff were significantly lower among case-patients than among control-patients (mean ± SD score 81.9 ± 15.6 vs. 90.7 ± 7.8, respectively; p<0.001), as were scores for trust in and rapport with physicians (56.0 ± 11.5 vs. 62.2 ± 5.4, respectively; p<0.001), trust in and rapport with nurses (21.8 ± 3.7 vs. 23.8 ± 2.4, respectively; p<0.001), and information and support received from healthcare center staff (7.5 ± 1.7 vs. 8.4 ± 1.2, respectively; p<0.001).

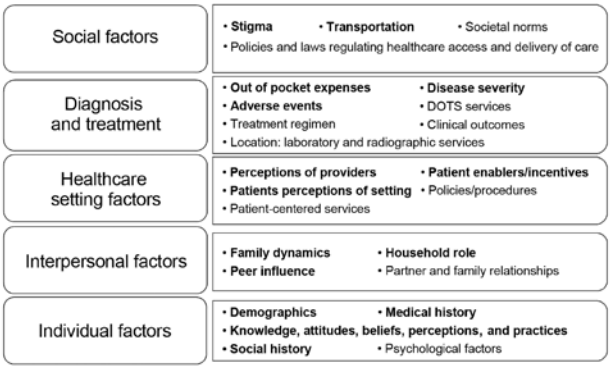

Figure 2. Social ecologic model used to identify factors influencing loss to follow-up during treatment for multidrug resistant tuberculosis in the Philippines, 2012–2014. Boldface indicates data collected through patient interviews and medical record abstractions. DOTS, directly observed therapy.

Among diagnosis and treatment factors, frequency of certain adverse drug reactions reported by patients did not differ significantly, except for less frequently reported diarrhea among case-patients (OR 0.49, 95% CI 0.29–0.85). However, scores for self-reported severity of adverse drug reactions were significantly higher among case-patients; reactions included vomiting (mean ± SD 5.23 ± 3.72 vs. 4.02 ± 3.46; p = 0.008), dizziness (5.48 ± 3.51 vs. 4.55 ± 3.14; p = 0.029), and fatigue or extreme tiredness (5.18 ± 3.56 vs. 4.14 ± 3.25; p = 0.017) (Tables 5, 6). Reported cost of travel to the treatment center during the intensive phase of treatment was significantly higher among case-patients than among control-patients (mean ± SD 98 ± 104 pesos vs. 71 ± 57 pesos, respectively, p = 0.035).

Univariate analysis indicated that social factors significantly associated with loss to follow-up were self-reported lack of time to go to the treatment facility (OR 2.23, 95% CI 1.1–4.54) and absence of someone to accompany the patient to the treatment facility during the intensive phase of treatment (OR 1.97, 95% CI 1.06–3.65). Scores reflecting patient self-stigmatization among case-patients and control-patients did not differ significantly (p = 0.10) (Table 7).

Independent factors positively associated with loss to follow-up were alcohol abuse (OR 2.84, 95% CI 1.39–5.80) and patient higher self-rating of vomiting severity (OR 1.10, 95% CI 1.01–1.21, per 1 point in severity rating score). Factors protective against loss to follow-up were receipt of any type of assistance from the TB program (OR 0.09, 95% CI 0.03–0.25); better general TB knowledge (OR 0.97, 95% CI 0.95–0.99, per 1 point in cumulative score); and higher levels of trust in, rapport with, and support from physicians and nursing staff (OR 0.93, 95% CI 0.90–0.96, per 1 point in cumulative score) (Table 8).

Table 1. Calculation of summary scores in study of loss to follow-up during treatment for multidrug-resistant TB, the Philippines, 2012–2014*

Score type	Score calculation	Score interpretation
General TB knowledge, including understanding of severity of the disease and susceptibility to the disease	Participants were asked 15 questions that focused on 1) the severity of the TB problem in their community, 2) TB transmission and morbidity/mortality, and 3) TB treatment. Each item answered correctly was awarded 1 point. Incorrect answers or "Not sure" responses received 0 points. The summary score was extrapolated onto a scale of 100 and reported as a percentage by using the following formula: General TB knowledge score = (total points earned/15) × 100 (i.e., score is calculated on a scale of 0–100%).	A higher score may indicate greater TB knowledge and greater perceived severity and susceptibility to the disease.
Expectations related to TB and its treatment	Participants were asked 5 interview questions aimed at determining their concerns for passing TB to loved ones, relapsing, and developing worsening drug resistance. Possible range of scores 5–15.	A higher score may indicate greater concerns or an expectation that TB could cause problems in the future. In addition to factors such as knowledge, attitudes, and beliefs, expected outcomes can determine a person's actions. These expectations may be derived from 1) previous experiences, 2) observing or hearing about others in similar situations, 3) persuasive conversations, or 4) emotional or physical responses.
Self-efficacy (or confidence) to adhere to treatment at the time treatment was about to begin	Eight interview questions were included in the self-efficacy questionnaire. "Very confident" = 3 points, "A little confident" = 2 points, "unsure" = 1 point, and "I knew I could not do this" = 0 points. The score for each item would be added together to calculate a cumulative self-efficacy score. Possible range of scores 0–24 points.	A higher score may indicate a high degree of self-reported self-efficacy for adhering to treatment regimen, coping with the treatment, and meeting with DOT staff when about to start treatment.
Social support from family and friends	Score was based on responses to 3 interview questions with possible range of scores 3–15.	Lower scores may indicate less support.
Trust in, rapport with, and support from physicians and nursing staff	An overall score was based on 22 items grouped together. Possible range of scores 22–110.	A higher score may indicate a greater level of trust, rapport, and perceived support. Items were separated by topic, and separate scores were also calculated for participants' 1) trust in, and rapport with physicians (13 questions); 2) trust in, and rapport with nurses (5 questions); and 3) perceived support from health center staff (4 questions).
Patient self-stigmatization	A cumulative score for stigma was based on 2 interview questions. Possible range of scores 1–10.	A higher score may indicate less stigma.

*DOTS, directly observed therapy; TB, tuberculosis.

The primary reason for stopping treatment, most commonly reported by case-patients, was medication side effects or the fear of side effects, reported by 52 (58%) of 89 case-patients who responded to this question. The 2 other most commonly self-reported reasons for loss to follow-up were need to work and financial problems, reported by 25 (28%) of 89 patients, and lack of money for transportation to the treatment facility, reported by 18 (20%) of 89 patients.

Discussion

This large study, guided by a 5-level theoretical social ecologic model, demonstrated that loss to follow-up from MDR TB treatment in the Philippines was independently associated with 2 individual factors (general TB knowledge and alcohol abuse), 2 healthcare setting factors (receiving any type of assistance from the TB program and levels of trust in, rapport with, and support from physicians, nursing staff and other healthcare workers in the treatment facilities), and 1 diagnosis and treatment factor (higher self-rated severity of vomiting as an adverse drug reaction). Multivariable analysis did not identify any interpersonal or social factors associated with loss to follow-up. The most commonly reported primary reason for loss to follow-up was medication side effects or fear of side effects.

This study demonstrated that general TB knowledge was significantly lower among case-patients than among control-patients. Although nonadherence to treatment rarely results from patient apathy, patients' lack of knowledge of their medical condition and its treatment is associated with poor health outcomes (*23*). For this reason, patient education is a valuable component of TB control. A recent systematic review and meta-analysis demonstrated that provision of patient education was a strategy associated with lower rates of loss to follow-up (*24*). Among TB patients, interventions to improve general TB knowledge are significantly associated with better outcomes (*25*). Knowledge of the bacterial causation of TB, mode of transmission, diagnostic testing, meaning of test results,

Table 2. Univariate analysis of Individual factors associated with loss to follow-up during treatment for multidrug-resistant TB, the Philippines, 2012–2014*

Factor	Total†	Case-patients‡	Control-patients‡	Odds ratio (95% CI)	p value
Data from review of medical records					
Demographics					
Sex					
M	164	60 (65.9)	104 (57.1)	1.45 (0.86–2.45)	0.16
F	109	31 (34.1)	78 (42.9)	1.00	
Age	273	41.6 (13.2)§	38.0 (12.5)§	1.02 (1.00–1.04)¶	**0.028**
Social history					
Tobacco smoking					
Current, past	153	65 (73)	88 (48.6)	2.86 (1.65–4.97)	**<0.001**
Never	117	24 (27)	93 (51.4)	1.00	
Alcohol abuse					
Current, past	175	66 (75)	109 (60.9)	1.93 (1.09–3.4)	**0.02**
Never	92	22 (25)	70 (39.1)	1.00	
Drug abuse					
Current, past	52	19 (22.4)	33 (18.6)	1.26 (0.67–2.37)	0.48
Never	210	66 (77.6)	144 (81.4)	1.00	
Clinical information					
No. previous TB episodes	273	1.71 (0.90)§	1.62 (1.07)§	1.10 (0.86–1.40)¶	0.449
BMI					
<18.5	146	51 (56)	95 (52.2)	1.17 (0.7–1.94)	0.55
≥18.5	127	40 (44)	87 (47.8)	1.00	
Cavitary TB disease					
Yes	97	31 (44.3)	66 (41)	1.14 (0.65–2.02)	0.64
No	134	39 (55.7)	95 (59)	1.00	
Smear-positive at treatment start					
Yes	219	70 (82.4)	149 (85.1)	0.81 (0.41–1.63)	0.56
No	41	15 (17.6)	26 (14.9)	1.00	
Data from patient interviews					
Total no. persons residing in household	272	5.12 (2.79)§	5.53 (2.95)§	1.94 (0.63–5.99)¶	0.27
Residence, comparison 1					
Rural area	40	15 (23.4)	25 (24.5)	0.94 (0.45–1.96)	0.88
Urban slum	126	49 (76.6)	77 (75.5)	1.00	
Residence, comparison 2					
Urban area	105	26 (34.7)	79 (50.6)	0.52 (0.29–0.91)	**0.02**
Urban slum	126	49 (65.3)	77 (49.4)	1.00	
Paid employment before starting treatment					
Yes	126	49 (54.4)	77 (42.3)	1.63 (0.98–2.71)	0.06
No	146	41 (45.6)	105 (57.7)	1.00	
Employed before starting treatment but had to quit#					
Yes	90	35 (79.5)	55 (83.3)	0.78 (0.29–2.07)	0.61
No	20	9 (20.5)	11 (16.7)	1.00	
Employed before starting treatment but fired/asked to take leave of absence#					
Yes	10	5 (35.7)	5 (31.3)	1.22 (0.27–5.59)	0.80**
No	20	9 (64.3)	11 (68.8)	1.00	
Family sold belongings or household items (assets) to help pay expenses during TB treatment					
Yes	93	28 (31.8)	65 (35.7)	0.84 (0.49–1.44)	0.53
No	177	60 (68.2)	117 (64.3)	1.00	
Family borrowed money to cover costs due to TB illness					
Yes	181	60 (74.1)	121 (70.8)	1.18 (0.65–2.14)	0.58
No	71	21 (25.9)	50 (29.2)	1.00	
General TB knowledge††	272	67.81 (16.31)§	74.25 (13.78)§	0.97 (0.95–0.99)¶	**<0.001**
Expectations related to TB and TB treatment	272	11.01 (1.87)§	10.76 (1.55)§	1.10 (0.94–1.28)¶	0.28
Self-efficacy (or confidence) to adhere to treatment at the time treatment was about to begin	272	4.91 (5.28)§	2.44 (3.77)§	1.13 (1.06–1.19)¶	**<0.001**

*Boldface indicates significance. BMI, body mass index; TB, tuberculosis.
†Total reflects number of patients for whom data or responses for each respective category were available.
‡No. (%) unless noted otherwise.
§Mean (SD).
¶Odds ratio is per 1 unit increase.
#Of 126 patients who had paid employment before starting treatment, 90 reported that they subsequently "had to quit" and 10 reported that they had subsequently been "fired/asked to take leave of absence."
**Fisher exact test.
††Such as understanding of disease severity, susceptibility, scale 0%–100%).

Table 3. Univariate analysis of interpersonal factors associated with loss to follow-up during treatment for multidrug-resistant TB, the Philippines, 2012–2014*

Characteristic, from data from patient interviews	Total	Case-patients†	Control-patients†	Odds ratio (95% CI)	p value
Head of household‡					
Yes	91	37 (41.1)	54 (29.7)	1.65 (0.98–2.8)	0.06
No	181	53 (58.9)	128 (70.3)	1.00	
In charge of household budget					
Yes	96	32 (35.6)	64 (35.2)	1.02 (0.6–1.72)	0.95
No	176	58 (64.4)	118 (64.8)	1.00	
Social support from family and friends	271	12.07 (3.35)§	12.87 (2.99)§	0.92 (0.85–1.00)¶	**0.047**

*Boldface indicates significance. TB, tuberculosis.
†No (%) unless noted otherwise.
‡Provided more than half the cost of keeping up a home the year before becoming sick with TB.
§Mean (SD).
¶Odds ratio is per 1 unit increase.

rationale for prolonged treatment, and effect of treatment interruptions should be clearly explained to patients and their loved ones. It is also crucial to educate patients about expected adverse events before starting treatment. Patient education that addresses commonly held misperceptions about TB may also discourage patients from consulting traditional healers, thereby avoiding delayed diagnosis and treatment (*26*).

Alcohol abuse was recorded in medical records at a significantly higher frequency for case-patients than for control-patients. The association between alcohol abuse or alcohol use disorders and loss to follow-up during MDR TB treatment has been demonstrated in multiple studies (*13–15*,*27*); 1 small randomized clinical trial demonstrated improved TB outcomes for patients in groups randomly assigned to receive naltrexone or behavioral counseling integrated into TB care (*28*). This finding, when combined with similar findings in other studies, calls for additional studies to assess the effect of using standard assessment tools to screen for alcohol dependence and of managing alcohol use as part of TB clinical services.

Receiving assistance from the TB program, including such measures as covering the cost of transportation, food, and housing, was associated with improved treatment adherence. However, if the process of applying for financial assistance is long and difficult and if this assistance is not given in a timely manner or regularly, patients may abandon their efforts to adhere to treatment. Decentralization of treatment (i.e., the transfer of care from a centralized MDR TB treatment center to a community DOTS facility) was protective; odds of being lost to follow-up decreased by 10 times (*12*). This finding suggests that the decentralization of care into multiple treatment facilities closer to patients' homes, which was used as part of the PMDT scale-up scheme in the Philippines, is a valid strategy for improving TB treatment patient retention. In addition to decentralization, the National TB Control Program began piloting the

Table 4. Univariate analysis of healthcare setting factors associated with loss to follow-up during treatment for multidrug-resistant TB, the Philippines, 2012–2014*

Characteristic, from data from patient interviews	Total	Case-patients†	Control-patients†	Odds ratio (95% CI)	p value
Treatment center provided financial assistance or other items to facilitate treatment adherence					
Yes	245	69 (76.7)	176 (96.7)	0.11 (0.04–0.29)	**<0.001**
No	27	21 (23.3)	6 (3.3)	1.00	
Types of assistance provided					
Food products					
Yes	114	27 (29.7)	87 (47.8)	0.46 (0.27–0.79)	**0.004**
No	159	64 (70.3)	95 (52.2)	1.00	
Housing assistance					
Yes	15	6 (6.6)	9 (4.9)	1.36 (0.47–3.94)	0.57
No	258	85 (93.4)	173 (95.1)	1.00	
Free medications to treat side effects from anti-TB drugs					
Yes	196	49 (53.8)	147 (80.8)	0.28 (0.16–0.48)	**<0.001**
No	77	42 (46.2)	35 (19.2)	1.00	
Money for transportation					
Yes	224	59 (64.8)	165 (90.7)	0.19 (0.1–0.37)	**<0.001**
No	49	32 (35.2)	17 (9.3)	1.00	
Trust in, rapport with, and support from physicians and nursing staff	273	81.88 (15.56)‡	90.73 (7.79)‡	0.93 (0.91–0.96)§	**<0.001**
Information and support from healthcare center staff	272	7.53 (1.68)‡	8.43 (1.22)‡	0.64 (0.52–0.78)§	**<0.001**

*Boldface indicates significance. TB, tuberculosis.
†No (%) unless noted otherwise.
‡Mean (SD).
§Odds ratio is per 1 point increase in cumulative score.

Community-Based PMDT Care Initiative in 2014 to provide a more accessible venue for management of MDR TB in the patient's home. The effectiveness of this initiative in improving treatment adherence should be rapidly evaluated, and the evaluation results should be used for future program planning. All modalities for addressing patient barriers should be considered. Adherence to MDR TB treatment might be improved by providing sufficient and timely financial assistance to patients (especially for transportation) by augmenting current enablers and providing livelihood programs during and after MDR TB treatment through strategic multisectoral partnership.

Table 5. Univariate analysis of diagnosis and treatment factors associated with loss to follow-up during treatment for multidrug-resistant TB, the Philippines, 2012–2014*

Characteristic, data from patient interviews	Total	Case-patients†	Control-patients†	Odds ratio (95% CI)	p value
Side effects during MDR TB treatment‡					
Nausea					
Yes	230	74 (81.3)	156 (85.7)	0.73 (0.37–1.42)	0.35
No	43	17 (18.7)	26 (14.3)	1.00	
Vomiting					
Yes	210	74 (81.3)	136 (74.7)	1.47 (0.79–2.75)	0.22
No	63	17 (18.7)	46 (25.3)	1.00	
Diarrhea					
Yes	195	56 (61.5)	139 (76.4)	0.49 (0.29–0.85)	**0.01**
No	78	35 (38.5)	43 (23.6)	1.00	
Headache					
Yes	212	68 (75.6)	144 (79.1)	0.82 (0.45–1.48)	0.50
No	60	22 (24.4)	38 (20.9)	1.00	
Sleep disturbances					
Yes	231	75 (83.3)	156 (85.7)	0.83 (0.42–1.67)	0.61
No	41	15 (16.7)	26 (14.3)	1.00	
Tingling/pain in hands or feet					
Yes	187	55 (61.1)	132 (72.5)	0.6 (0.35–1.02)	0.06
No	85	35 (38.9)	50 (27.5)	1.00	
Hearing problems					
Yes	202	68 (75.6)	134 (73.6)	1.11 (0.62–1.98)	0.73
No	70	22 (24.4)	48 (26.4)	1.00	
Dizziness					
Yes	231	75 (83.3)	156 (85.7)	0.83 (0.42–1.67)	0.61
No	41	15 (16.7)	26 (14.3)	1.00	
Nervousness/anxiety					
Yes	160	51 (56.7)	109 (59.9)	0.88 (0.53–1.46)	0.61
No	112	39 (43.3)	73 (40.1)	1.00	
Pain in joints					
Yes	221	68 (75.6)	153 (84.1)	0.59 (0.31–1.09)	0.09
No	51	22 (24.4)	29 (15.9)	1.00	
Vision problems					
Yes	155	53 (58.9)	102 (56)	1.12 (0.67–1.87)	0.66
No	117	37 (41.1)	80 (44)	1.00	
Fatigue/extreme tiredness					
Yes	208	70 (77.8)	138 (75.8)	1.12 (0.61–2.04)	0.72
No	64	20 (22.2)	44 (24.2)	1.00	
Participant travel expenses					
Cost to travel to treatment center, intensive phase of treatment	239	97.58 (103.64)§	70.83 (57.24)§	1.00 (1.00–1.01¶	**0.035**
Cost to travel to treatment center, continuation phase of treatment	138	67.93 (65.24)§	52.46 (39.37)§	1.01 (0.00–1.02)¶	0.383
Source of funds to travel to/from treatment center, intensive phase of treatment					
Own/personal money					
Yes	203	68 (74.7)	135 (74.2)	1.03 (0.58–1.83)	0.92
No	70	23 (25.3)	47 (25.8)	1.00	
TB program funds					
Yes	183	48 (52.7)	135 (74.2)	0.39 (0.23–0.66)	**<0.001**
No	90	43 (47.3)	47 (25.8)	1.00	
Borrowed money					
Yes	99	38 (41.8)	61 (33.5)	1.42 (0.85–2.39)	0.18
No	174	53 (58.2)	121 (66.5)	1.00	

*Boldface indicates significance. TB, tuberculosis.
†No. (%) unless noted otherwise.
‡Participants were asked to rate severity of medication side effects on a scale of 1–10 (0 = absence of symptoms; 1 = very mild, 10 = extremely severe).
§Mean (SD).
¶Odds ratio is per 1 point increase in cost unit.

Table 6. Rating of the severity of medication side effects experienced during treatment for multidrug-resistant TB, the Philippines, 2012–2014*

Side effect	Total	Case-patients, mean (SD) score	Control-patients, mean (SD) score	Odds ratio (95% CI)†	p value
Nausea	273	5.05 (3.51)	4.42 (3.18)	1.06 (0.98–1.14)	0.136
Vomiting	273	5.23 (3.72)	4.02 (3.46)	1.10 (1.02–1.18)	**0.008**
Diarrhea	273	3.34 (3.41)	3.74 (3.18)	0.96 (0.89–1.04)	0.345
Headache	272	4.37 (3.36)	4.20 (3.23)	1.02 (0.94–1.10)	0.689
Sleep disturbances	272	5.23 (3.59)	5.31 (3.26)	0.99 (0.92–1.07)	0.854
Tingling/pain in hands or feet	272	3.58 (3.53)	4.14 (3.56)	0.96 (0.89–1.03)	0.218
Hearing problems	272	4.49 (3.47)	4.13 (3.46)	1.03 (0.96–1.11)	0.417
Dizziness	272	5.48 (3.51)	4.55 (3.14)	1.09 (1.01–1.18)	**0.029**
Nervousness/anxiety	272	3.26 (3.34)	3.01 (3.16)	1.02 (0.95–1.11)	0.548
Skin problems or rash	272	2.70 (3.26)	2.69 (3.22)	1.00 (0.93–1.08)	0.975
Joint pain	272	5.14 (3.57)	5.49 (3.34)	0.97 (0.90–1.04)	0.427
Vision problems	272	3.10 (3.07)	2.80 (3.05)	1.03 (0.95–1.12)	0.45
Fatigue/extreme tiredness	272	5.18 (3.56)	4.14 (3.25)	1.10 (1.02–1.18)	**0.017**

*Boldface indicates significance. TB, tuberculosis.
†Odds ratio is per 1 point increase in severity rating score.

We found that levels of trust in, rapport with, and support from physicians, nursing staff, and other caregivers in the treatment facilities were significantly lower among patients lost to follow-up than among control-patients. A higher degree of trust, good rapport, and support from providers has been shown to be associated with patients' adherence to medical recommendations and with improvements to self-reported and objective measures of health (*29–31*). In a study by Holtz et al. in South Africa, the strongest individual risk factor for nonadherence to MDR TB treatment was having an unsatisfactory opinion about the attitude of the healthcare workers (*32*). Interventions focused on enhancing provider–patient mutual trust and respect are needed. Provider training with regard to active listening, health literacy, message-framing, motivational interviewing, communication skills for trust-building and sensitivity should be considered.

We found that patients' higher rating of the severity of their vomiting was independently associated with loss to follow-up. Moreover, the most commonly reported reason for stopping treatment (58%) was medication side effects. However, frequency of adverse drug reactions reported by patients in interviews did not differ significantly between case-patients and control-patients (except that diarrhea symptoms were reported significantly less often by case-patients than by control-patients). Still, case-patients reported significantly higher subjectively perceived severity of vomiting, dizziness, and fatigue as medication side effects. These symptoms affect quality of life and interfere with the capacity to work and the ability to engage in activities of daily living. A study of 583 MDR TB patients in the Philippines who had undergone treatment during 1999–2006 showed that taking >5 drugs was significantly associated with loss to follow-up compared with taking 2–3 drugs (*12*). The authors interpreted the association between a higher drug burden and loss to follow-up as being related to more extensive drug resistance and competing risk for death among those patients. However, it also might be related to experiencing more side effects by the patients who received a higher number of toxic second-line drugs, which more likely led to stopping treatment, than by patients who received a lower number of drugs. Our study suggests that strict monitoring for, and appropriate treatment of, adverse drug reactions may help improve treatment adherence. Ancillary drugs must be included in procurement plans and made widely available at treatment facilities. Initial and refresher trainings for healthcare providers about management of adverse drug reactions and patient education about expected adverse drug reactions before treatment initiation may also help improve treatment adherence. Patients should understand the need for strict monitoring for adverse drug reactions and the availability of effective and free treatment for those reactions, especially reactions that are subjectively difficult for patients (e.g., nausea, vomiting, dizziness, and extreme fatigue).

Our study is subject to several limitations. As with any case–control study, it provides relatively weak empirical evidence. Because patient interviews were part of the protocol, deceased patients were excluded, which might have introduced survivor biases into our results. Almost a third of selected control-patients could not be located, which could bias the results in favor of selecting control-patients with a stronger relationship to the clinics. The retrospective nature of interviews is also subject to recall and exposure misclassification biases. Interview responses may reflect socially desirable answers rather than true thoughts and experiences. Some patients who were adherent to treatment at the time of the interview may be lost to follow-up at a later time (in this study, 66% of control-patients were still receiving treatment at the time of the interview); thus, outcome misclassification is possible. Identification of alcohol abuse was based on

the records in medical charts without standardized assessment for each patient (such as the Alcohol Use Disorder Identification Test) (*33*). Previous studies have demonstrated that loss to follow-up from treatment was less in smaller cohorts (*24*) and was more with program scale-up (*34*). Thus, increased loss to follow-up may be related to healthcare setting structural factors such as insufficient number of facilities and providers or limited experience with management of MDR TB, but our study did not address those factors.

Despite these limitations, our study provides useful data. The interviews captured patients' perspectives and provided nuances that retrospective cohort studies lack (*11*,*12*,*15*–*17*). These data, along with medical record data, afford program leaders greater insight for improving services and designing patient-centered interventions to reduce loss to follow-up during MDR TB treatment in the Philippines. A revision of the PMDT strategy should address identified barriers to completing MDR TB treatment and implement actions that support patients' adherence to treatment.

Table 7. Univariate analysis of social factors associated with loss to follow-up during treatment for multidrug-resistant TB, the Philippines, 2012–2014*

Category	Total	Case-patients†	Control-patients†	Odds ratio (95% CI)	p value
Distance between participant's home and treatment center, intensive phase of treatment					
Comparison 1					
0 to <1 km	23	7 (23.3)	16 (31.4)	0.67 (0.24–1.87)	0.44
1 to <5 km (referent)	58	23 (76.7)	35 (68.6)	1.00	
Comparison 2					
5 to <10 km	46	13 (36.1)	33 (48.5)	0.6 (0.26–1.37)	0.23
1 to <5 km (referent)	58	23 (63.9)	35 (51.5)	1.00	
Comparison 3					
≥10 km	104	38 (62.3)	66 (65.3)	0.88 (0.45–1.7)	0.69
1 to <5 km (referent)	58	23 (37.7)	35 (34.7)	1.00	
Comparison 4					
Not sure/don't know	42	10 (30.3)	32 (47.8)	0.48 (0.2–1.15)	0.10
1 to <5 km (referent)	58	23 (69.7)	35 (52.2)	1.00	
Usual mode of transportation/transportation used to cover the greatest distance traveling to the treatment center, intensive phase of treatment					
Walk					
Yes	37	19 (20.9)	18 (9.9)	2.4 (1.19–4.85)	**0.01**
No	236	72 (79.1)	164 (90.1)	1.00	
Public transportation					
Yes	239	79 (86.8)	160 (87.9)	0.91 (0.43–1.92)	0.80
No	34	12 (13.2)	22 (12.1)	1.00	
Personal vehicle					
Yes	16	6 (6.6)	10 (5.5)	1.21 (0.43–3.45)	0.72
No	257	85 (93.4)	172 (94.5)	1.00	
Major challenges when traveling to the treatment center, intensive phase of treatment					
The center was far away					
Yes	141	52 (57.8)	89 (48.9)	1.43 (0.86–2.38)	0.17
No	131	38 (42.2)	93 (51.1)	1.00	
Did not always have money for transportation					
Yes	193	71 (78)	122 (67.4)	1.72 (0.96–3.08)	0.07
No	79	20 (22)	59 (32.6)	1.00	
Did not have the time to go for treatment					
Yes	36	18 (19.8)	18 (9.9)	2.23 (1.1–4.54)	**0.02**
No	236	73 (80.2)	163 (90.1)	1.00	
Going for treatment caused problems with work or school					
Yes	81	33 (36.3)	48 (26.5)	1.58 (0.92–2.71)	0.10
No	191	58 (63.7)	133 (73.5)	1.00	
Did not have anyone to go with					
Yes	52	24 (26.4)	28 (15.4)	1.97 (1.06–3.65)	**0.03**
No	221	67 (73.6)	154 (84.6)	1.00	
The center's hours were not convenient					
Yes	23	11 (12.1)	12 (6.6)	1.95 (0.82–4.6)	0.12
No	250	80 (87.9)	170 (93.4)	1.00	
Minutes to travel from home to treatment center, intensive phase of treatment	272	51.00 (43.56)‡	54.16 (45.03)‡	1.00 (0.99–1.00)§	0.583
Minutes to travel from home to treatment center, continuation phase of treatment	198	22.25 (15.93)‡	31.07 (36.94)‡	0.99 (0.97–1.01)§	0.057
Patient self-stigmatization	272	6.20 (2.76)‡	5.66 (2.44)‡	1.09 (0.98–1.20)§	0.104

*Boldface indicates significance. TB, tuberculosis.
†No. (%) unless noted otherwise.
‡Mean (SD).
§Odds ratio is per 1 point increase in unit.

Table 8. Multivariable analysis of factors associated with loss to follow-up during treatment for multidrug-resistant TB, the Philippines, 2012–2014*

Social ecologic model level, factor	Odds ratio (95% CI)	p value
Personal factors		
Score TB knowledge	0.97 (0.95–0.99)†	0.003
Alcohol abuse	2.84 (1.39–5.80)	0.004
Healthcare setting factors		
Received assistance from TB program	0.09 (0.03–0.25)	<0.001
Score trust/rapport with healthcare worker	0.93 (0.90–0.96)†	<0.001
Diagnosis and treatment factors		
Self-rated severity of vomiting as adverse drug reaction	1.10 (1.01–1.21)‡	0.03

*TB, tuberculosis.
†Odds ratio is per 1 point increase in cumulative score.
‡Odds ratio is per 1 point increase in severity rating score.

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Dr. Tupasi is a founding president of the Tropical Disease Foundation based in Metro Manila, the Philippines. Her interests include tuberculosis and other infectious diseases, along with establishment of the first Green Light Committee–approved Programmatic Management of Drug-Resistant Tuberculosis Program.

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Address for correspondence: Thelma E. Tupasi, Tropical Disease Foundation, Inc., Amorsolo St and Urban Ave, Barangay, Pio Del Pilar, Makati City 1230, the Philippines; email: tetupasi@yahoo.com

Far East Scarlet-Like Fever Caused by a Few Related Genotypes of *Yersinia pseudotuberculosis*, Russia

Nelly F. Timchenko,[1] Ruslan R. Adgamov, Alexander F. Popov, Ekaterina K. Psareva, Konstantin A. Sobyanin, Alexander L. Gintsburg, Svetlana A. Ermolaeva[1]

We used multivirulence locus sequence typing to analyze 68 *Yersinia pseudotuberculosis* isolated in Russia during 1973–2014, including 41 isolates from patients with Far East scarlet-like fever. Four genotypes were found responsible, with 1 being especially prevalent. Evolutionary analysis suggests that epidemiologic advantages could cause this genotype's dominance.

Far East scarlet-like fever (FESLF), a rare and poorly studied disease caused by *Yersinia pseudotuberculosis*, was first described in 1959, when an outbreak involving >300 hospitalized patients occurred in the city of Vladivostok, Russia, on the coast of the Pacific Ocean (*1*). Since the 1960s, multiple outbreaks and sporadic cases of FESLF, mainly associated with consumption of contaminated vegetables, have been reported from far eastern and northern parts of Russia and other countries in Eurasia (*2–4*).

Comparing clinical patterns of FESLF and pseudotuberculosis showed that FESLF is not just a form of pseudotuberculosis but is an independent infectious disease that was unknown until the 1960s (*4*). FESLF is an acute disease with a cyclic course that includes severe fever and early signs such as rash that covers the body, particularly the face, neck, toes, and hands; these signs have become known as "hood," "gloves," and "socks" (online Technical Appendix Figure 1, http://wwwnc.cdc.gov/EID/article/22/3/15-0552-Techapp1.pdf). Typical features of FESLF include a "raspberry tongue" and well-defined nipples. Erythema nodosum can occur with relapse; lamellar or defurfuration on earlobes, hands, palms, feet, and trunk appears during the recovery period. We sought to determine clonal relationships of *Y. pseudotuberculosis* strains responsible for cases of FESLF reported in Russia during 1973–2014 and environmental strains found in vegetables and small rodents.

Author affiliations: Institute of Epidemiology and Microbiology named after G.P. Somov, Vladivostok, Russia (N.F. Timchenko, E.K. Psareva); Gamaleya Institute of Epidemiology and Microbiology, Moscow, Russia (R.R. Adgamov, K.A. Sobyanin, A.L. Gintsburg, S.A. Ermolaeva); Pacific State Medical University, Vladivostok (A.F. Popov); Pirogov Russian National Research Medical University, Moscow (S.A. Ermolaeva)

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The Study

Our study examined 68 isolates collected in Russia during 1973–2014, including 17 outbreak and 24 sporadic isolates from humans and 15 rodent and 11 vegetable isolates (online Technical Appendix Table 1, Figure 2). All but 3 isolates belonged to the O1b serotype; these 3 isolates belonged to the O3 serotype. The most recent FESLF isolates (from 2014) came from a patient who showed typical signs of FESLF, including a cyclic course, fever, and "raspberry tongue." A comparison of clinical signs and symptoms in historical versus recent patients suggested that the disease had not evolved since its first description.

The isolates were kept frozen until the experiment started. To characterize clonal relationships of the strains, we applied the multilocus sequence typing (MLST) scheme developed by Laukkanen-Ninios et al. (*5*). PCR products were obtained with primers and conditions listed at the *Yersinia pseudotuberculosis* MLST database (University of Warwick, Coventry, UK; http://mlst.ucc.ie/mlst/dbs/Ypseudotuberculosis).

We found three MLSTs among FESLF isolates: MLST2 (n = 33), MLST26 (n = 5), and MLST32 (n = 3); this MLST was specific for serotype O3 (Table 1). All but 1 vegetable isolate belonged to MLST2, which was also found in 9 (60%) of 15 rodent isolates. MLST2 prevailed among isolates from all sources.

MLST analysis was complemented with sequencing of 4 virulence genes involved in critical steps of generalized infection: intestine barrier crossing (*inv* and *yadA*) (*6,7*) and macrophage activity regulation (*yopE* and *cnf*) (*8,9*) (Tables 1, 2). The genes *inv* and *cnf* are chromosomal, whereas *yopE* and *yadA* are encoded on the virulence plasmid of *Yersinia* (pYV). Sequences from this study have been deposited into GenBank (accession nos. KR028003–KR028011). A total of 4 distinct virulence sequence types (VSTs) were found (Table 1).

Combining MLST and VST gave rise to 6 multivirulence locus sequence types (MVLSTs) (Table 1). The sequences of 10 MVLST genes (excluding *cnf*) were

[1]These authors were co–principal investigators.

Table 1. Combined genotypes of the *Yersinia pseudotuberculosis* strains in study of Far East scarlet-like fever caused by a clonal group of *Y. pseudotuberculosis*, Russia*

MVLST	Source of isolated strains, no.			MLST†	VST‡	Plasmid profiles		
	FESLF	Rodents	Vegetables			pYV§	pYpsIP31758.1¶	pVM4.4#
1**								
A	29	9	10	2	1	+	+	–
B	4	0	0	2	1	+	–	+
2	5	0	0	26	1	+	–	–
3	3	1	0	32	2	+	–	–
4	0	0	1	14	3	+	–	–
5	0	3	0	42	4	+	–	–
6	0	2	0	64	2	+	–	–

*Multivirulence locus sequence typing (MVLST) types found in Far East scarlet–like fever (FESLF) isolates are in bold. MLST, multilocus sequence typing; VST, virulence sequence types; +, positive; –, negative.
†MLST types are provided in the *Y. pseudotuberculosis* MLST database (http://mlst.ucc.ie/mlst/dbs/Ypseudotuberculosis).
‡VSTs are determined on the basis of alleles of the virulence genes *inv*, *cnf*, *yadA*, and *yopE*.
§Plasmid designated pYV was evidenced by PCR with primers specific to *yadA* and *yopE* (online Technical Appendix Table 2, http://wwwnc.cdc.gov/EID/article/22/3/15-0552-Techapp1.pdf) and confirmed by agarose gel electrophoresis.
¶Plasmid designated pYpsIP31758.1, which is also called pVM82 (*3*), was confirmed by PCR with primers specific to *dotA* (online Technical Appendix Table 2) and was confirmed by agarose gel electrophoresis.
#Plasmid designated pVM4.4 was confirmed with agarose gel electrophoresis.
**The subtypes had different plasmid profiles.

used to build a maximum-likelihood tree with MEGA6 (*10*). We excluded the *cnf* gene from the analysis because the dominant allele carries a nonsense mutation that interrupts the polypeptide after Asn181. The maximum-likelihood tree divided into 2 subclades (Figure 1). One subclade united MVLSTs found in FESLF isolates and MVLST6, which was found only in rodent isolates. The second subclade united MVLSTs found in rodent and vegetable isolates.

The diversity of virulence genes was analyzed with DnaSP software version 5.10 (*11*; Table 2). A noticeable feature of virulence genes was the predominance of nonsynonymous substitutions, whereas basic parameters of nucleotide diversity were similar in virulence and housekeeping genes (Table 2). Positive selection was confirmed for *yopE* by the Tajima neutrality test implemented in MEGA6. The diversity was especially low among strains from the FESLF subcluster. MVLST1 and MVLST2 shared the VST1 type (Table 1). MVLST3 shared VST2 with MVLST6 found in rodent strains.

Plasmids, particularly the pYV plasmid, are central to the virulence of *Yersiniae* (*12*). The pYV-specific markers *yopE* and *yadA* were found in all strains. The presence of the additional plasmid pVM82/pYpsIP31758.1 was screened with PCR specific to the *dotA* gene (*3*), which was found in all but 4 MVLST1 strains but not in other genotypes.

Table 2. Polymorphism of housekeeping and virulence genes in study of Far East scarlet-like fever caused by a clonal group of *Yersinia pseudotuberculosis*, Russia*

Target gene†	Fragment length, bp‡	Alleles, no.	Indels, no.	Polymorphic sites/parsimony informative	Substitutions, no.		Results of positive selection test, probability/dN – dS§	Nucleotide diversity
					S	N		
adk	387	1	0	0/0	NA	NA	NA	NA
argA	357	1	0	0/0	NA	NA	NA	NA
aroA	354	1	0	0/0	NA	NA	NA	NA
glnA	336	3	0	2/0	10	2	1.000/–2.734	0.01974
thrA	339	3	0	2/0	2	0	1.000/–1.455	0.00390
tmk	372	4	0	7/0	7	0	1.000/–2.832	0.00941
trpE	465	2	0	1/0	1	0	1.000/–1.021	0.00215
Concatemers MLST¶	2,610	6	0	22/5	20	2	1.000/–3.937	0.00289
inv	603	2	0	3/0	1	2	1.000/–0.494	0.00498
yopE	540	3	0	3/0	0	3	**0.034/1.835**	0.00368
yadA	651	3	6	9/2	2	7	**0.408/0.238**	0.00930
Concatemers virulence genes#	1,794	3	6	15/4	3	12	**0.109/1.241**	0.00457
Concatemers MVLST**	4,404	6	6	37/9	23	14	1.000/–3.023	0.00311

*Positive values are shown in bold. dN, number of nonsynonymous substitutions per site; dS, number of synonymous substitutions per site; MLST, multilocus sequence typing; MVLST, multivirulence locus sequence typing; NA, not applicable.
†Housekeeping genes are included in the MLST scheme.
‡Length of fragments included in the sequence analysis.
§The probability of rejecting the null hypothesis of strict neutrality (dN = dS) in favor of the alternative hypothesis (dN is >dS) and the test statistics (dN – dS) are shown. Values were calculated by using MEGA6 *(10)*.
¶Concatemers of sequences included in the MLST scheme. The sequences were cut off to start and finish an analysis with the first and third codon positions, respectively. Concatemers were gathered manually.
#Concatemers of virulence gene fragments.
**Concatemers of 7 housekeeping and 3 virulence gene fragments.

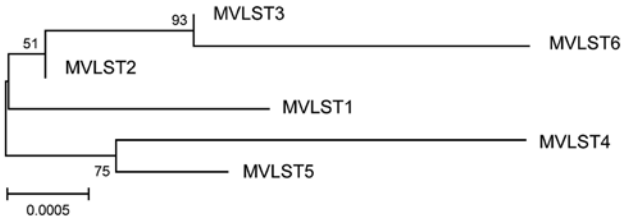

Figure 1. Maximum-likelihood tree generated with concatenated multivirulence locus sequence type (MVLST) sequences for study of Far East scarlet-like fever caused by a clonal group of *Yersinia pseudotuberculosis*, Russia. Reliability values for the branching nodes are indicated. Branch lengths and scale bar indicate distances measured in terms of the proportion of nucleotide substitutions between sequences.

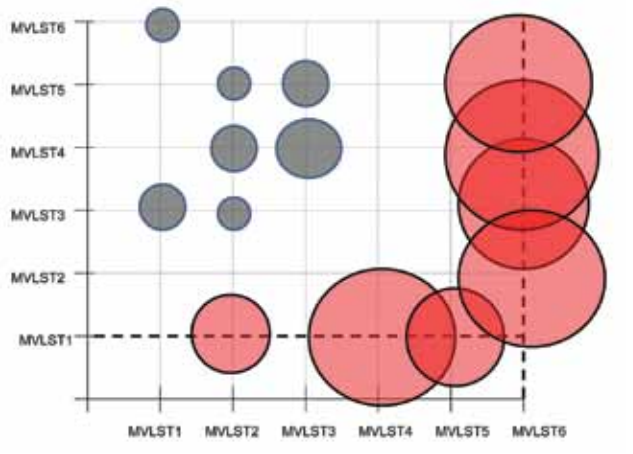

Figure 2. Graphic representation of the evolutionary analysis that tested the hypothesis of equality of evolutionary rates between multivirulence locus sequence type (MVLST) genotypes for study of Far East scarlet-like fever caused by a clonal group of *Yersinia pseudotuberculosis*, Russia. The χ^2 test statistic was applied for the pairwise comparison of concatenated sequences of MVLST markers, with the *Y. pestis* sequence being used as an outgroup. Circles indicate values of the χ^2 test statistic of the pairwise comparison calculated in MEGA6 (*10*); diameters correspond to values of rejection of the null hypothesis that states the equality of evolutionary rates between pairs of concatenated sequences. Statistically significant values are shown in red.

Plasmid purification (*13*) confirmed results obtained from PCR-based screening (data not shown). An additional small plasmid was found in MVLST1 strains that lacked pVM82/pYpsIP31758.1. Consequently, plasmid profiling divided MVLST1 into 2 subtypes, MVLST1a and MVLST1b, without changing other MVLSTs (Table 1).

Our findings show that FESLF clinical manifestations are caused by strains belonging to at least 4 distinct genotypes, with predominance of MVLST1a (MLST2/VST1/pVM82). We consider the MVLST1a genotype to be generally dominant among strains responsible for FESLF in Russia, a suggestion supported by the finding that MVLST1a appears to be the only genotype that carries the pVM82/pYpsIP31758.1 plasmid. A body of epidemiologic data has shown that most epidemic and many sporadic FESLF strains carry this plasmid (*3,13*).

The fact that full FESLF symptomatology is caused by several distinct genotypes supports the view that specific virulence traits are characteristic of FESLF-associated strains (*2,3*) and suggests that the dominance of the MVLST1a genotype could be caused by its epidemiologic advantages rather than its pathogenic traits. The prevalence of MVLST1a among all isolate sources suggests the genotype's wider dissemination in the region we studied, which supports the possibility that this clone has epidemiologic advantages.

To further address this question, we used an evolutionary analysis implemented in MEGA6 (*10*) to test the hypothesis of equality of evolutionary rates by using the χ^2 test for pairwise comparison of concatenated sequences of MVLST markers, with the *Y. pestis* sequence used as an outgroup. The hypothesis of equal rates between MVLST1 and other genotypes was rejected ($p<0.05$; Figure 2). The molecular clock test performed with MEGA6 by comparing the maximum-likelihood values with and without molecular clock constraints under the Tamura-Nei model supported this conclusion. The inequality of evolutionary rates favors the idea of more effective reproduction and growth of MVLST1 strains in the environment, possibly because of better adaptation to environmental niches. Another clone with divergent evolutionary rates was the rare MVLST6 (MLST64/VST2) genotype, which has been isolated from small rodents in the Far East of Russia (i.e., in this study and according to data on the isolation of MlST64, listed in the *Y. pseudotuberculosis* MLST database).

Conclusions

FESLF, a relatively new disease, is caused by the bacterium that evolved into the causative agent of plague (*14*). The evolution of *Y. pestis* is linked to loss of functionality of some factors that are active in *Y. pseudotuberculosis* and to the acquisition of additional factors of both plasmid and chromosomal origin; these alterations enable the organism to adapt and occupy new environmental niches (*14*). The FESLF causative agent lost at least 2 chromosomally encoded virulence loci, *cnf* and HPI; its most successful clone, MVLST1a, acquired an additional plasmid. The geographic region where the first outbreaks of FESLF were registered seems close, if not identical, to the region where *Y. pestis* emerged. Overall, our data support the view of *Y. pseudotuberculosis* as a rapidly developing pathogenic species, whereas its wide dissemination in the environment promotes selection of clones that are potentially hazardous for humans (*2–4,15*).

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Dr. Timchenko is a leading scientist at the Laboratory of Molecular Epidemiology and Microbiology at the Institute of Epidemiology and Microbiology named after G.P. Somov, Vladivostok, Russia. Her research interests include microbiology and pathogenic traits of *Yersinia pseudotuberculosis*.

Dr. Ermolaeva is a head of the Laboratory of Ecology of Pathogenic Bacteria at Gamaleya Institute of Epidemiology and Microbiology, Moscow, Russia. Her main research interests are in the field of molecular ecology of pathogenic bacteria.

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Address for correspondence: Svetlana A. Ermolaeva, Gamaleya Institute of Epidemiology and Microbiology, Gamaleya St 18, Moscow 123098, Russia; email: drermolaeva@mail.ru

Highly Pathogenic Avian Influenza A(H5N8) Viruses Reintroduced into South Korea by Migratory Waterfowl, 2014–2015

Jung-Hoon Kwon,[1] Dong-Hun Lee,[1] David E. Swayne, Jin-Yong Noh, Seong-Su Yuk, Tseren-Ochir Erdene-Ochir, Woo-Tack Hong, Jei-Hyun Jeong, Sol Jeong, Gyeong-Bin Gwon, Chang-Seon Song

Highly pathogenic avian influenza A(H5N8) viruses were isolated from migratory waterfowl in South Korea during fall 2014–winter 2015, a recurrence after initial introduction in winter 2014. These reappeared viruses were phylogenetically distinct from isolates circulating in poultry farms in South Korea.

Since the Asian-lineage subtype H5 highly pathogenic avian influenza (HPAI) virus was first detected in China in 1996, outbreaks of infection caused by this virus in poultry have been continuous. The HPAI (H5) viruses have evolved and continue to evolve into many genetic lineages and multiple clades (*1*). In January 2014, novel reassortant HPAI viruses of subtype H5N8, clade 2.3.4.4, were detected in poultry and wild bird carcasses in South Korea (*2*). Closely related viruses were also detected in Japan (*3*) and China (*4*). Genetic analysis showed that this virus was generated by reassortment of HPAI viruses of eastern China. Subsequently, HPAI (H5N8) viruses spread to Europe and North America and were then reintroduced into South Korea and Japan (*5*). The HPAI (H5N8) viruses identified in South Korea in early 2014 were divided into groups A (A/Baikal teal/Korea/Donglim3/2014 strain-like) and B (A/breeder duck/Korea/Gochang1/2014-like). Group A viruses further evolved into 3 distinct subgroups: icA1 (Europe/Japan), icA2 (North America/Japan), and icA3 (South Korea/Japan) (*5*). Wild birds were suspected of being a source of intercontinental transmission because the timing and direction of the outbreak coincided with the migratory route of wild birds (*5*,*6*). We sequenced and genetically analyzed the complete genomes of 11 HPAI (H5N8) viruses isolated from wild migratory waterfowl in South Korea during December 2014 and February 2015 and compared these isolates with other HPAI (H5N8) isolates, including isolates identified from South Korea poultry farms in late 2014.

Author affiliations: Konkuk University, Seoul, South Korea (J.-H. Kwon, J.-Y. Noh, S.-S. Yuk, T.-O. Erdene-Ochir, W.-T. Hong, J.-H. Jeong, S. Jeong, G.-B. Gwon, C.-S. Song); US Department of Agriculture, Athens, Georgia, USA (D.H. Lee, D.E. Swayne)

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The Study

A total of 11 HPAI (H5N8) viruses were isolated from 980 samples of wild bird feces and 102 swab samples collected from wild bird habitats in South Korea where active surveillance was conducted during December 2014 and February 2015 (Table). Eight of 65 fecal samples (K14-362–K14-374) collected on December 2014, one of the 50 fecal samples (N15-99) collected on February 2015, one of the 17 swab samples from healthy common teals (KU-12) collected on January 2015, and one of the 13 swab samples from healthy mallards (KU3-2) collected on February 2015 were positive for influenza A virus by egg inoculation and matrix (M) gene real-time reverse transcription PCR performed as described (*8*). The hosts of the influenza A virus–positive fecal samples were identified as mandarin ducks, greater white-fronted geese, and mallards by DNA barcoding techniques, as described (*7*). Full-genome sequencing was performed by next-generation sequencing using the Ion Torrent Personal Genome Machine system (Thermo Fisher Scientific, Grand Island, NY, USA) (online Technical Appendix 1, http://wwwnc.cdc.gov/EID/article/22/3/15-1006-Techapp1.pdf). The viruses were subtyped as H5N8 by using a BLAST search, and the multibasic cleavage site of the hemagglutinin (HA) gene (PLRERRRKR/GLF) was detected.

For phylogenetic analysis, we constructed a maximum-likelihood tree in MEGA6 software (http://www.megasoftware.net) using the Hasegawa-Kishino-Yano (HKY) model. A median-joining phylogenetic network was constructed by using NETWORK version 4.613 (www.fluxus-engineering.com), and Bayesian analysis was performed by using BEAST version 1.8.1 (http://beast.bio.ed.ac.uk). A maximum clade credibility tree was generated for each dataset by using TreeAnnotator in BEAST (online Technical Appendix 1).

Each genome segment of 11 HPAI (H5N8) viruses shared high nucleotide sequence identities ranging from 99.1% to 100%: polymerase basic protein 2, 99.4%–100%; polymerase basic protein 1, 99.3%–100%; polymerase acidic protein, 99.5%–100%; HA, 99.1%–100%;

[1]These authors contributed equally to this article.

Table. HPAI (H5N8) isolates and total wild bird samples collected in South Korea, December 2014–February 2015*

Collection date	Location	Sample type	No. HPAI (H5N8) positive/no. total	Host†	Strain
2014					
Sep 26	36°44′ N, 127°07′ E	Feces	0/55		
Sep 27	36°37′ N, 126°21′ E	Feces	0/110		
Nov 6	36°37′ N, 126°21′ E	Feces	0/335		(1 LPAI)
Nov 7	36°44′ E, 127°07′E	Feces	0/105		
Nov 22	36°44′ N, 127°07′ E	Feces	0/260		(3 LPAI)
Dec 24	36°44′ N, E127°07′ E	Feces	**8/65**	Mandarin duck	K14-363-1
					K14-366-1
					K14-367-1
				Greater white-fronted goose	K14-367-4
					K14-369-3
					K14-371-4
					K14-372-2
					K14-374-1
2015					
Jan 22	36°47′ N, 127°03′ E	Swab	**1/17**	Common teal	KU-12
Jan 29	35°18′ N, 128°40′ E	Swab	**1/13**	Mallard	KU3-2
			0/30	Northern pintail	(1 LPAI)
Feb 6	37°32′ N, 127°01′ E	Feces	1/50	Mallard	N15-99
Feb 11	36°42′ N, 126°27′ E	Swab	0/14	Mallard	
Feb 25	37°23′ N, 129°14′ E	Swab	0/13	Black-tailed gull	
Mar 16	35°53′ N, 127°01′ E	Swab	0/15	Eurasian wigeon	
Total			11/1,082		

*HPAI, highly pathogenic influenza virus; LPAI, low pathogogenicity avian influenza; bold, period of study.
†The hosts of the HPAI-positive fecal samples were identified by using DNA barcoding techniques as described (7).

nucleoprotein, 99.6%–100%; neuraminidase, 99.2%–100%; M protein, 99.4%–100%; and nonstructural protein, 99.2%–100%. Phylogenetic analysis showed that the 4 different subtype H5N8 virus clusters, icA 1–3 and the South Korea poultry farm cluster, most likely evolved from H5N8 virus identified from South Korea in early 2014. All H5N8 isolates collected in South Korea during winter 2014–15 identified in this study clustered with isolates from Japan, including the A/chicken/Miyazaki/7/2014 strain, and were characterized as subgroup icA3. Isolates obtained from South Korea poultry farms in late 2014 were phylogenetically distinct from isolates in other subgroups (Figure 1; online Technical Appendix 1 Figures 1, 2).

Group A (H5N8) viruses have been detected on South Korea poultry farms since the first outbreak in January 2014, including during the summer season. A second wave of the HPAI (H5N8) outbreak started in September 2014. Although the growing HPAI outbreak in September 2014 coincided with the fall migration of migratory waterfowl, phylogenetic analyses suggest that the HPAI (H5N8) viruses detected on South Korean poultry farms in late 2014 are not related to the icA3 viruses carried by wild waterfowl but have instead evolved from viruses circulating on poultry farms or among resident wild birds in South Korea since early 2014.

By the beginning of the fall 2014 migration of migratory waterfowl, new subgroups of H5N8 viruses (icA1, icA2, icA3) were detected in wintering sites of migratory waterfowl, including South Korea and Japan, in late 2014 and early 2015 (*5*,*9*). The icA1 subgroup is composed of HPAI (H5N8) viruses from Europe, South Korea, and Japan, whereas the icA2 subgroup is composed of HPAI (H5N8) viruses from North America, Taiwan, and Japan and the icA3 subgroup is composed of HPAI (H5N8) viruses isolated in South Korea and Japan. Markov chain Monte Carlo analyses showed that the substitution rates estimated for HPAI (H5N8) viruses identified from South Korea are 9.23×10^{-3} (95% highest posterior density range 7.43×10^{-3} to 1.11×10^{-2}) nt substitutions/site/year, which is higher than previous estimates for the HA gene of H5N1 viruses from China from 1996 through 2012 (4.378×10^{-3} nt substitutions/site/year] (*10*). The interval estimated from most recent common ancestor of the icA3 cluster from South Korea and Japan was 0.44 years (95% highest posterior density range 0.33–0.55 months, corresponds to August 2014) (Figure 2, http://wwwnc.cdc.gov/EID/article/22/3/15-1006-F2.htm).

Conclusions

These results suggest that HPAI (H5N8) viruses circulated in wild bird populations and evolved into subgroups during the breeding season. Detection of subtype H5N8 viruses in healthy wild birds (*12,13*; this study) and subclinical infection with viral shedding among migratory waterfowl experimentally infected with HPAI (H5N8) viruses (*11*) support the theory of long-term circulation of HPAI (H5N8) viruses in wild bird population.

This study also found that subtype icA3 viruses, derived from HPAI (H5N8) viruses from South Korea and reintroduced by migratory waterfowl, were genetically distinct from the HPAI (H5N8) viruses that continued to circulate

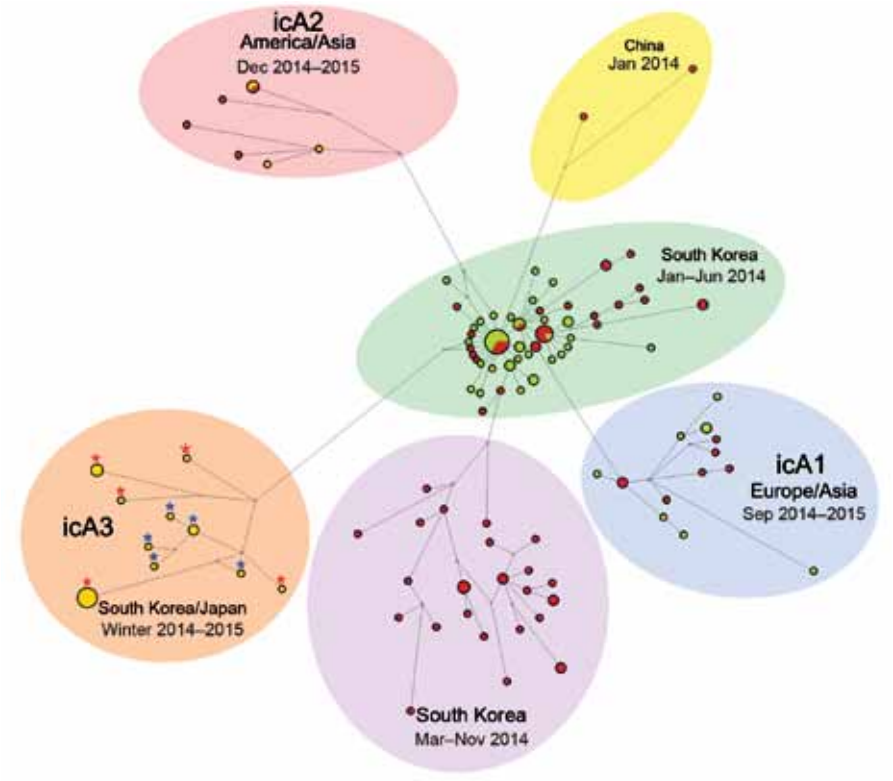

Figure 1. Median-joining phylogenetic network of highly pathogenic avian influenza A(H5N8) viruse isolates identified in South Korea during 2014–2015 showing relationships with other virus isolates. The median-joining network was constructed from the hemagglutinin gene and includes all the most parsimonious trees linking the sequences. Each unique sequence is represented by a circle sized relative to its frequency in the dataset. Branch length is proportional to the number of mutations. Isolates are colored according to the origin of the sample: red inner circle, poultry farm isolates; yellow inner circle, wild bird isolates. Red asterisks indicate isolates from South Korea and blue asterisks indicate isolates from Japan identified during December 2014–February 2015.

in poultry farms. In the previous 4 HPAI (H5N8) virus outbreaks in South Korea and Japan, migratory waterfowl were identified as the source of HPAI outbreaks (*14,15*); however, related HPAI viruses were not reintroduced into South Korea and Japan after the initial outbreak season. The phylogenetic analysis described here shows that HPAI (H5N8) viruses isolated from migratory wild birds in the winter of 2014–15 are phylogenetically distinct from isolates from South Korean poultry farms. HPAI (H5N8) viruses thus independently evolved in wild bird populations and poultry farms in South Korea until late 2014.

Our results indicate that HPAI (H5N8) viruses have been circulating in wild waterfowl population since early 2014. Enhanced global active surveillance is needed to monitor the spread of these viruses through wild birds. Such efforts could clarify the epidemiology of HPAI virus and facilitate early recognition of novel genotypes.

GISAID (Global Initiative on Sharing All Influenza Data) acknowledgment tables for laboratory contributions are shown in online Technical Appendix 2 (http://wwwnc.cdc.gov/EID/article/22/3/15-1006-Techapp2.xlsx).

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Mr. Kwon is a Ph.D. candidate at Konkuk University, Seoul, South Korea. His primary research interest is the epidemiology of highly pathogenic avian influenza viruses in wild birds. Dr. Lee is a postdoctoral researcher at the Southeast Poultry Research Laboratory, USDA Agricultural Research Service, Athens, Georgia, USA. His research interests include molecular epidemiology and host–pathogen interaction of avian influenza viruses.

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Address for correspondence: Chang-Seon Song, Avian Disease Laboratory, College of Veterinary Medicine, Konkuk University, 1 Hwayang-dong, Gwangjin-gu, Seoul, 143-701, South Korea; email: songcs@konkuk.ac.kr

Treatment of *Mycobacterium abscessus* Infection

Shannon A. Novosad, Susan E. Beekmann, Philip M. Polgreen, Kate Mackey, Kevin L. Winthrop; *M. abscessus* Study Team[1]

Mycobacterium abscessus is often resistant to multiple antimicrobial drugs, and data supporting effective drugs or dosing regimens are limited. To better identify treatment approaches and associated toxicities, we collected a series of case reports from the Emerging Infections Network. Side effects were common and often led to changing or discontinuing therapy.

Mycobacterium abscessus infections are challenging to treat because multidrug resistance necessitates prolonged intravenous (IV) therapy and side effects are perceived to be common. For the best chance of pulmonary disease cure, guidelines from the American Thoracic Society/Infectious Diseases Society of America (ATS/IDSA) recommend multidrug macrolide-based therapy based on susceptibility testing results and surgical resection. However, these guidelines also state that there are no drug combinations with proven efficacy (*1*). Similarly for extrapulmonary disease, macrolide-based treatment regimens based on susceptibility testing results are recommended (*1*,*2*). Inducible macrolide resistance in many strains of *M. abscessus* further complicates treatment (*3*). Given the lack of evidence-based therapies, we hypothesized that treatment regimens have no clear pattern and that medication changes and toxicities occur frequently.

The Study

The Emerging Infections Network (EIN) gathers information about emerging infectious diseases in North America (*4*) and is frequently used for case collection. The EIN is funded through a cooperative agreement between the Centers for Disease Control and Prevention and IDSA. To learn more about treatment regimens and associated side effects, during March–December 2013, we asked EIN physician members to report recent cases of *M. abscessus* via an emailed electronic data collection form.

A total of 65 cases were reported from 16 states; patient mean age was 53.6 years. Most cases were in white, nonsmoking women. Concurrent conditions included cystic fibrosis (n = 9, 14%), cancer (n = 7, 11%), and chronic obstructive pulmonary disease (n = 6, 9%). Ten (15%) patients had used immunosuppressive medications in the 3 months before diagnosis. Most (36 [55%]) organisms were reported as *M. abscessus* complex, 27 (42%) as *M. abscessus*, and 2 (3%) as *M. massliense*. According to available records, at the time of case report, 55 (85%) patients had started or finished antimicrobial drug therapy.

Of the 65 patients, 41 (63%) had pulmonary *M. abscessus* infection; 19 isolates were from bronchoalveolar lavage fluid and 16 from ≥2 sputum samples. Of these 41 patients, 34 (83%) started antimicrobial drug therapy. Among those not starting therapy, 2 opted for monitoring only, 1 died before therapy was started, and 4 had no reason reported. A total of 21 initial medication combinations were reported (Table 1). The most commonly reported medications were IV amikacin (n = 22, 65%) and azithromycin (n = 24, 71%). The most commonly used regimen was IV amikacin, a second IV agent, and a macrolide (n = 15, 44%). Only 5 patients received no IV agents. Twenty-eight (82%) patients required a change in therapy (because of side effects, lack of effectiveness, or need for suppressive regimen); 3 underwent surgical therapy, and 12 stopped therapy (median duration 12 months, interquartile range [IQR] 9–18 months).

Of the 24 patients with extrapulmonary disease (median age 50 years, IQR 42–66 years), most (17 [71%]) had skin or soft tissue infections. Also reported were 2 corneal, 1 peritoneal, 1 catheter-related, and 1 pacemaker pocket infection plus 1 case each of endocarditis and osteomyelitis. Medical therapy had been started by 21 (88%) patients. Reasons for not starting therapy included being lost to follow-up, declining therapy, or being referred for surgery without antimicrobial drugs. The most commonly used agents were IV amikacin (n = 9, 43%), macrolides (n = 18, 86%), and imipenem (n = 7, 33%) (Table 1). Regimens that contained ≥1 IV agent were administered to 12 (57%) patients; IV amikacin–based regimens with a macrolide and 1 other IV agent were administered to 5 (24%). Change from the initial therapeutic regimen was needed by 14 (67%) patients. Among the 15 patients who stopped therapy, median duration of therapy was 6 months (IQR 4–8 months); 14 (93%) stopped therapy because of improvement or presumed cure. Fourteen (58%) of the 24 patients with extrapulmonary disease underwent surgery.

Author affiliations: Oregon Health and Science University, Portland, Oregon, USA (S.A. Novosad, K. Mackey, K.L. Winthrop); University of Iowa Carver College of Medicine, Iowa City, Iowa, USA (S.E. Beekmann, P.M. Polgreen)

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[1]Members of the study team are listed at the end of this article.

Table 1. Initial drug regimens for pulmonary and extrapulmonary *Mycobacterium abscessus* infection and therapy-modifying/ending side effects*

Regimen, no. patients	Total no. (%) patients	Therapy-modifying/ending side effect, no. (%) patients
Pulmonary disease, 33†		
Non-IV agents	5 (15)	2 (40)
Clarithromycin, linezolid, 1		
Azithromycin, inhaled amikacin, ethambutol, rifampin, 2		
Azithromycin, inhaled amikacin, ethambutol, moxifloxacin, 1		
Azithromycin, ethambutol, linezolid, 1		
Single IV agent	3 (9)	1 (33)
Amikacin, azithromycin, levofloxacin, 1		
Tigecycline, inhaled amikacin, clofazimine, 1		
Cefoxitin, azithromycin, inhaled amikacin, 1		
Dual IV agents	24 (73)	15 (63)
Amikacin/macrolide-based regimens	19 (57)	13 (68)
Amikacin, macrolide, and 1 IV agent, in addition to amikacin	15 (45)	10 (67)
Amikacin, azithromycin, tigecycline, 7		
Amikacin, clarithromycin, cefoxitin, 2		
Amikacin, azithromycin, cefoxitin, 4		
Amikacin, azithromycin, imipenem, 2		
Amikacin, macrolide, 1 IV agent in addition to amikacin, and other oral agents	4 (12)	3 (75)
Amikacin, azithromycin, cefoxitin, moxifloxacin, 1		
Amikacin, clarithromycin, cefoxitin, moxifloxacin, 1		
Amikacin, azithromycin, imipenem, ethambutol, rifampin, 1		
Amikacin, clarithromycin, cefoxitin, other, 1		
Other amikacin-based regimens	1 (3)	0
Amikacin, cefoxitin, 1		
Regimens without IV amikacin	4 (12)	2 (50)
Azithromycin, imipenem, tigecycline, 1		
Clarithromycin, tigecycline, imipenem, 1		
Clarithromycin, moxifloxacin, tobramycin, cefoxitin, 1		
Azithromycin, inhaled amikacin, cefoxitin, imipenem, 1		
Triple IV agents	1 (3)	1 (100)
Amikacin, macrolide, and 2 IV agent, in addition to amikacin	1 (3)	1 (100)
Amikacin, azithromycin, tigecycline, cefoxitin, 1		
Extrapulmonary disease, 21		
No IV agents	9 (43)	1 (11)
Clarithromycin, other, 1		
Clarithromycin, doxycycline, 1		
Moxifloxacin, tobramycin drops, azithromycin drops, other, 1		
Azithromycin, linezolid, 1		
Tobramycin drops, azithromycin, moxifloxacin drops, azithromycin topical, 1		
Levofloxacin, doxycycline, 1		
Ciprofloxacin, minocycline, 1		
Clarithromycin, minocycline, 1		
Azithromycin, moxifloxacin, 1		
Single IV agent	5 (24)	2 (40)
Amikacin, azithromycin, clofazimine, 1		
Cefoxitin, azithromycin, 1		
Amikacin, ethambutol, 1		
Imipenem, azithromycin, moxifloxacin, 1		
Imipenem, azithromycin, ciprofloxacin, 1		
Dual IV agents	6 (29)	5 (83)
Amikacin-based regimens	6 (29)	5 (83)
Amikacin, macrolide and one IV agent, in addition to amikacin	5 (24)	4 (80)
Amikacin, azithromycin, imipenem, 2		
Amikacin, clarithromycin, cefoxitin, 1		
Amikacin, clarithromycin, imipenem, 2		
Amikacin, macrolide,1 IV agent, in addition to amikacin, and other oral agents	1 (5)	1 (100)
Amikacin, clarithromycin, cefoxitin, moxifloxacin, linezolid, 1		
Triple IV agents	1 (5)	1 (100)
Amikacin, macrolide, 2 IV agents, in addition to amikacin, and oral agent	1 (5)	1 (100)
Amikacin, clarithromycin, imipenem, tigecycline, clofazimine, 1		

*IV, intravenous.
†Therapy was started for 34 patients, but 1 initial regimen was unknown.

Side effects were common; 74 side effects were documented among 34 (62%) of 55 patients who received treatment. Most common were nausea/vomiting (n = 17, 31%) and skin changes (n = 11, 20%) (Table 2). When the specific medication causing a side effect was known, it was most commonly amikacin (22 [30%]) or tigecycline (13 [18%]). Of the 9 reported episodes of renal insufficiency, 7 were attributed to amikacin. IV agents were commonly associated with side effects that often required dosage adjustment or discontinuation. Among those receiving amikacin and tigecycline, 51% and 36% of patients, respectively, had to adjust or stop medication because of side effects. Intermittent dosing of amikacin seemed to cause fewer side effects than daily dosing (42% vs. 77%, respectively). Among patients with renal insufficiency attributed to amikacin, 71% were receiving it daily.

Among patients with pulmonary infection, antimicrobial drug therapy was completely discontinued for 4 because of side effects. No patients with extrapulmonary disease completely stopped therapy because of side effects. Overall, ≥54 medication changes among 30 patients were made because of side effects or intolerance.

At the time of data collection, 8 patients had died: 6 with pulmonary and 2 with extrapulmonary disease. Of these 8 patients, 6 died while receiving therapy (5 pulmonary, 1 extrapulmonary).

Conclusions

Our series showed a wide range of treatment strategies for *M. abscessus* infection; most consisted of prolonged antimicrobial drug therapy. Side effects were common, and therapy often needed to be changed or stopped. Amikacin, the most commonly used IV agent, was associated with multiple side effects; amikacin therapy was stopped or adjusted for 51% of patients.

Heterogeneity of initial treatment regimens was less among those with pulmonary disease than among those with extrapulmonary disease, but regimens still varied widely. However, despite the guidelines, surgical therapy was uncommon for patients with pulmonary disease; only 3 patients in this series underwent surgery.

In a retrospective analysis of 41 patients with *M. abscessus* pulmonary disease in South Korea, 18 (43.9%) patients experienced side effects (*5*). This percentage is lower than what we found (62%), possibly because a large percentage of patients in our series received amikacin or a regimen with >1 IV agent. In our series, tigecycline was used, but often as a secondary agent. A recent study of 52 patients who received tigecycline-containing salvage regimens reported improvement in 60% of patients but side effects (most commonly nausea/vomiting) in 94%; 23% of side effects were directly associated with tigecycline (*6*). Side effects from tigecycline were also common among patients in our series.

Our study had several limitations, including unknown specific subspecies of *M. abscessus*. Most isolates were reported as *M. abscessus* complex (55%) or *M. abscessus* (42%), and it is unclear if these were ever correctly identified to the subspecies level (such as *M. abscessus abscessus*). Given increasing evidence regarding varying antimicrobial drug susceptibility patterns of different subspecies, knowing if treatment patterns or side effect profiles differed between subspecies would be helpful. Incomplete information regarding duration of therapy with specific agents limited our ability to report information such as median time to any side effect or a side effect severe enough to require therapy alteration for individual medications. Although we did collect information regarding outcomes, this study was not powered to evaluate outcomes associated with individual regimens or medications. Because only EIN members could submit cases, selection bias is possible. Their treatment practices may differ from those of non-EIN members if members follow ATS/IDSA guidelines more closely.

Our survey revealed that therapeutic regimens for *M. abscessus* infection vary widely. Side effects are common and often lead to changing or discontinuing therapy. Given these findings and increasing rates of nontuberculous mycobacterial infections (*7*,*8*), prospective studies requiring cooperation across multiple centers are needed to better define appropriate treatment regimens that will maximize effectiveness while minimizing side effects.

Table 2. Side effects associated with antimicrobial drug therapy for *Mycoobacterium abscessus* infection, March–December 2013*

Side effect	No. (%)
Gastrointestinal†	23 (41.8)
Skin changes‡	11 (20.0)
Renal insufficiency	9 (16.4)
Hearing loss	7 (12.7)
Tinnitus	6 (10.9)
Loss of balance	4 (7.3)
Transaminitis	4 (7.3)
Shortness of breath or airway irritation	3 (5.5)
Neutropenia or thrombocytopenia	2 (3.6)
Other§	5 (9.1)

*Data for 55 patients; some patients reported the same adverse event >1 time and attributed it to different medications. Here, each adverse event is reported only 1 time.
†Nausea/vomiting, abdominal pain, diarrhea.
‡Rash, pruritis, discoloration.
§Anxiety, failure to thrive, fatigue, oral and genitourinary candidiasis.

Members of the *M. abscessus* Study Team: Lilian Abbo, Philip Brachman, Shingo Chihara, Daniel Gluckstein, K. V. Gopalakrishna, Donald R Graham, Alex Granok, R. Gordon Huth, Michael Klevay, James Leggett, Sarah Mooney, David Mushatt, Steven Norris, Lisa Oakley, Brian Petroelje, Hari Polenakovik, Susan Rhee, Kamla Sanasi-Bhola, Paul Southern, Mingquan Suksanong, Gregory Valainis, Mark Wallace, and Regina Won.

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Dr. Novosad is a medical doctor and Pulmonary/Critical Care Fellow at Oregon Health and Science University. Her research focuses on the epidemiology and natural history of chronic pulmonary infections.

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Address for correspondence: Shannon Novosad, 3181 SW Sam Jackson Park Rd, UHN67, Portland, OR 97239, USA; email: novosad@ohsu.edu

Middle East Respiratory Syndrome Coronavirus during Pregnancy, Abu Dhabi, United Arab Emirates, 2013

Asim Malik, Karim Medhat El Masry, Mini Ravi, Falak Sayed

As of June 19, 2015, the World Health Organization had received 1,338 notifications of laboratory-confirmed infection with Middle East respiratory syndrome coronavirus (MERS-CoV). Little is known about the course of or treatment for MERS-CoV in pregnant women. We report a fatal case of MERS-CoV in a pregnant woman administered combination ribavirin–peginterferon-α therapy.

As of June 19, 2015, a total of 1,338 laboratory-confirmed cases of Middle East respiratory syndrome coronavirus (MERS-CoV) and 475 associated deaths had been reported to the World Health Organization (http://www.who.int/csr/disease/coronavirus_infections/risk-assessment-19june2015/en/). Payne et al. (*1*) reported stillbirth in a pregnant patient for whom MERS-CoV serologic testing was retrospectively positive, suggesting that surveillance for and treatment of MERS-CoV in pregnant women may differ from that for nonpregnant persons. We report a fatal case of MERS-CoV in a pregnant woman administered ribavirin–peginterferon-α therapy.

Case Report

On November 19, 2013, a 32-year-old woman residing in Abu-Dhabi, United Arab Emirates, sought medical care for fever and back pain of 4 days' duration. The woman, a school teacher from Jordan, was 32 weeks pregnant; she reported 3 earlier pregnancies (2 live births) and no concurrent conditions. Emergency department (ED) and obstetric physicians suspected urinary tract infection. The patient declined admission but returned to the ED on November 22 with worsening fever, cough, and shortness of breath. She denied recent travel, sick contacts, or animal exposure within the previous 2 weeks. Lung examination results were within normal limits; the patient had no signs of active labor or fetal distress. Chest computed tomography scan revealed bilateral consolidation; pulmonary embolus was not seen.

The patient was admitted to the medical unit with suspected community-acquired pneumonia. Ceftriaxone and azithromycin were initiated. Her condition deteriorated, and on November 23, she was transferred to the intensive care unit (ICU) because of respiratory failure and hypotension. On November 24, acute respiratory distress syndrome developed, requiring respiratory and hemodynamic support. Empiric oseltamivir and vancomycin were added to the treatment regimen. Later that day, the baby was delivered by caesarean section because the patient was persistently hypoxemic while on maximal ventilator support. Transient improvement in oxygenation was noted after the delivery. The newborn, who was noted to be healthy and had Apgar scores of 6 and 8 at 1 and 5 minutes, respectively, had no contact with the mother after birth.

Nasopharyngeal aspirate samples were tested for influenza A(H1N1)pdm09 virus and MERS-CoV by real-time reverse transcription PCR (*2*), and multiple other laboratory and culture tests were conducted (Table). Most yielded negative results, but on November 25, the regional laboratory reported the MERS-CoV real-time reverse transcription PCR results were positive. Laboratory testing was done by qualitative assay, using the 2012 novel human CoV (human coronavirus–Erasmus Medical Center). The assay, performed according to a previously described method (*3*), contains reagents and enzyme for specific amplification of the region upstream of the envelope gene in the CoV genome.

On November 26, oral ribavirin (400 mg and 600 mg morning and evening, respectively) and subcutaneous peginterferon-α (180 μg 1×/wk) were initiated. On November 27, ribavirin was increased to 1,200 mg every 8 hours, and meropenem was begun. Septic shock developed in the patient, requiring maximal vasopressors and ventilator support. Despite intensive support, the patient's condition continued to deteriorate; she died on December 2. Cultures of blood, tracheal aspirate, and urine obtained on the day of death showed no growth; a chest radiograph revealed improvement in pulmonary edema and consolidation.

On November 21, MERS-CoV pneumonia developed in the patient's husband. He had no concurrent conditions and fully recovered after receiving an antimicrobial drug regimen similar to his wife's at a different facility. The husband subsequently reported that he and his wife had visited a cattle farm (goats, sheep, and camels) 10 days before becoming sick (*4*) (Figure) but had not consumed camel meat or milk. In addition, a mild cough without fever or other symptoms developed in the patient's 8-year-old son; MERS-CoV PCR testing of nasopharyngeal aspirate from the boy was positive. He recovered uneventfully without intervention. The younger sibling and newborn remained asymptomatic and tested negative for MERS-CoV.

Patients infected with MERS-CoV typically show signs of respiratory illness (*2*) and sometimes diarrhea.

Author affiliation: Mafraq Hospital, Abu Dhabi, United Arab Emirates

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Table. Laboratory and imaging investigation for a pregnant patient with MERS-CoV infection, Abu Dhabi, United Arab Emirates, 2013*

Test	Finding						
	22 Nov	24 Nov	25 Nov	26 Nov	27 Nov	28 Nov	29 Nov
Urine culture						Neg	Neg
Blood culture	Neg	Neg				Neg	Neg
High vaginal swab sample	Neg						
Sputum culture		Neg					
Tracheal aspirate culture							Neg
MDR screen		Neg					
MRSA screen		Neg					
Legionella		Neg					
Influenza A		Neg					
Influenza B		Neg					
H1N1 PCR		Neg					
MERS-CoV rRT-PCR, specimen		1 Neg, 1 Pos, C_t 34.10, naso aspirate	Pos, C_t 22.02, tracheal aspirate		Pos, tracheal aspirate		Neg, tracheal aspirate
Leukocytes, × 10^9 cells/L	5.03	6.76	21.57	18.22	10.51	15.69	9.52
Chest imaging, modality	Bilateral consolidation, contrast CT		Bilateral consolidation, radiograph	Resolving consolidation and edema, radiograph		Worsening edema, radiograph	Improved edema and consolidation, radiograph

*Specimen processing was performed by using the EZ1 Advanced XL instrument (QIAGEN, Hilden, Germany) with the LightCycler (Roche Diagnostics, Indianapolis, IN, USA) or Rotor-Gene (QIAGEN) instrument for automated amplification and detection. The detection limit of the assay is 3.4 copies per reaction. Blank cells indicate no testing was done on that day. CT, computed tomography; C_t, cycle threshold; H1N1, influenza A(H1N1)pdm09 virus; MDR, multidrug resistance; MERS-CoV, Middle East respiratory syndrome; MRSA, methicillin-resistant *Staphylococcus aureus*; naso aspirate, nasopharyngeal aspirate; Neg, negative; Pos, positive; rRT-PCR, real-time reverse transcription PCR.

Complications include acute renal failure and acute respiratory distress syndrome with shock. Immunocompromised patients may have atypical signs and symptoms. Furthermore, several issues are relevant to MERS-CoV and other infectious diseases acquired during pregnancy: 1) pregnancy is associated with immunologic changes that may alter susceptibility to and severity of infectious diseases; 2) the effects of infection upon the fetus are not fully understood; and 3) prophylaxis and treatment appropriate for the general population might not be appropriate for pregnant women (*5*–*8*). When the pregnant patient in our study sought medical care, she had atypical symptoms (fever and back pain, followed later by cough and shortness of breath). It is unclear if the delay in initiating antimicrobial therapy may have contributed to the fatal outcome.

MERS-CoV infection developed in 2 of the patient's 4 other family members, an outcome compatible with the description of other small clusters among household members and close contacts. The spectrum of illness and symptomatology among the affected family is also noteworthy: the young child was mildly sick, whereas the pregnant mother died. This discrepancy in disease severity correlates with findings from other reports (*9*,*10*).

No MERS-CoV transmission was documented between the patient and hospital staff; thus, the staff's use of contact and droplet precautions, including airborne precautions while performing aerosol-generating procedures, seems to have been effective (*11*,*12*). Of note, these precautions were implemented after the patient was transferred to the ICU. Thirty-six hospital personnel were screened: 11 ED, 13 obstetrics, 1 operating room, and 11 ICU staff. No symptoms developed, and all staff tested negative for MERS-CoV infection. The lack of cross transmission in the exposed healthcare workers before implementation of protective measures supports the benefit of using standard precautions and the fact that transmission of MERS-CoV between humans remains limited (*13*).

Although, MERS-CoV infection and pregnancy were a fatal combination for the patient in our study, virus shedding ceased in the patient during therapy with ribavirin and peginterferon-a. Knowledge about the use of these drugs is limited; thus, further studies are needed to understand the possible safety, efficacy, and optimal dosage and duration of this regimen. Few data exist regarding the use of ribavirin in pregnant humans; however, the drug is generally contraindicated in pregnancy (*14*,*15*) because of evidence of teratogenic and embryocidal effects in animal studies. The potential role of cyclosporine, intravenous immune globulin, and high-frequency ventilation for treatment of MERS-CoV during pregnancy needs evaluation.

After the baby was delivered, the patient showed transient improvement, with improved oxygenation, followed by progressive worsening of clinical status and death. It is unclear whether there is a pattern of delayed release of chemokines and activation of inflammatory cascades leading to delayed worsening of the clinical condition. The patient was maintained on broad-spectrum antibacterial drugs with excellent pharmacokinetics. All cultures and screening for resistant pathogens remained negative, making it unlikely that the patient succumbed to a superimposed bacterial infection.

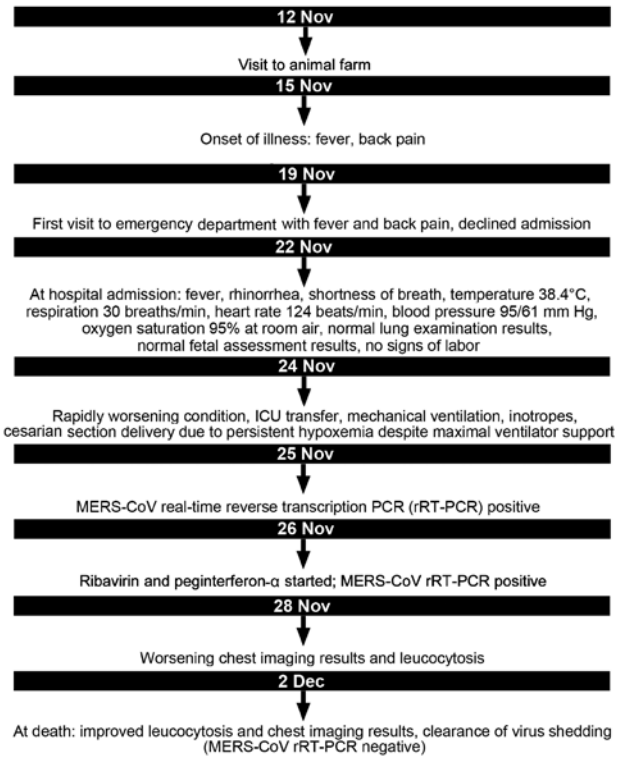

Figure. Timeline of clinical events in a pregnant patient with Middle East respiratory syndrome coronavirus (MERS-CoV) infection, Abu Dhabi, United Arab Emirates, 2013. ICU, intensive care unit.

Conclusions

Pregnant women who seek medical care for pneumonia, influenza-like illness, or sepsis on the Arabian Peninsula may benefit from screening for MERS-CoV to ensure early diagnosis and management of this sometimes fatal disease. The immunologic and chemokine response to the infection needs close examination to help define the potential therapeutic role of antiinflammatory agents in this disease.

MERS-CoV infection and pregnancy were a fatal combination in this case. Death occurred despite treatment with a combined ribavirin and interferon regimen and despite clearance of virus shedding and radiographic evidence of improvement at death. Thus, this regimen needs to be further studied in pregnant patients with MERS-CoV infection.

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Dr. Malik is Chief of Infectious Diseases Service and the Chairman of Infection Control at Mafraq Hospital, Abu Dhabi, United Arab Emirates. His interests include healthcare-associated infections, multidrug-resistant pathogens, and emerging infectious diseases.

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Address for correspondence: Asim Malik, Mafraq Hospital, Infection Control Box 2951, Abu Dhabi, United Arab Emirates; email: asmalik@mafraqhospital.ae

Preliminary Favorable Outcome for Medically and Surgically Managed Extensively Drug-Resistant Tuberculosis, France, 2009–2014

Benoît Henry,[1] Matthieu Revest, Nathalie Dournon, Loïc Epelboin, Guillaume Mellon, Guillaume Bellaud, Pierre Mordant, Damien Le Dû, Nicolas Véziris, Christine Bernard, Sébastien Morel, Stéphane Jauréguiberry, Christian Michelet, François Bricaire, Pierre Tattevin,[2] Éric Caumes[2]

We report 20 cases of extensively drug-resistant tuberculosis managed in France. Treatment was individualized and included bedaquiline and linezolid for most patients and surgery in 8 patients. At last follow-up (22 months), 19 patients had achieved conversion from positive to negative on culture testing. These promising results of comprehensive management obtained in a small series deserve confirmation.

Multidrug-resistant tuberculosis (TB) and extensively drug-resistant TB (XDR TB) are among the most difficult infections to treat and are major public health concerns worldwide (*1*). In France, the number of imported XDR TB cases has dramatically increase recently, especially cases originating from countries of the former Union of Soviet Socialist Republics (*2*).

The Study

During 2009–2014, we identified 20 persons who were admitted to 2 tertiary-care hospitals in Rennes and Paris, France, with culture-positive XDR TB infections. Patients were identified through hospital database searches; patient data were extracted from medical charts. For each patient, we performed direct examination of sputum smears; cultures on Lowenstein-Jensen medium; genotypic resistance profiling (GenoType MTBDR Plus; HAIN Lifescience, Nehren, Germany); and in vitro drug susceptibility testing (DST) on Lowenstein-Jensen medium, according to the proportions method.

All patients were isolated in negative-pressure rooms until their respiratory sample culture results converted from positive to negative (hereafter referred to as culture conversion). Medical and surgical therapeutic options were determined during multidisciplinary meetings involving infectious diseases, respiratory diseases, microbiology, and thoracic surgery departments. In agreement with World Health Organization guidelines (*3*), we selected anti-TB drug therapy on the basis of results from previously used agents or genotypic and phenotypic DST. Throughout hospitalization, treatment toxicity was carefully monitored through clinical assessments, routine laboratory tests, therapeutic drug monitoring, audiograms for patients on aminoglycosides, and weekly electrocardiograms. Treatment efficacy was monitored through thoracic imaging and monthly examination of respiratory samples. For patients with nondisseminated pulmonary TB, surgery was considered at the initiation of medical treatment if success of the treatment was deemed unlikely because of extensive lesions or after 3 months of optimized medical treatment if sputum conversion was not achieved. In agreement with procedures implemented by the French Information Protection Commission, all data were anonymized and collected on a standardized form.

The 20 XDR TB patients (Table 1) had recently arrived in France from Georgia (n = 17), Armenia (n = 2), and the Russian Federation (n = 1); median duration between arrival and hospitalization was 2 (interquartile range [IQR] 1–7) days. Median delay from admission to initiation of any anti-TB treatment was 18 (IQR 11–25) days.

During the intensive phase of treatment, each patient was given 4–9 presumably active anti-TB agents (Table 2). All patients required long-term central venous access for administration of amikacin or carbapenems. Duration of medical treatment was individualized but continued a

Author affiliations: Centre Hospitalier Universitaire Pitié Salpêtrière, Paris, France (B. Henry, N. Dournon, L. Epelboin, G. Mellon, G. Bellaud, N. Véziris, C. Bernard, S. Morel, S. Jauréguiberry, F. Bricaire, E. Caumes); Université Pierre et Marie Curie, Paris (B. Henry, L. Epelboin, N. Véziris, S. Morel, F. Bricaire, E. Caumes); Centre Hospitalier Universitaire Pontchaillou, Rennes, France (M. Revest, C. Michelet, P. Tattevin); Hôpital Européen Georges Pompidou, Paris (P. Mordant); Université Paris Descartes, Paris (P. Mordant); Centre Hospitalier de Bligny, Briis-sous-Forges, France (D. Le Dû); Centre National de Référence des Mycobactéries et de la Résistance des Mycobactéries aux Antituberculeux, Paris (N. Véziris, C. Bernard); Centre d›Immunologie et des Maladies Infectieuses, Paris (N. Véziris, S. Jauréguiberry); Université Rennes 1, Rennes (C. Michelet, P. Tattevin)

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[1]Current affiliation: Université Paris Descartes, Sorbonne Paris Cité, APHP, Hôpital Necker Enfants Malades, Centre d'Infectiologie Necker Pasteur, Institut Imagine, Paris, France.

[2]These senior authors contributed equally to this article.

Table 1. Clinical and demographic characteristics of 20 persons with extensively drug-resistant TB, France, 2009–2014*

Characteristic	Value†
Age, y (range)	37.1 (32–40.1)
Sex, no. patients	
M	18
F	2
Country of origin, no. patients	
Georgia	17
Armenia	2
Russian Federation	1
Body mass index, kg/m^2 (range)	19.8 (17.7–22.7)
Past imprisonment, no. patients/no. total (%)	4/18 (22.2)
Past or present intravenous drug use, no. patients/no. total (%)	10/20 (50)
Previous history of TB, no. patients/no. total (%)	19/20 (95)
Previous anti-TB treatment, no. patients/no. total (%)	19/20 (95)
Previous thoracic surgery for TB, no. patients/no. total (%)	3/20 (15)
HIV infection, no. patients/no. total (%)	2/20 (10)
Hepatitis C virus infection, no. patients/no. total (%)	12/20 (60)
Duration of TB symptoms before current admission, y (range)	2.4 (0.5–7)
Organs involved, no. patients	
Lungs	20
Epididymis	1
Weight loss, no. patients/no. total (%)‡	15/17 (88.2)
Prolonged fever, no. patients/no. total (%)§	8/16 (50)
Hemoptysis, no. patients/no. total (%)	13/16 (81)
Serum albumin, g/L (range)	30 (26.5–32.7)
Cavitary lesions on chest radiographs or CT scan images, no. patients/no. total (%)	19/19 (100)
Multilobar radiological involvement, no. patients/no. total (%)	18/19 (94.7)

*CT, computed tomography; TB, tuberculosis.
†Quantitative data are median (interquartile range); qualitative data are no. patients/no. with data available (%).
‡Loss of >5% of total bodyweight.
§Body temperature >38°C during at least 3 weeks.

minimum of 12 months after culture conversion. Median duration of treatment was 24 (range 18–51) months. Median length of stay in the acute-care setting was 67 (IQR 55–124) days.

The following grade 3 or 4 treatment-associated adverse events (Common Terminology Criteria for Adverse Events, http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf) were reported in 18 patients: digestive side effects (14 patients), hepatitis (1 patient), peripheral neuropathy (7 patients), neuropsychiatric side effects (3 patients), and hearing impairment (3 patients). Candidemia developed in 5 patients. No grade 3 or 4 nephrotoxicity or cytopenia or significant QT interval prolongation were reported. Adverse events related to anti-TB agents were managed by a wide range of symptom treatments.

Thoracic surgery was performed in 8 patients (lobectomy in 5, pneumonectomy in 3), of whom 2 had severe postsurgical complications. Six of these patients were culture-positive before surgery, and their cultures converted a median of 25 days postsurgery (IQR 20–33 days).

After a median follow-up of 22 (IQR 15–27) months, 18 patients were alive; 2 had died of causes considered unrelated to TB. Sputum cultures had converted for 19 patients, 4 of whom had completed treatment and a median of 38 (IQR 29–40) months of posttreatment follow-up. Median time from treatment initiation to culture conversion was 100 (IQR 75–114) days. One patient is still under treatment and has not experienced culture conversion. No patients were lost to follow-up.

Conclusions

This small series of XDR TB cases managed in a high-income country illustrates the potential safety and efficacy of multidisciplinary and individualized treatment with a multidrug regimen that includes new anti-TB agents (e.g., bedaquiline); innovative use of older agents (e.g., linezolid); and the combination of imipenem plus amoxicillin/clavulanate. The series also emphasizes difficulties faced by healthcare professionals caring for XDR TB patients.

The final outcome could not be ascertained for most study patients because they are still receiving antimycobacterial therapy; however, the 90% survival rate after a median follow-up of 22 months after treatment initiation is reassuring and compares favorably with survival rates of 66%, 54%, and 38% in the United States (*4*), South Africa (*5*), and the United Kingdom (*6*), respectively. The high rate of microbiologic conversion in our study (95%) also reflects the potentially achievable treatment efficacy, even in the context of previously treated XDR TB cases.

Our findings should be interpreted cautiously because of the small number of patients and the relatively short follow-up at the time of this writing. However, the

Table 2. Anti-TB agents used in the treatment of persons with extensively drug-resistant TB, France, 2009–2014*

Drug	No. patients previously treated with drug/no. with available data (%)	No. patients with resistant strains†/no. with available data (%)	No. study patients treated with drug/no. with available data (%)
Rifampin	12/15 (80)	20/20 (100)	0/20
Isoniazid	13/15 (86.7)	20/20 (100)	0/20
Pyrazinamide	14/15 (93.3)	15/17 (88.2)	9/20 (40)
Ethambutol	13/14 (92.9)	17/20 (85)	5/20 (25)
Streptomycin	6/14 (42.9)	19/20 (95)	0/20
Amikacin	3/14 (21.4)	10/20 (50)	12/20 (60)
Kanamycin	5/14 (35.7)	19/20 (95)	0/21
Capreomycin	9/15 (60)	16/20 (80)	2/20 (10)
Ofloxacin	4/14 (28.6)	20/20 (100)	0/20
Levofloxacin	3/12 (25)	NA	NA
Moxifloxacin	4/14 (28.6)	14/19 (73.7)	7/20 (35)
Ethionamide	9/15 (60)	17/20 (85)	5/20 (25)
Linezolid	0/14	0/20	20/20 (100)
p-aminosalicylate	14/17 (82.4)	4/20 (20)	16/20 (80)
Amoxicillin/clavulanate	3/14 (21.4)	NA	19/20 (95)
Imipenem	0/14	NA	19/20 (95)
Cycloserine	12/15 (80)	16/21 (76.2)	13/20 (65)
Clarithromycin	2/13 (15.4)	NA	0/21
Clofazimine	2/13 (15.4)	NA	9/18 (50)
Bedaquiline	0/20	NA	16/20 (80)

*N/A, no available data; TB, tuberculosis.
†Determined by in vitro susceptibility testing at admission.

overall figures of culture conversion are more satisfactory than those previously reported in high-income countries, where conversion rates ranged from 46.7% to 76.1% (*4*,*6*–*10*), and in high-prevalence settings, where conversion rates are lower (*5*,*11*,*12*). Preliminary results from a study conducted in South Africa were more favorable: samples from 48 (76%) of 63 patients with 6 months follow-up were culture-negative (*13*). Postsurgery sputum conversion was rapidly achieved for patients in our study; thus, pulmonary resection surgery, although risky, may also have contributed substantially to treatment successes.

Numerous difficulties were encountered during the study. Patients were referred to our centers soon after arriving in France, causing communication difficulties for patients with a limited understanding of French and English. Medical histories were long and complex, and most patients were in advanced stages of pulmonary TB.

The numerous side effects observed during prolonged anti-TB regimens must be optimally and intensively managed; otherwise, patients may not complete treatment. Because of the limited number of potentially active anti-TB agents, drugs with documented long-term toxicities must also be included in multidrug regimens. The high incidence of breakthrough candidemia cases (5/20 patients [25%]) was not anticipated, although prolonged exposure to broad-spectrum antimicrobial drugs and long-term central venous access are acknowledged risk factors for candidemia. This risk must be taken into account when considering treatment of XDR TB with carbapenems and amoxicillin/clavulanate. The overall good tolerability of linezolid may be a result of the low dosage (routinely, 600 mg/d initially, decreased to 300 mg/d if toxicity is suspected, even with limited evidence).

Financial, social, and cultural aspects of the management of vulnerable and marginalized patients are also essential and time-consuming. Prolonged hospital stays were necessary for patients in our study, resulting in high healthcare costs, as previously reported in South Africa (*14*). Limited resources and vulnerability are risk factors for noncompliance and disease progression. However, failure to adequately address these issues would translate into additional XDR TB transmission in the community and increased illness and death, which could result in a much higher societal burden. Previous experience in high-income countries has documented that comprehensive care of TB patients is cost-effective, even in the most vulnerable and marginalized populations, especially when multidrug-resistant or XDR TB are involved (*15*).

Our results reflect the situation in a high-income setting with free access to all potentially active drugs, extensive investigation of responsible strains (e.g., using DST and genotypic tests), daily monitoring of adverse events, regular multidisciplinary meetings to tailor treatment to any new event and evaluate the need for thoracic surgery in selected cases, dedicated medical and paramedical staff, and psychosocial support. Unfortunately, the situation may not be the same in the countries most affected by XDR TB.

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Dr. Henry is a chief resident of infectious diseases at Université Paris Descartes, Sorbonne Paris Cité, APHP, Hôpital Necker Enfants Malades, Centre d'Infectiologie Necker Pasteur, Institut Imagine, Paris, France. His primary research interests include tuberculosis, prosthetic joint infections, and infections in immunocompromised hosts.

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Address for correspondence: Pierre Tattevin, Service des maladies infectieuses et réanimation médicale, Centre Hospitalier Universitaire Pontchaillou, rue Le Guilloux, 35000 Rennes, France; email pierre.tattevin@chu-rennes.fr

Lyme Disease in Hispanics, United States, 2000–2013

Christina A. Nelson, J. Andrew Starr, Kiersten J. Kugeler, Paul S. Mead

Hispanics comprise a growing portion of the US population and might have distinct risk factors for tickborne diseases. During 2000–2013, a total of 5,473 Lyme disease cases were reported among Hispanics through national surveillance. Hispanics were more likely than non-Hispanics to have signs of disseminated infection and onset during fall months.

Lyme disease (LD) is caused by the spirochete *Borrelia burgdorferi*, transmitted to humans through the bite of infected *Ixodes scapularis* and *I. pacificus* ticks. Early localized infection typically manifests as erythema migrans with concomitant fever and malaise; disseminated infection can lead to facial palsy, carditis, arthritis, or neuropathy (*1*).

Outdoor workers in LD-endemic areas have increased odds of occupational exposure to ticks and a rate of LD seropositivity substantially higher than that of the general population (*2*,*3*). In the United States, Hispanics comprise 43.6% of grounds maintenance workers and 43.4% of workers in the farming, fishing, and forestry industries, potentially placing this population at greater risk for LD from occupational exposures (*4*).

An estimated 9 million Hispanics live in the 13 states with the highest reported incidence of LD, all of which are located in the Northeast, upper Midwest, and mid-Atlantic regions (*5*,*6*). Little is known, however, about the epidemiology of LD in the rapidly growing and diverse US Hispanic population. Improved understanding of LD in Hispanics could aid prevention efforts by public health practitioners and diagnosis by clinicians. The objective of this study was to describe the epidemiology of LD in the US Hispanic population and identify differences between Hispanics and non-Hispanics with LD by using national surveillance data.

The Study

LD is a nationally notifiable condition, and cases are reported by state and local health departments to the Centers for Disease Control and Prevention (CDC) through the National Notifiable Diseases Surveillance System in accordance with previously established protocols (*7*). LD cases reported during 2000–2007 were confirmed cases only. In 2008, a revised case definition was implemented that altered the laboratory criteria and distinguished between confirmed and probable cases; cases reported during 2008–2013 included both categories (*8*).

We used 2010 US Census population data to calculate incidence rates (*5*,*9*). Weighting was applied to state- and county-specific numbers of cases to account for variations in completeness of ethnicity data. Descriptive statistics and comparisons were calculated by using SAS version 9.3 (SAS Institute, Cary, NC, USA). We compared median age of Hispanics and non-Hispanics with LD using the Kolmogorov-Smirnov 2-sample test. Risk ratios (RRs) were used to compare categorical data.

CDC human subjects review of the protocol determined it was not research involving human subjects. Thus, Institutional Review Board approval was not required.

During 2000–2013, a total of 374,338 LD cases were reported to CDC, of which 148,444 (39.7%) reports contained information about ethnicity and were included in this analysis. Among these, 5,473 (3.7%) persons self-identified as being of Hispanic ethnicity. Most (54.8%) Hispanics with LD were male; median age was 32 years (interquartile range 15–46 years).

Annual incidence of reported LD among Hispanics was 0.8 cases/100,000 persons, compared with 4.0/100,000 among non-Hispanics. During 2000–2001, Hispanics comprised 2.8% of all persons with LD, whereas during 2009–2013, Hispanics comprised 3.7%–4.9% of persons with reported LD. In comparison, the proportion of Hispanics in the US population increased slightly during this period, from 13% in 2000 to 16% in 2010 (*5*).

Although a bimodal age distribution was evident among both Hispanics and non-Hispanics with LD, the peak in children was less pronounced among Hispanics (Figure). Highest incidence among Hispanic children was in boys 10–14 years of age, whereas among non-Hispanic children, incidence was highest in boys 5–9 years of age. In adults, highest incidence among both Hispanics and non-Hispanics was in men 65–74 years of age.

Hispanics were significantly less likely than non-Hispanics to have disease onset during the summer months (RR 0.85, 95% CI 0.83–0.88) and more likely to have disease onset during the fall months (RR 1.15, 95% CI 1.07–1.24) (Table 1). Although erythema migrans was the most commonly

Author affiliations: Centers for Disease Control and Prevention, Fort Collins, Colorado, USA (C.A. Nelson, K.J. Kugeler, P.S. Mead); University of Virginia School of Medicine, Charlottesville, Virginia, USA (J.A. Starr)

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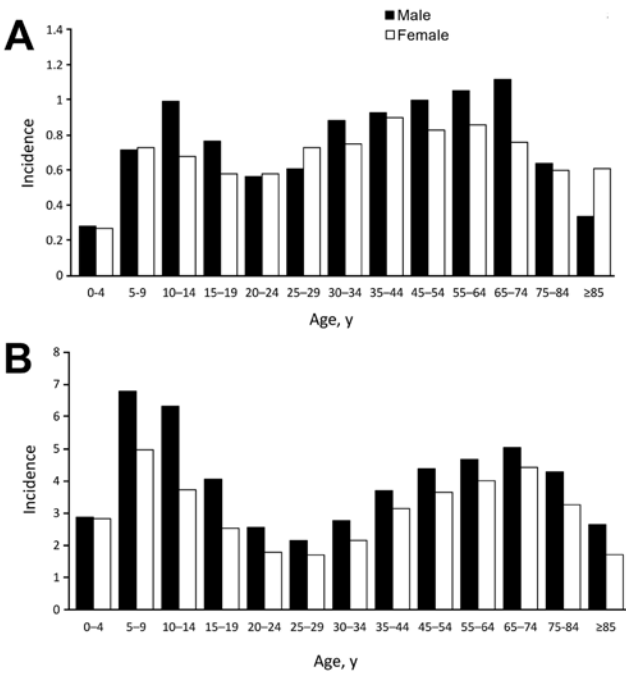

Figure. Age- and sex-specific incidence of Lyme disease among Hispanics (A) and non-Hispanics (B), United States, 2000–2013. For persons ≥35 years, age categories are collapsed into 10-year intervals. Incidence is cases per 100,000 persons.

reported clinical feature for both groups, it was less commonly reported among Hispanics than non-Hispanics (RR 0.83, 95% CI 0.80–0.86). Manifestations of disseminated disease, such as arthritis and facial palsy, were more commonly reported among Hispanics than non-Hispanics (Table 1).

As expected, >90% of LD cases overall were reported from high-incidence states, although Hispanics with LD were slightly less likely to report residence in a high-incidence state (RR 0.90, 95% CI 0.82–0.98). All of the statistical associations were similar when analysis was restricted to confirmed cases only, with the exception of residence in a high-incidence state, which became nonsignificant (RR 0.99, 95% CI 0.89–1.10).

After weighting, nearly half of all estimated cases of LD among Hispanics were from New York or New Jersey (Table 2). Among counties with at least 75 estimated LD cases among Hispanics during the study period, highest incidence among Hispanics occurred in Columbia County, New York (170.4 cases/100,000 persons); Sussex County, New Jersey (111.4/100,000); and Hunterdon County, New Jersey (106.3/100,000).

Conclusions

Overall, the epidemiology of LD among Hispanics was similar to that among non-Hispanics: bimodal age distribution, slight predilection in males, and clustering in states to which LD is highly endemic were apparent (*10*). However, we identified several important differences. Most notably, Hispanics with LD were significantly more likely than non-Hispanics with LD to have signs of disseminated infection and symptom onset during fall months.

Although the overall incidence of LD in Hispanics was lower than that in non-Hispanics, additional research is needed to determine the reasons underlying these differences and the extent of any LD underdiagnosis in the Hispanic population. Inadequate healthcare access, language barriers, and lack of LD awareness could cause both underdiagnosis and delays in diagnosis in the Hispanic population. During 2009–2013, a total of 41.5% of Hispanics lacked health insurance, compared with 15.1% of non-Hispanic whites; 15.5% of Hispanics described delay in or nonreceipt of medical care because of cost (*11*). Furthermore, whether the predilection toward symptom onset in the fall

Table 1. Demographic and clinical characteristics of Hispanics and non-Hispanics with LD, United States, 2000–2013*

Characteristic	Hispanic, n = 5,473	Non-Hispanic, n = 142,971	RR (95% CI)	p value
Male sex†	2,982 (54.8)	78,417 (55.0)	1.00 (0.97–1.02)	
Median age, y (IQR)	32 (15–46)	42 (16–58)		**0.0001‡**
Disease onset				
Total with known date of disease onset	3,826 (69.9)	116,600 (82.6)	–	
Summer months, Jun–Aug	2,170 (56.7)	77,548 (66.5)	**0.85 (0.83–0.88)**	
Fall months, Sep–Nov	637 (16.7)	16,821 (14.4)	**1.15 (1.07–1.24)**	
Clinical features				
Total with information on clinical features	2,696 (49.3)	90,180 (63.1)	–	
Erythema migrans	1,605 (59.5)	64,660 (71.7)	**0.83 (0.80–0.86)**	
Arthritis	854 (31.7)	25,647 (28.4)	**1.11 (1.05–1.18)**	
Facial palsy	391 (14.5)	7,529 (8.4)	**1.74 (1.58–1.91)**	
Atrioventricular block	36 (1.3)	952 (1.1)	1.26 (0.91–1.76)	
Meningitis	36 (1.3)	1,026 (1.1)	1.17 (0.84–1.63)	
Residence in high-incidence state§	4,937 (90.2)	130,305 (91.1)	**0.90 (0.82–0.98)**	

*Values are no. (%) unless otherwise indicated. Statistically significant differences between the comparison groups are in bold. IQR, interquartile range; LD, Lyme disease; RR, risk ratio.
†Percentage of persons with LD for whom sex is known (n = 5,442 Hispanics, n = 142,625 non-Hispanics).
‡The substantial difference in median age between the US Hispanic population (27 y) and the US non-Hispanic population (42 y) most likely accounts for the difference seen here.
§Defined as 1 of the 13 highest-incidence states that accounted for 95% of all reported confirmed cases of LD in 2010: Connecticut, Delaware, Maine, Maryland, Massachusetts, Minnesota, New Hampshire, New Jersey, New York, Pennsylvania, Vermont, Virginia, and Wisconsin.

Table 2. Locations with the highest number of estimated cases and incidence of LD among Hispanics, United States, 2000–2013*

Location	No. reported cases among Hispanics	% Total reported cases with ethnicity information	Estimated total no. cases†	% Total estimated no. Hispanics with LD	No. annual estimated cases/100,000 Hispanics	Counties with highest estimated incidence among Hispanics‡
All states	5,473	39.7	13,786	100	0.8	–
New York	1,825	52.8	3,456	25.1	3.6	Columbia (170.4), Putnam (61.3), Dutchess (47.4)
New Jersey	474	14.2	3,331	24.2	7.6	Sussex (111.4), Hunterdon (106.3), Warren (41.3)
Connecticut	986	50.6	1,950	14.1	14.5	Windham (45.6), New London (30.8), Fairfield (11.9)
Massachusetts	491	36.0	1,364	9.9	7.8	Plymouth (17.3), Norfolk (13.1), Middlesex (8.5)
Pennsylvania	356	28.8	1,238	9.0	6.1	Bucks (18.3), Northampton (16.3), Chester (14.3)
Maryland	253	35.7	708	5.1	5.4	Howard (16.0), Baltimore (14.1), Anne Arundel (8.8)

*LD, Lyme disease.
†After correcting for missing ethnicity data. Calculated as follows: (no. reported cases)/x = (% with ethnicity information)/100, where x is the weighted number of cases.
‡Incidence calculated as number of annual estimated cases in county/100,000 Hispanic residents in county. Only counties with a substantial number of cases were included in this comparison. Seventy-five weighted cases was chosen as the cutoff based on distribution.

months for Hispanics results from delays in medical care or other factors, such as seasonal outdoor labor patterns, is unclear. Lastly, because a larger proportion of Hispanics than the overall US population live in urban areas (*12*), the risk for LD might be differentially diluted in Hispanics.

Our findings were subject to several limitations. First, we had to exclude more than half of reported LD cases because of missing ethnicity data. Although we have no reason to believe that case reports with missing ethnicity data differed otherwise from those included in this study, we cannot exclude this possibility. Ethnicity reporting is also subject to error. Finally, surveillance data are limited by underreporting and reporting bias, which might differ by state and between Hispanic and non-Hispanic populations.

Reaching at-risk populations with culturally and linguistically appropriate prevention education is essential. Although some educational materials about prevention of tickborne diseases have been translated to Spanish (*13*,*14*), additional translations and modifications to address cultural differences would be helpful. Furthermore, targeted educational campaigns could enhance use of these materials and improve the reach, retention, and overall impact of prevention education.

We identified specific risk groups and patterns of LD within the US Hispanic population. Direct and more in-depth assessments regarding prevention practices, knowledge, and LD epidemiology on local and national scales will further the understanding of LD risk in this population and guide future targeted prevention and education efforts.

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Dr. Nelson is a medical epidemiologist at CDC's Bacterial Diseases Branch, Division of Vector-Borne Diseases, National Center for Emerging and Zoonotic Infectious Diseases, Fort Collins, Colorado. Her primary research interests are the epidemiology and clinical manifestations of LD, *Bartonella* infections, tularemia, and plague.

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Address for correspondence: Christina A. Nelson, Centers for Disease Control and Prevention, 3156 Rampart Rd, Mailstop P02, Fort Collins, CO 80521, USA; email: wje1@cdc.gov

Association between Severity of MERS-CoV Infection and Incubation Period

Victor Virlogeux, Minah Park, Joseph T. Wu,[1] Benjamin J. Cowling[1]

We analyzed data for 170 patients in South Korea who had laboratory-confirmed infection with Middle East respiratory syndrome coronavirus. A longer incubation period was associated with a reduction in the risk for death (adjusted odds ratio/1-day increase in incubation period 0.83, 95% credibility interval 0.68–1.03).

The incubation period of an infectious disease is the time from the moment of exposure to an infectious agent until signs and symptoms of the disease appear (*1*). This major biological parameter is part of the case definition and is used to determine duration of quarantine and inform policy decisions when mathematical modeling is used (*2*). Incubation periods vary from person to person, and their distribution tends to be right-skewed and unimodal (*3*). Variability in incubation periods for infection with Middle East respiratory syndrome coronavirus (MERS-CoV) has been described (*4*–*8*). Previous studies have not examined whether the length of the incubation period in a person has any correlation with subsequent clinical outcomes.

In 2015, South Korea had the largest outbreak of MERS-CoV infections outside the Arabian Peninsula (*6*). In a previous study, we reported that patients who died of severe acute respiratory syndrome (SARS) coronavirus infection had a shorter incubation period compared with infected patients who survived (*9*). The objective of this study was to examine the association between severity of MERS-CoV illness and length of incubation period.

The Study

We retrieved publicly available data from the Korea Center for Disease Control and Prevention, the Korean Ministry of Health and Welfare, the World Health Organization, and local news reports in South Korea to compile a list of all confirmed cases that had been reported by July 26, 2015 (*6*). Exposure data were available for 109 (64%) of 170 patients. For most cases, information on exposure was recorded as intervals ≥2 days during which infection was believed to have occurred, rather than exact dates of presumed infection. For the subset of patients without available exposure data, we assumed that their incubation time was 0–21 days because 21 days was the longest incubation period reported (*9,10*). Data for patients is provided in online Technical Appendix 1 (http://wwwnc.cdc.gov/EID/article/22/3/15-1437-Techapp1.xlsx).

To estimate incubation period distribution, we fitted a gamma distribution that enabled interval censoring (*6*) by using Markov Chain Monte Carlo methods in a Bayesian framework (online Technical Appendix 2, http://wwwnc.cdc.gov/EID/article/22/3/15-1437-Techapp2.pdf) (*9*). In this analysis and analyses described below, we specified flat priors for each parameter and drew 10,000 samples from the posterior distributions after a burn-in of 5,000 iterations.

To evaluate potential factors, such as age and sex, that could be associated with length of incubation period, we fitted a multiple linear regression model to the data with the log incubation period as response variable and age and sex as explanatory variables. To determine the association between incubation period and severity of disease, we first estimated the difference in mean incubation period between patients who died and those who survived. However, this analysis could not account for potential confounders. Therefore, we specified a multivariable logistic regression model in which death was the binary response variable and predictors included age, sex, and the incubation time for each patient (*9*). We performed this analysis by using an exact likelihood approach and incubation times resampled from the 10,000 posterior samples in each iteration (online Technical Appendix). All analyses were conducted by using R version 3.0.2 (R Foundation for Statistical Computing, Vienna, Austria). Raw data and R syntax enabling reproduction of results are available from the Dryad Digital Repository (http://dx.doi.org/10.5061/dryad.v3456).

Of 170 patients in this study, 36 (21%) died. Mean patient age was 54.6 years, and 98 (58%) were male. Patients who died were significantly older than patients who survived (68.9 years vs. 50.8 years; $p<0.001$). No differences regarding age, sex, and case-fatality risk were observed between patients with or without recorded exposure data. We estimated a mean incubation period of MERS-CoV in all 170 patients of 6.9 days (95% credibility interval [CrI] 6.3–7.5 days) by using a gamma distribution. Age and sex had no associations with incubation period.

The mean incubation period was 6.4 days (95% CrI 5.2–7.9 days) for 36 patients who died compared with 7.1

Author affiliations: Ecole Normale Supérieure de Lyon, Lyon, France (V. Virlogeux); The University of Hong Kong, Hong Kong, China (V. Virlogeux, M. Park, J.T. Wu, B.J. Cowling)

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[1]These senior authors contributed equally to this article.

days (95% CrI 6.3–7.8 days) for 134 patients who survived (Figure). The difference in means was 0.62 days (95% CrI -0.99 to 2.04 days). In the multivariable logistic regression model, we found that a longer incubation period was associated with a marginally reduced risk for death (odds ratio 0.83/1-day increase in incubation period, 95% CrI 0.68–1.03/day) after adjustment for age and sex (online Technical Appendix 2 Table 2).

To examine sensitivity of our results, we also fitted the logistic regression models by using 3 categories for the incubation period. We observed similar results and a reduced risk for death associated with longer incubation periods (online Technical Appendix Table 2). Results were also consistent in the subset of 109 patients with recorded exposure intervals (online Technical Appendix Table 2).

Conclusions

We estimated the incubation period of MERS-CoV cases during the recent MERS outbreak in South Korea and found that patients who died had a shorter incubation period than patients who survived. In a previous study, we found that the length of incubation period in patients infected with SARS coronavirus was also correlated with severity of the disease, with a shorter incubation period for patients who died (*9*). The pathogenesis of MERS-CoV and SARS coronavirus infection is similar (*11*), with a rapid progression to respiratory failure and intubation occurring ≈1 week after onset of symptoms and up to 5 days earlier in MERS patients than in SARS patients (*4*,*12*). Moreover, high rates of hemoptysis were observed in patients infected with MERS-CoV, which suggests severe lung injury (*4*).

MERS-CoV also has higher replication rates and shows broader cell tropism in the lower human respiratory tract than severe acute respiratory syndrome coronavirus (*13*). These results suggest that a shorter incubation period could be related to a higher initial infective dose and consequently to faster or greater pathogen replication. This finding could lead to a more severe disease induced by more aggressive and damaging inflammatory responses (*14*). Closer monitoring of patients who have a shorter incubation period could be considered during such outbreaks.

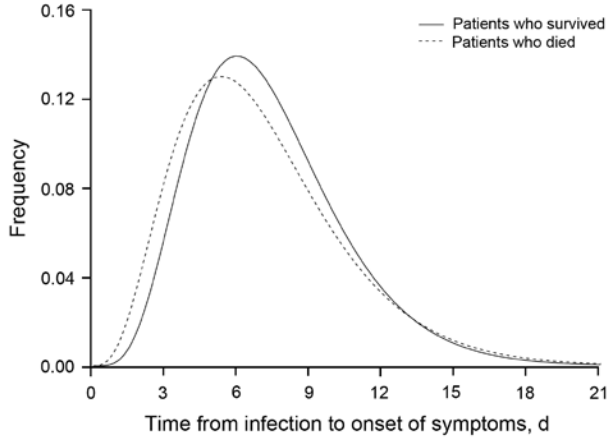

Figure. Parametric estimates of incubation period distribution for patients who died of infection with Middle East respiratory syndrome coronavirus (dashed line) and patients who survived infection (solid line), South Korea, 2015.

Another potential explanation for our findings is that patients with longer incubation periods were identified and infection confirmed more quickly. This improvement in time to identification and admission to a hospital led to improved prognosis. Although longer incubation periods were correlated with shorter delays from onset to laboratory confirmation, we did not find evidence of a strong mediating effect of delay from onset to laboratory confirmation on the risk for death. However, with the small sample size, there was limited statistical power to detect a small-to-moderate effect.

Our study had some limitations. Our estimates of the incubation period were based on self-reported exposure data, which could be affected by recall bias. Moreover, 61 patients (36%) included in our main analysis had missing exposure data, and inclusion in a Bayesian framework with a wide interval of 0–21 days was necessary. Both of these limitations could have reduced the statistical power of our study to identify an association. Finally, we did not have information on underlying medical conditions or the geographic location of cases, or the treatments that were given to cases, and these variables could have been associated with clinical outcomes.

In conclusion, we found an association between shorter incubation periods among patients with MERS-CoV infection and a higher risk of death subsequently, similar to the association previously reported for severe acute respiratory syndrome coronavirus (*9*). This association might occur because the duration of the incubation period is an early reflection of disease pathogenesis.

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Dr. Virlogeux is a MD/PhD student at the University of Medicine Lyon-Est and at the Ecole Normale Superieure in Lyon, France. His research interests include infectious disease epidemiology, and the mathematical modelling of epidemic dynamics.

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Address for correspondence: Benjamin J. Cowling, World Health Organization Collaborating Centre for Infectious Disease Epidemiology and Control, School of Public Health, Li Ka Shing Faculty of Medicine, The University of Hong Kong, 21 Sassoon Rd, Pokfulam, Hong Kong, China; email: bcowling@hku.hk

Liver Abscess Caused by Infection with Community-Acquired *Klebsiella quasipneumoniae* subsp. *quasipneumoniae*

Sebastien Breurec, Benedicte Melot, Bruno Hoen, Virginie Passet, Kinda Schepers, Sylvaine Bastian, Sylvain Brisse

We report a case of pyogenic liver abscess caused by community-acquired *Klebsiella quasipneumoniae* subsp. *quasipneumoniae*. The infecting isolate had 2 prominent features of hypervirulent *K. pneumoniae* strains: the capsular polysaccharide synthesis region for K1 serotype and the integrative and conjugative element ICE*Kp1,* which encodes the virulence factors yersiniabactin, salmochelin, and RmpA.

The syndrome of pyogenic liver abscess caused by community-acquired *Klebsiella pneumoniae* (CA-KLA) infection has been described mainly in Asia, particularly in Taiwan. Infection is caused by hypervirulent strains of particular clonal groups (CG); prominent among the clonal groups is CG23 of capsular serotype K1 (*1*,*2*). Although intestinal colonization is probably a prerequisite for disease, the gate of entry leading to infection and mechanism by which it occurs are unknown (*3*). A novel species of the genus *Klebsiella* closely related to *K. pneumoniae*, *K. quasipneumoniae*, was recently described (*4*); the species is divided into 2 subspecies, but its pathogenicity is not well known. Until now, *K. quasipneumoniae* has only been isolated from persons with hospital-acquired infections or carriage (*4*–*6*). We report a case of liver abscess caused by community-acquired *K. quasipneumoniae* subsp. *quasipneumoniae*.

On June 21, 2014, a 65-year-old man was admitted to the medical center of Basse-Terre, Guadeloupe (French West Indies), with a history of fever, vomiting, and joint pain. He also had a history of coronary heart disease, type 2 diabetes, and essential hypertension. The patient had not previously been hospitalized in 2014. He was given analgesic drugs and was discharged. Five days later, he again visited the medical center with persistent fever. Clinical examination showed a painful, red left eye; congestive heart failure; and a tender, enlarged spleen. Laboratory analysis showed elevated biological values for serum C-reactive protein (328 mg/L), serum procalcitonin (18 mg/L), leukocytes (21.5 cells/mL), polymorphonuclear leukocytes (20.5 cells/mL), platelets (30 cells/mL), aspartate aminotransferase (8 × the upper limit of normal [U/L]), alanine aminotransferase (3.5 × U/N), total bilirubin (43 μmol/L), and serum creatinine (170 μmol/L. Urine and blood cultures were negative, and findings of chest radiograph and abdominal ultrasound were unremarkable. Treatment was begun with intravenous amoxicillin/clavulanate.

On June 28, the patient was transferred to the university medical center at Pointe-à-Pitre, Guadeloupe. Ophthalmic examination revealed uveitis in the left eye. The diagnoses of leptospirosis with ocular involvement and bacterial sepsis were considered, and the antimicrobial agent was changed to ceftriaxone daily. On July 4, the diagnosis of leptospirosis was regarded as most likely, and antimicrobial drug therapy was narrowed to amoxicillin. However, on July 8, the eye condition (endophthalmitis and orbital cellulitis) worsened, and the antimicrobial drugs were switched to ceftazidime and levofloxacin.

On July 17, the patient's general condition had improved, although endophthalmitis persisted. All microbiological samples remained negative, as did all test results for *Leptospira* spp. Drug treatment was stopped. On July 28, because the patient reported recurring/constant abdominal pain in the right upper quadrant of the abdomen, a computed tomography scan was performed; it showed a 35 × 35 × 60 mm abscess in liver segments 5 and 6. The abscess was drained on July 30, yielding pus that, when cultured, grew *K. pneumoniae* (API 20NE system strip; bioMérieux, Marcy-l'Etoile, France). The patient responded well and was treated as an outpatient with oral moxifloxacin (400 mg/d) for an additional 2 weeks. He recovered, albeit with permanent monocular blindness.

To determine the genotypic characteristics of the *Klebsiella* isolate (SB4935), we obtained a genomic sequence using a 2 × 300 nt paired-end protocol on an MiSeq instrument (Illumina, San Diego, CA. USA). Reads were assembled using a CLCbio assembler (Aarhus, Denmark) into 66 contigs of an average coverage depth of 47 of high-quality nucleotides. The draft genome sequence was 5.2 Mb in

Author affiliations: Institut Pasteur de la Guadeloupe, Les Abymes, France (S. Breurec); Centre Hospitalier Universitaire de Pointe-à-Pitre/les Abymes, Pointe-à-Pitre, France (S. Breurec, B. Melot, B. Hoen, K. Schepers, S. Bastian); Université des Antilles, Faculté de Médecine Hyacinthe Bastaraud, Pointe-à-Pitre (S. Breurec, B. Hoen); Inserm CIC 14-24, Pointe à Pitre (B. Melot, B. Hoen, K. Schepers); Institut Pasteur, Paris, France (V. Passet, S. Brisse); Centre National de la Recherche Scientifique, Paris (V. Passet, S. Brisse)

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length and 57% rich in guanine-cytosine content. The genomic sequence was submitted to the European Nucleotide Archive (accession no. PRJEB9601). Multilocus sequence typing (MLST) (*7*), core genome MLST, antimicrobial drug resistance, and virulence genes were searched by using the BIGSdb *Klebsiella* genome database (http://bigsdb.web.pasteur.fr/klebsiella) (*8*). Capsular typing was performed by slide agglutination. Susceptibility to antimicrobial drugs was determined by disk diffusion. The genome was annotated by using the RAST server (*9*). Comparison with genome of NTUH-K2044 (*10*) was performed with the Artemis Comparison Tool (http://www.sanger.ac.uk/software/artemis/ACT).

Phylogenetic analysis of the 7-gene MLST sequences showed that isolate SB4935 belongs to *K. quasipneumoniae* subsp. *quasipneumoniae* (sequence type 446) (*4*). Notably, the strain possessed a capsular polysaccharide synthesis (*cps*) region, typical of strains of capsular serotype K1 (*3*). Comparison with the *cps* region of *K. pneumoniae* K1 reference strain NTUH-K2044 showed complete conservation of genes across the entire *cps* cluster (from genes *galF* to *uge*, with 92% to 100% protein identity, depending on the gene). Strain SB4935 reacted against anti-K1 serum. Thus, horizontal transfer of the entire K1 *cps* region had occurred, either between *K. pneumoniae* and *K. quasipneumoniae* or from another unidentified lineage.

Furthermore, the SB4935 genome comprised a 76-kb DNA genomic island that displayed features typical of a horizontally acquired region: 1) chromosomal insertion into the asn-tRNA locus, 2) an integrase gene, and 3) flanking 16-bp direct repeats. This genomic island was highly similar (89%–100% protein identity) to the integrative and conjugative element (ICE) ICE*Kp1* of *K. pneumoniae* NTUH-K2044 (*11*) and coded for the following virulence factors: a yersiniabactin iron-uptake system, the regulator of mucoid phenotype RmpA, and salmochelin (*iroBCDN* cluster). In addition, genes for the conjugative transfer of the island were present. The insertion was found at the same location in NTUH-K2044 and SB4935 genomes; that is, immediately downstream of a tRNA-Asn locus adjacent to gene KP1_3578 coding for a sodium:proton antiporter. These results indicate horizontal gene transfer of the ICE*Kp1* at the same location in both strains.

Strain SB4935 harbored other typical virulence factors of *K. pneumoniae*. The *iutA* gene, which codes for the ferric aerobactin receptor, was present, but not *iucABCD,* which is involved in aerobactin biosynthesis. This finding suggests that isolate SB4935 can benefit from the production of aerobactin by neighboring strains (*12*). In addition, the genome harbored clusters *mrkABCDFHIJ* for type III fimbriae, involved in adhesion and biofilm formation, and *fimABCDEFGHI,* coding for type 1 fimbriae involved in urinary tract adhesion (*3*). No resistance gene was detected in the SB4935 genome other than bla_{OKP}, the β-lactamase gene of *K. quasipneumoniae* (*13*). This finding was consistent with the antimicrobial drug susceptibility profile (resistance only to ampicillin, ticarcillin, and piperacillin).

The clinical features of this case were similar to those of other published cases of CA-KLA (*14*). The pathogen causing endophthalmitis was not cultured, however. Although uncommon, endogenous endophthalmitis, which occurs by hematogenous dissemination, has been reported as a complication of hypervirulent *K. pneumoniae* liver abscess (*2*,*3*). In addition, because the patient did not receive antimicrobial drugs when blood cultures were obtained, the cultures' negative results might be due to low-level bacteremia.

The isolate we identified had several prominent features of hypervirulent *K. pneumoniae* strains, including the *cps* cluster for K1 capsule synthesis and ICE*Kp1*-encoding yersiniabactin, salmochelin, and RmpA. Serotype K1 is the most frequent capsular type of *K. pneumoniae* associated with CA-KLA (*1*,*3*). ICE*Kp1* has been more prevalent in strains associated with CA-KLA than in non–tissue invasive strains (*11*). Yersiniabactin is one of the most prominent features associated with invasive *K. pneumoniae* strains (*6*), and animal models support its strong pathologic contribution (*15*). Thus, horizontal transfer of high pathogenicity features into multidrug-resistant *K. pneumoniae* strains is concerning (*3*,*8*). Conjugative transfer of ICE*Kp1* from NTUH-K2044 to *Escherichia coli* and *K. pneumoniae* has been demonstrated (*11*).

Although we could not establish the history of transfer events, we identified high-virulence features in a close phylogenetic neighbor of *K. pneumoniae*. Further work is needed to clarify reservoirs of high pathogenicity elements and the mechanisms of transfer that contribute to the emergence of highly virulent *Klebsiella* strains.

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Dr. Breurec is a medical microbiologist at the Faculty of Medicine, the University Medical Center, and the Institut Pasteur of Pointe-à-Pitre, Guadeloupe. His research interests include genomic epidemiology, resistance, and virulence determinants of bacterial pathogens.

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Address for correspondence: Sylvain Brisse, Institut Pasteur, Microbial Evolutionary Genomics, 28 rue du Dr Roux, 75724 Paris, France; email: Sylvain.Brisse@pasteur.fr

Signs or Symptoms of Acute HIV Infection in a Cohort Undergoing Community-Based Screening

Martin Hoenigl, Nella Green, Martha Camacho, Sara Gianella, Sanjay R. Mehta, Davey M. Smith, Susan J. Little

We analyzed signs and symptoms in 90 patients diagnosed with acute HIV infection in a community-based program that offered universal HIV-1 nucleic acid amplification testing. Forty-seven (52%) patients reported ongoing signs or symptoms at the time of testing. Another 25 (28%) reported signs or symptoms that had occurred during the 14 days before testing.

The detection of acute HIV infection (AHI) is critical to HIV prevention and treatment strategies (*1*). Clinical diagnosis of AHI is difficult, however, because the signs and symptoms that occur during seroconversion are frequently not recognized as an indicator of AHI (*2*–*4*). Although screening programs that rely on point-of-care HIV antibody testing will reliably identify persons with established infection, these tests fail to detect AHI (*1*,*5*). The Centers for Disease Control and Prevention began addressing this problem by updating recommendations for the laboratory diagnosis of HIV in healthcare settings to include initial fourth generation HIV-1 p24 antigen–based immunoassays (*6*). However, previous studies indicate that sensitivity of p24 antigen detection for AHI might not exceed 80% (*7*). In addition, most testing programs in nonhealthcare settings continue to rely on routine antibody testing alone, with specific testing for AHI conducted only for persons with signs or symptoms.

Although previous studies focused on retrospective evaluation of AHI symptoms in persons diagnosed with early seropositive HIV infection (*8*,*9*) or cases identified by symptom-based AHI screening, the actual proportion of persons with AHI who are symptomatic at the time of testing remains unknown. We investigated the proportion of persons with AHI who have ongoing or recent signs or symptoms at the time of their diagnostic test in a cohort undergoing community-based universal AHI screening.

Author affiliations: Medical University of Graz, Graz, Austria (M. Hoenigl); University of California San Diego, San Diego, California, USA (M. Hoenigl, N. Green, M. Camacho, S. Gianella, S.R. Mehta, D.M. Smith, S.J. Little); Veterans Affairs Healthcare System, San Diego (S.R. Mehta, D.M. Smith)

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The Study

We analyzed AHI signs and symptoms in 90 patients given a diagnosis of AHI during 2007–2014. As part of this confidential HIV testing program, routine, individual donation, HIV nucleic acid amplification testing (NAT) has been provided to all rapid antibody–negative participants since June 2007 (samples for NAT are obtained at the time of rapid antibody testing) (*7*,*10*,*11*). AHI was defined as having a negative or indeterminate HIV antibody test result in the presence of detectable HIV-1 RNA, corresponding to Fiebig stages I–II, with a mean estimated date of infection within the previous 10 days (95% CI 7–14 days) (*12*). Dates of infection were estimated for all recently infected patients using previously published criteria on the basis of serologic and virologic test results (*13*).

At each patient's first visit after documentation of AHI diagnosis (median 4 days, interquartile range [IQR] 3–6 days after AHI testing), we obtained blood samples for CD4 and viral load testing and collected detailed information regarding occurrence, duration, and start and stop dates for 11 signs and symptoms associated with AHI (*5*,*14*). Participants were also asked to specify any other symptoms. In addition, patients who participated during 2007–2011 were asked if they had sought medical attention for any signs or symptoms. Typical AHI (i.e., ≥2 signs/symptoms) was defined according to criteria described by Braun et al. (*14*).

For statistical analysis, SPSS version 21 (SPSS, Inc., Chicago, IL, USA) was used. For analysis on signs or symptoms compatible with AHI, signs or symptoms that started ≥5 days before the estimated date of infection (i.e., before the 7–14 day 95% CI) were excluded. The University of California San Diego's Human Research Protections Program approved the study protocol, consent process, and all study-related procedures.

All 90 participants were male and self-identified as men who have sex with men (MSM). Median age was 29 (range 18–67) years. Half (50%) of participants reported white race; 29% reported Hispanic ethnicity. Median number of male partners reported for the previous 12 months was 20 (IQR 14–31). A total of 72 (80%) patients had signs or symptoms associated with AHI that occurred within 2 weeks before undergoing NAT; of these 72 patients, 47 (52% of the study population) had ongoing signs or symptoms, while signs or symptoms had resolved by the time of testing for 25 (28% of the study population). Twelve (13%) reported signs or symptoms starting after testing, while 6 (7%) reported the absence of signs or symptoms (Table 1). A total of 66 patients (73% of the study population)

Table 1. Comparison of AHI stage, characteristics of signs or symptoms, CD4+ cell count, and viral load between persons with signs or symptoms before and at the time of NAT versus persons without, San Diego, California, USA, 2007–2014*

Characteristic	Total no. persons	Symptoms before NAT†	Asymptomatic before NAT	p value	Ongoing symptoms at NAT	Absence of symptoms at NAT	p value
No. persons	90	72	18		47	43	
Overall no. signs/symptoms in those symptomatic, median (IQR)	5 (3–7); n = 84	5 (4–7)	5 (2–6); n = 12	NS	6 (4–8)	5 (3–6); n = 37	NS
Duration of symptoms, d, median (IQR)	9 (5–13); n = 79	10 (6–13); n = 67	4 (3–7); n = 12	<0.01	11 (8–14); n = 43	6 (3–9); n = 36	<0.01
CD4+ cell count, cells/μL, median (IQR)	435 (298–597)	435 (302–586)	448 (257–615)	NS	424 (299–592)	445 (295–610)	NS
Viral load, $\log_{10}$ RNA, median (IQR)	5.4 (4.5–6.3)	5.8 (4.8–6.4)	4.5 (3.2–5.0)	<0.01	5.6 (4.8–6.4)	5.0 (3.8–6.1)	0.07

*AHI, acute HIV infection; IQR, interquartile range; NAT, nucleic acid amplification testing; NS, not significant.
†Most frequently observed signs or symptoms that occurred during the 14 days before NAT or were ongoing at the time of NAT were fatigue (53 persons, 59% of the study population), fever (51, 57%), myalgia (48, 53%), headache (41, 46%), night sweats (35, 39%), pharyngitis (32, 36%), and gastrointestinal symptoms (29, 32%).

reported headache, pharyngitis, or myalgia occurring during the 14 days before AHI testing.

Overall, 69 patients (77%) reported signs or symptoms that met criteria of compatibility with AHI (Table 2). Onset of signs or symptoms compatible with AHI occurred at a median of 5 days (IQR 0–8, range –4 to 15 days) after the estimated date of infection. Neither viral load nor CD4 count correlated with duration or actual number of signs or symptoms.

Data on whether a patient sought medical attention because of signs or symptoms were available for 42 (47%) of 90 patients; of these, 12 (29%) reported that they sought medical attention because of their signs or symptoms and 30 (71%) did not. Significantly higher viral loads were observed for those who sought medical attention compared with those who did not (median 6.1 [IQR 5.7–6.7] log copies/mL vs. 4.7 [IQR 3.4–5.5] log copies/mL; p<0.01).

Overall, 70 (78%) of the 90 patients fulfilled criteria for having typical AHI and 20 (22%) did not (of the latter, 14 had only 1 sign or symptom, and 6 were asymptomatic). Patients with typical AHI had significantly higher viral loads compared with patients without (p<0.01). A total of 61 (85%) of 72 patients with signs or symptoms before NAT testing fulfilled criteria for having typical AHI. In addition, 40 (85%) of 47 patients who had ongoing signs or symptoms at the time of AHI testing fulfilled criteria for having typical AHI at that time.

Conclusions

We characterized signs or symptoms relative to the date of AHI diagnosis among persons seeking HIV testing in a program offering universal AHI screening. Two findings are notable: 1) 52% of participants reported ongoing signs or symptoms at the time of AHI testing, and 2) 80% reported signs or symptoms occurring within 2 weeks before undergoing testing.

These findings may have major clinical implications for community-based settings that restrict AHI testing to persons with ongoing signs or symptoms. This practice

Table 2. Signs or symptoms occurring in 69 persons with AHI, San Diego, California, USA, 2007–2014*

Characteristic	Signs/symptoms: Compatible with AHI†	Signs/symptoms: Resolved before NAT	Signs/symptoms: Ongoing at NAT	p value	Signs/symptoms: Persons seeking medical attention	Signs/symptoms: Persons not seeking medical attention	p value
No. persons reporting symptoms	69	16	41		10	22	
Duration of symptoms, d, median (IQR)	8 (5–11)	7 (4–8)	11 (7–13)	<0.01	13 (10–17)	10 (4–11)	0.01
No. (%) persons with typical AHI	64 (93)	15 (94)	36 (86)	NS	10 (100)	18 (82)	NS
Signs or symptoms, no. (%)							
Fever	53 (77)	11 (69)	34 (83)	NS	9 (90)	16 (73)	NS
Myalgia	48 (70)	13 (81)	28 (68)	NS	8 (80)	12 (55)	NS
Fatigue	48 (70)	13(81)	29 (71)	NS	9 (90)	13 (59)	NS
Headache	42 (61)	10 (63)	27 (66)	NS	8 (80)	11 (50)	NS
Night sweats	38 (55)	5 (31)	26 (63)	0.04	7 (70)	9 (41)	NS
Pharyngitis	34 (49)	9 (56)	17 (41)	NS	4 (40)	11 (50)	NS
GI symptoms‡	29 (42)	3 (19)	22 (54)	0.02	5 (50)	7 (32)	NS
Rash	19 (28)	6 (38)	12 (29)	NS	5 (50)	5 (23)	NS
Weight loss§	15 (22)	2 (13)	12 (29)	NS	6 (60)	3 (14)	0.01
Arthralgia	14 (20)	3 (19)	8 (20)	NS	4 (40)	1 (5)	0.02

*AHI, acute HIV infection; GI, gastrointestinal; IQR, interquartile range; NAT, nucleic acid amplification testing; NS, not significant.
†Defined as having started ≤4 days before estimated date of infection or after the estimated date of infection.
‡General GI symptoms (e.g., nausea, vomiting, and diarrhea).
§Weight loss >2.5 kg.

may be relatively insensitive in settings where MSM undergo HIV screening frequently (*11*). Our results show that expansion of AHI screening to include those with signs or symptoms during the 2 weeks before the test may increase the yield of AHI diagnoses by more than half.

Although our results may allow for estimation of sensitivity of signs and symptoms for AHI in persons seeking HIV testing, specificity of signs and symptoms remains unknown (in this study, signs and symptoms were not assessed in those who tested negative, and no control group was available). Estimates on frequency of signs and symptoms in HIV-negative persons (i.e., specificity) ranged widely in previous studies. Although in one study a specificity of 65% was estimated for influenza illness–like symptoms (*15*), specificities ranging from 38% to 91% for recent symptoms were estimated in another study (*5*). Limitations of those studies include the fact that exact time frames for occurrence of signs or symptoms (e.g., ongoing at the time of testing or occurring within the last 14 days) have not been evaluated, which makes comparison of results difficult. A limitation in our study is that all cases of AHI occurred among MSM. Therefore, our results may not be applicable to populations other than MSM, although previous studies have reported that clinical features of AHI may not differ by sex and age of patients (*4*).

In summary, HIV diagnostic testing strategies that limit AHI testing to patients with ongoing signs or symptoms may fail to identify many persons with AHI. In contrast, HIV NAT provided for MSM who report signs or symptoms during the preceding 2 weeks (representing 80% of AHI diagnoses) may increase the yield of AHI diagnoses by more than half.

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Dr. Hoenigl has been an Infectious Diseases Fellow at the Medical University of Graz, Austria, for 5 years and is currently working as a postdoctoral scholar at the Antiviral Research Center of the University of California, San Diego. His primary research interests are acute HIV infection, HIV prevention, mycology, and multidrug-resistant gram-negative bacterial infections.

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Address for correspondence: Martin Hoenigl, Antiviral Research Center, Division of Infectious Diseases, Department of Medicine, University of California, San Diego, 200 W Arbor Dr, Mail Code 8208, San Diego, CA 92103, USA; email: mhoenigl@ucsd.edu

Patient Diagnostic Rate as Indicator of Tuberculosis Case Detection, South Africa

Mareli M. Claassens, Cari van Schalkwyk, Rory Dunbar, Helen Ayles, Nulda Beyers

To address the uncertainty of the indirectly measured tuberculosis case detection rate, we used survey data stratified by HIV status to calculate the patient diagnostic rate, a directly measurable indicator, in 8 communities in South Africa. Rates were lower among HIV-negative than HIV-positive persons. Tuberculosis programs should focus on HIV-negative persons.

The accuracy of the indirectly measured tuberculosis (TB) case detection rate is uncertain. A directly measurable indicator for TB case detection has been proposed (*1*) and subsequently used in analyses (*2*,*3*) from prevalence surveys. This indicator, the patient diagnostic rate (PDR), is defined as the rate at which prevalent case-patients are recruited by TB programs (*1*). It is estimated by dividing the notification rate (number of newly notified cases/100,000 population/year) by the prevalence (number of all new cases/100,000 population). To focus TB program efforts for case detection, PDR can be stratified by patient variables such as smear positivity, age, sex, and HIV status. To investigate differences in the rate at which cases are detected, we used data from the Zambia South Africa Tuberculosis and AIDS Reduction (ZAMSTAR) trial (*4*) prevalence survey. Before beginning the study, we obtained approval from the Health Research Ethics Committees of Stellenbosch University, the University of Zambia, and the London School of Hygiene and Tropical Medicine.

The Study

The 2010 ZAMSTAR survey measured prevalence of culture-positive TB after 3 years of interventions in communities with a high TB/HIV burden. We selected communities that had a TB notification rate of ≥400 cases/100,000 population/year, were served by a healthcare facility offering TB diagnosis and treatment, and had a catchment area of ≥25,000 persons. Standard census enumerator areas were randomly selected within communities, and all adults (≥18 years of age) from all households within the selected areas were asked to participate.

After obtaining written informed consent, we collected 1 sputum sample from each adult and offered HIV testing with 2 rapid HIV tests (Determine HIV-1/2, Alere, San Diego, CA, USA; and Uni-Gold, Trinity Biotech, Bray, Ireland). Participants who self-reported themselves as HIV positive were asked to be retested, but if they refused, they were not tested and were assumed to be HIV positive. Sputum samples were inoculated onto manual mycobacterial growth indicator tubes (Becton Dickinson, Franklin Lakes, NJ, USA), and identification of *Mycobacterium tuberculosis* isolates was confirmed by 16SrRNA sequencing (*4*).

Community notification rates were determined by using 2010 notification data from the electronic TB register of the community facility for the number of all newly notified cases (numerator) and 2011 census data for the estimated population size (denominator). To estimate the number of persons living with HIV, we stratified notification rates by using HIV data from the TB register for notified cases and by splitting the population per community into HIV positive or negative according to prevalence survey HIV results (Table 1). Prevalence rates were standardized by age and sex according to the 2011 census age/sex distribution per community. Prevalence data were stratified by HIV by using a survey variable that captured HIV test results combined with self-reported HIV status (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/22/3/15-1618-Techapp1.pdf). The PDR per community, stratified by HIV status, was calculated by dividing notification rate by prevalence (Table 2).

We assumed that the 2011 census would give an accurate estimate of the community population in 2010 and that the HIV prevalence in the community population would be similar to that in the survey population. We varied these assumptions according to estimated national population growth and national adult HIV prevalence in a sensitivity analysis; the effect was minimal (data not shown).

Overall, the PDR was 0.34 (95% CI 0.29–0.39) per person-year for the HIV-negative population and 1.53 (95% CI 1.27–1.79) per person-year for the HIV-positive population. In all 8 communities, the PDR was lower for the HIV-negative than the HIV-positive population (Table 2).

Study limitations included selection bias, which could have been introduced by sampling of areas with high

Author affiliations: Desmond Tutu TB Centre and Department of Paediatrics and Child Health at Stellenbosch University, Tygerberg, South Africa (M.M. Claassens, R. Dunbar, N. Beyers); The South African Department of Science and Technology/National Research Foundation Centre of Excellence in Epidemiological Modelling and Analysis at Stellenbosch University, Stellenbosch, South Africa (C. van Schalkwyk); London School of Hygiene and Tropical Medicine, London, UK (H. Ayles); Zambart, Lusaka, Zambia (H. Ayles)

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Table 1. Tuberculosis notification rates per ZAMSTAR community in the Western Cape of South Africa, stratified by HIV status, 2010*

		Population, no. ‡		Notifications, no. (%)§			Notification rate¶	
Community	Total†	HIV–	HIV+	HIV–	HIV+	Missing HIV data	HIV–	HIV+
A	22,830	19,791	3,039	191	251	2 (0.5)	9.7	82.6
B	16,824	14,699	2,125	45	176	43 (16.3)	3.1	82.8
C	32,342	26,842	5,500	156	303	12 (2.6)	5.8	55.1
D	20,418	16,910	3,508	63	127	12 (4.9)	3.7	36.2
E	91,380	77,086	14,294	202	410	33 (5.1)	2.6	28.7
F	39,357	33,925	5,432	189	319	8 (1.6)	5.6	58.7
G	34,765	27,812	6,953	276	440	28 (3.8)	9.9	63.3
H	24,030	19,804	4,226	141	332	12 (2.5)	7.1	78.6
Total	281,946	236,869	45,077	1,263	2,358	150 (4.0)	5.3	52.3

*ZAMSTAR, Zambian South African Tuberculosis and AIDS Reduction trial; HIV–, HIV-negative; HIV+, HIV positive.
†Split into HIV-positive and HIV-negative according to ZAMSTAR proportions.
‡Total adult (≥18 years of age) population, data from 2011 census.
§Data from 2010 electronic tuberculosis register.
¶Notification rate expressed per 1,000 persons per year.

notification rates. High notification rates could indicate a well-functioning reporting system, in contrast to areas with lower notification rates and possibly poorer reporting systems where similar prevalence rates could have been obtained. Similar prevalence rates with lower notification rates would have further decreased PDR. The uncertainty around HIV prevalence was not accounted for in the analysis. HIV status of survey participants combined self-reported data and HIV tests performed as part of the survey, but HIV status for a large number of participants remained unknown. Accurate data would have narrowed the 95% CIs. HIV results were sometimes missing from notification data, but unknown HIV status was not included in the analysis. HIV status information was missing specifically for 1 of the 8 communities (community B) and could have biased the results if a higher proportion of missing results were for HIV-positive and TB-negative persons, which would have meant a lower TB prevalence in the HIV-positive group and therefore a higher PDR. Few TB cases were diagnosed, leading to a small sample size, especially when the number of missing HIV results was considered. The assumption that the community size and age/sex distribution was the same in 2010 as in the 2011 census could have influenced the results.

Table 2. Patient diagnostic rate per ZAMSTAR community in the Western Cape of South Africa, stratified by HIV status, 2010*

	Patient diagnostic rate†	
Community	HIV– (95% CI)	HIV+ (95% CI)
A	0.74 (0.4–1.09)	1.63 (1.01–2.26)
B	0.30 (0.14–0.46)	4.16 (0.81–7.51)
C	0.28 (0.18–0.38)	1.61 (0.67–2.55)
D	0.27 (0.15–0.38)	0.81 (0.55–1.08)
E	0.23 (0.12–0.34)	1.09 (0.55–1.62)
F	0.33 (0.21–0.45)	2.01 (0.83–3.19)
G	0.61 (0.37–0.86)	2.64 (1.51–3.76)
H	0.35 (0.24–0.47)	1.99 (1.22–2.76)
Total	0.34 (0.29–0.39)	1.53 (1.27–1.79)

*ZAMSTAR, Zambian South African Tuberculosis and AIDS Reduction trial; HIV–, HIV-negative; HIV+, HIV positive.
†Rate at which prevalent case-patients are recruited by tuberculosis programs, per person-year, calculated by dividing the notification rate by prevalence.

Conclusions

In the absence of HIV infection, a PDR of >0.84 per person-year corresponds to the World Health Organization goal of detecting >70% incident cases according to the original Styblo model (*5*), assuming a disease duration of 2 years. However, when taking HIV status into account, disease durations of 3 years for HIV-negative and 0.93 years for HIV-positive persons are assumed (*6*), meaning that a case detection rate of >70% would correspond to a PDR of 0.78 among HIV-negative and 2.51 among HIV-positive persons. Our analysis showed that TB cases were detected at a lower rate among HIV-negative than among HIV-positive persons. None of the communities detected HIV-negative cases at a sufficient rate to limit transmission. Our results are specific to the ZAMSTAR communities in the Western Cape, which has many facilities with integrated TB/HIV programs and might not be representative of other non-ZAMSTAR settings, although TB/HIV integration is included in the South African National Tuberculosis Management Guidelines (http://www.sahivsoc.org/upload/documents/NTCP_Adult_TB%20Guidelines%2027.5.2014.pdf). However, our findings are similar to those of a study in Kenya (*2*), indicating that the PDR seems a consistent and appropriate statistic for evaluating case detection by using prevalence survey data from countries with a high burden of TB and HIV. A study of miners in South Africa showed that the duration of confirmed TB before diagnosis (calculated by dividing point prevalence by incidence) was 0.80 (95% CI 0.42–1.35) years among HIV-positive and 2.39 (95% CI 1.37–4.21) years among HIV-negative persons (*7*). These findings are similar to those of this study. Prevalence estimates were used in conjunction with incidence to determine disease duration; we used notification rates instead of incidence, in conjunction with prevalence estimates, to determine the rate at which TB programs recruit patients.

Case detection efforts should not focus on HIV-positive persons only, who seek healthcare earlier (*8*), are smear negative (*9*,*10*), and contribute less to community

transmission (*10*); efforts should include strategies to detect HIV-negative patients who might contribute proportionally more to community transmission (*11*) and be accessing healthcare services already (*12*). HIV-negative persons who are more likely to be smear positive should undergo diagnostic testing for TB; for TB-positive persons, effective treatment should be started quickly (*13*).

Our findings should be validated with analyses from other settings. Given the current World Health Organization focus on prevalence surveys, data for such analyses should be available. To help TB programs to develop active case-finding strategies, future research could investigate HIV-negative persons who are at risk of having TB and being missed by the healthcare system.

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Dr. Claassens is a clinical epidemiologist at the Desmond Tutu TB Centre, Stellenbosch University. Her research interests include building local capacity to strengthen health systems and develop contextual solutions to health issues.

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Address for correspondence: Mareli M. Claassens, Desmond Tutu TB Centre, Faculty of Medicine and Health Sciences, Stellenbosch University, PO Box 241, Cape Town 8000, South Africa; email: mcla@sun.ac.za

Monitoring Therapy Adherence of Tuberculosis Patients by using Video-Enabled Electronic Devices

Alistair Story, Richard S. Garfein, Andrew Hayward, Valiantsin Rusovich, Andrei Dadu, Viorel Soltan, Alexandru Oprunenco, Kelly Collins, Rohit Sarin, Subhi Quraishi, Mukta Sharma, Giovanni Battista Migliori, Maithili Varadarajan, Dennis Falzon

A recent innovation to help patients adhere to daily tuberculosis (TB) treatment over many months is video (or virtually) observed therapy (VOT). VOT is becoming increasingly feasible as mobile telephone applications and tablet computers become more widely available. Studies of the effectiveness of VOT in improving TB patient outcomes are being conducted.

In 2014, 1.5 million people globally died from tuberculosis (TB) (*1*). Most TB patients are eminently curable by an affordable course of treatment, although this treatment currently takes a minimum of 6 months to complete and 2 years or longer for multidrug-resistant tuberculosis (MDR-TB) (*2*). Millions of patients begin TB treatment each year but face constant challenges to comply with daily medication, causing many to adhere inconsistently or to stop prematurely. Treatment interruption increases the risk for acquired drug resistance, treatment failure, disease progression, relapse and death; it also prolongs transmissibility (*3*). Loss to medical follow-up is higher when patients have a negative treatment experience, such as when access to care involves substantial travel time, lost earnings, and other patient expenditures; when adverse drug reactions are frequent or consequential; or conversely, when patients feel better and their motivation to finish treatment declines (*4*). For many, treatment is complicated by concomitant health conditions (e.g., HIV/AIDS) and destabilizing socio-structural factors (e.g., substance abuse, homelessness, poor health care access). New medicines currently under study bring renewed hope to TB patients of safer, simpler, and more effective regimens; however, all of these treatments still require several months to complete, making adherence a continuing concern for the future (*1*).

The need for close, regular contact between caregivers and TB patients receiving treatment has been long recognized and remains topical (*5*) (http://www.who.int/tb/post2015_TBstrategy.pdf). Direct observation of treatment (directly observed therapy, or DOT) was 1 of the 5 components of the strategy promoted by the World Health Organization (WHO) and public health advocates to address the global TB emergency declared in the early 1990s (*6*), (http://www.who.int/tb/dots/whatisdots/en/). Recently, innovative approaches have been piloted that bridge the gap between caregiver and patient and limit the cost and stress of frequent travel to health centers for DOT. Telephone video communication is an example, enabling health professionals to watch patients take their medication, address patients' concerns, and provide advice and support (*7,8*). Video (or virtually) observed therapy (VOT) was piloted by using videophones connected to telephone landlines and has more recently evolved toward video-enabled mobile cellular devices. Mobile telephones with video applications (smartphones) and tablet computers are becoming increasingly affordable and reliable in high- and low-income settings. Furthermore, geographic coverage of cellular and internet networks is increasingly available in places where telephone landline services had never existed or are facing obsolescence. Improved access to the technologies and infrastructure needed for VOT is foreseeable in both low- and middle-income countries in the coming years. These same countries have the greatest share of the global burden of TB and drug-resistant TB and are in urgent need of expanding their treatment programs. VOT shows promise as a new patient-centered option to support TB patients. It offers patients freedom to take their medications when and where they choose, and it engenders a more holistic approach to care.

Author affiliations: Find & Treat, University College London Hospitals, London, UK (A. Story); University of California San Diego School of Medicine, La Jolla, California, USA (R.S. Garfein, K. Collins); Farr Institute of Health Informatics, University College London, London (A. Hayward); World Health Organization Country Office, Minsk, Belarus (V. Rusovich); World Health Organization (WHO) Regional Office for Europe, Copenhagen, Denmark (A. Dadu); Center for Health Policies and Studies, Chisinau, Moldova (V. Soltan); United Nations Development Programme, Chisinau (A. Oprunenco); National Institute of TB & Respiratory Diseases (NITRD), New Delhi, India (R. Sarin); ZMQ, New Delhi (S. Quraishi); WHO Country Office, Bangkok, Thailand (M. Sharma); European Respiratory Society, Lausanne, Switzerland (G.B. Migliori); WHO Collaborating Centre, Tradate, Italy (G.B. Migliori); WHO Global TB Programme, Geneva, Switzerland (M. Varadarajan, D. Falzon)

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Efforts to use VOT for TB patient support are now gaining momentum worldwide (*9*; http://www.youtube.com/watch?v=is95C8tgOyo). Studies from the United States and Mexico show that smartphone VOT is acceptable, can save resources, and improves patient commitment to treatment even in highly mobile populations (*10*). In London, United Kingdom, where DOT is recommended for treatment of patients with multidrug-resistant TB and for other patients in unfavorable social circumstances, early findings from an ongoing trial of VOT against traditional DOT is showing potential to improve adherence. Reduced costs to patients and healthcare providers are expected even when the expense of providing hardware and cellular data connection to patients are factored in (*11*). For example, a smartphone used in this study costs less than 1 episode of face-to-face contact with local community nursing. VOT has been used successfully in TB patients in London since 2007, including among children, who tend to be proficient with this technology. Another trial has started in Moldova, a middle-income former Soviet Union country in Eastern Europe, to investigate the effectiveness of VOT by using the patient's desktop computer or a tablet computer provided by the study (*12*). A trial has also recently been launched in the United States (by R.S.G.) to compare the efficacy of VOT with traditional DOT for monitoring adherence to short-course treatment for latent TB infection. The early promise of VOT has led TB providers in Belarus, India, the United States, and elsewhere to start planning its implementation.

VOT should be viewed as a tool to facilitate patient/provider contact and not to supplant physical interaction between the patient and the healthcare professional. Patients would still need to visit clinics to collect medication, to submit samples to the laboratory, and for assessment of response to treatment. Establishing VOT also requires sound investment, including the training of patients and VOT observers.

VOT remains a relatively new and emerging technology, with limited knowledge about its effectiveness and limitations. To understand these effects and make the best use of precious public health resources, VOT must be evaluated under more diverse conditions and settings to define its function and compare it with other existing or emerging technologies geared for the same purposes within an evolving landscape (e.g., short message service [texting] communication and electronic medication monitors). Likewise, synergies between digital health and traditional approaches to improve patient treatment outcomes, and even between different digital health technologies, should be explored.

VOT may pose risks to patient confidentiality while data are transferred; however, these issues could be addressed through encryption and secure data management. Any residual risk for disclosure of disease status should be balanced against the likelihood for the same to happen when patients have to visit TB clinics regularly or to have a DOT observer visit their home or workplace every day, which is culturally inappropriate in many societies and could aggravate stigma for the patient's household.

In early 2015, a multi-partner collaboration, led by WHO and the European Respiratory Society, started to elaborate target product profiles (TPPs) for digital health products focused specifically on topical challenges in the implementation of the new End TB Strategy (*5,13*). A TPP describes the characteristics and requirements for a particular concept under development to help different stakeholders, including developers, define solutions to address specific problems. Participants in these discussions define the nature of the problem to be addressed, its relative priority compared with other pressing needs, and match the need to an existing or forthcoming digital solution. Mindful of the positive early results, but also the need for appropriate, evidence-based guidance on its use, the WHO/ERS initiative has identified VOT as one of the digital tools in support of treatment adherence to be followed closely with a TPP (*13*). We propose to take forward the TPP of VOT as a collaborative group of partners. This process will embrace a broad cross-section of representative users, developers, and policy makers. If evidence for the effectiveness of VOT continues to grow, technical details should be elaborated to guide further development and the eventual large-scale deployment of VOT. One of these is the model by which software will be made available, conceivably through open-source or socially responsible licensing (*14*,*15*). Whichever approach is adopted, a sustainable means to enable VOT interventions worldwide, such as through public funds or insurance systems, will be needed.

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Dr. Story, the founder and clinical lead of the pan-London Find & Treat Service, has core expertise in tackling tuberculosis and other communicable diseases among homeless people, drug and alcohol users, prisoners and destitute migrants. His research interests include outreach, integrating point of care diagnostics on the street, case management, and the use of mobile internet technologies to promote engagement with health services and treatment continuity.

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Address for correspondence: Dennis Falzon, Global TB Programme, World Health Organization, 20 Avenue Appia, CH-1211 Geneva 27, Switzerland; email: falzond@who.int

Tuberculosis Risk among Medical Trainees, Pune, India

Anita Basavaraj, Ajay Chandanwale, Akhil Patil, Dileep Kadam, Samir Joshi, Nikhil Gupte, Katie McIntire, Divyashri Jain, Hamza Dalal, Rohan Badave, Andrea DeLuca, Amita Gupta, Robert Bollinger, Vidya Mave

During 2012–2013, at a public hospital in Pune, India, 26 (3.9%) cases of tuberculosis were reported among 662 medical trainees, representing an estimated incidence of 3,279 cases/100,000 person-years. Three of these infections were isoniazid-resistant, 1 was multidrug-resistant, and 1 occurred in a trainee who had fulminant hepatitis after starting treatment for TB.

India has the world's largest burden of tuberculosis (TB) and multidrug-resistant (MDR) TB; estimated community TB incidence is 167 cases/100,000 person-years (*1*,*2*). Occupational TB risk is elevated worldwide; however, limited available data suggest that healthcare workers (HCWs), specifically medical trainees, may be at particularly high risk in India and other countries with high TB incidence (*3*–*5*). Although the World Health Organization (WHO) has long recommended infection control guidelines for TB prevention among HCWs, implementation globally has been suboptimal (*6*). To prioritize improved local implementation of WHO guidelines, documenting TB risk among HCWs and medical trainees is urgently needed. We estimated TB prevalence and TB incidence and investigated the frequency of key TB treatment outcomes among medical trainees at a public teaching hospital in India.

The Study

During June 2012–December 2013, we conducted a retrospective study among HCWs at Byramjee-Jeejeebhoy Government Medical College–Sassoon General Hospital (BJGMC-SGH), a 1,300-bed public teaching hospital in Pune in the state of Maharashtra, India. All major clinical and preclinical departments were made aware of the study objectives and referred HCWs who self-identified as having TB to the study team. After obtaining written consent, we entered demographic and clinical data from HCW interviews and clinical and laboratory data from medical records into case report forms. Medical trainees were defined as medical residents or interns.

Routine sputum acid-fast bacilli (AFB) smear and culture were performed at baseline for suspected pulmonary TB (PTB) and repeated at 2 months and, if positive, at 3 months and at the end of treatment. Participants with suspected extrapulmonary TB (EPTB) (e.g., pleural TB, meningitis, or lymphadenitis) underwent additional AFB smear, culture, or histopathologic evaluation of the affected sites as appropriate. All AFB cultures were performed on Lowenstein-Jenson media. Drug-susceptibility testing was performed routinely on all positive sputum and EPTB cultures at baseline and at each follow-up evaluation by using the proportion method (*7*). Routine HIV testing was performed under the national program (*2*). The BJGMC-SGH Institutional Ethics Committee approved all study methods.

Primary end points included estimated TB prevalence and TB incidence per 100,000 person-years among medical trainees. To estimate prevalence and incidence, we obtained the denominator (the total number of medical trainees) from employment records. TB prevalence was calculated by dividing the number of TB cases by the total number of medical trainees. Estimated incidence was calculated as the number of TB cases multiplied by 100,000 and divided by the duration of exposure and the total number of medical trainees. Duration of exposure was 18 months for medical residents and 12 months for interns.

Secondary end points included cure (smear- or culture-negative at the end of treatment), treatment failure (smear- or culture-positive at month 5 or later), death (of any cause during treatment), and treatment success (cured and completed treatment) (*1*). We also evaluated positive AFB smear or cultures at month 2 of treatment, resistance to any anti-TB drug, MDR TB, and adverse events. MDR TB was defined as resistance to at least isoniazid and rifampin (*3*). Descriptive statistics were used to measure secondary outcomes. All analyses were conducted by using Stata version 10 (Stata Corp LP, College Station, TX, USA).

Among the 1,886 HCWs assessed in the study, 47 cases of TB were identified (*8*); 26 cases (14 in residents and 12 in interns) were identified among 662 medical trainees, who had 793 person-years of follow-up. Overall among medical trainees, TB prevalence was 3.9%, and estimated TB incidence was 3,279 cases/100,000 person-years (Table). The

Author affiliations: Byramjee-Jeejeebhoy Medical College, Pune, India (A. Basavaraj, A. Chandanwale, A. Patil, D. Kadam, S. Joshi, H Dalal, R Badave); Byramjee-Jeejeebhoy Medical College Clinical Trials Unit, Pune (A. Basavaraj, A. Chandanwale, D. Kadam, S. Joshi, N. Gupte, D. Jain, A. Gupta, R. Bollinger, V. Mave); Johns Hopkins University School of Medicine, Baltimore, Maryland, USA (N. Gupte, K. McIntire, A. Gupta, R. Bollinger, V. Mave); Johns Hopkins Bloomberg School of Public Health, Baltimore (A. Deluca),

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Table. Estimated prevalence and incidence of TB among medical trainees at Byramjee-Jeejeebhoy Government Medical College–Sassoon General Hospital, Pune, India, June 2011–December 2013*

Category	All medical trainees, N = 662	Residents, n = 262	Interns, n = 400
No. TB cases (prevalence†)	26 (3.9)	14 (5.3)	12 (3.0)
Total person-years of exposure	793	393	400
Estimated TB incidence per 100,000 person-years‡ (95% Poisson CI)	3,279 (2,142–4,804)	3,562 (1,948–5,977)	3,000 (1,550–5,240)

*TB, tuberculosis.
†Number of TB cases divided by the total number of medical trainees.
‡Number of TB cases multiplied by 100,000 and divided by the duration of exposure and the total number of medical trainees.

median age of trainees with TB was 27 (interquartile range 26–28) years; 14 (54%) were male, 9 (35%) had PTB, 17 (65%) had EPTB (6 [35%] with microbiological confirmation), and 17 (65%) had presumptive TB. Most worked in the general medicine (46%) or radiology (15%) departments (Figure). All trainees with TB were HIV negative.

The frequency of MDR TB was 44% (4/9 cases of culture-positive TB); 3 trainees had TB with single-drug resistance to isoniazid and had their disease successfully treated with first-line anti-TB drugs, and 1 had MDR TB that was successfully treated with second-line anti-TB drugs. All other trainees with TB had their disease successfully treated with first-line anti-TB drugs, except 1 who experienced acute fulminant hepatic failure during TB treatment and required liver transplant and treatment with levofloxacin, ethambutol, and streptomycin. The frequency of adverse drug reactions was 62% (16/26), including 14 cases of gastritis. No cases of treatment failure or death were reported.

Conclusions

Our investigation found a 15-fold higher estimated incidence of TB among medical trainees at BJGMC-SGH (3,279 cases/100,000 person-years) than among that reported for the community in the study state of Maharashtra, India (167 cases/100,000 person-years) (*2*). Although our findings are consistent with those of previous studies showing substantially elevated risk among medical trainees, we demonstrate ≈2-fold higher TB incidence among medical trainees than that indicated by a decade-old study of medical trainees from India (*5*,*6*); this increase is likely attributable to increasing TB incidence in the community. Increased TB risk among medical trainees is probably a function of duration of exposure. Another study from India reported a 4-fold higher prevalence of TB infection among older medical students (i.e., >23 years of age) compared with younger medical students (18–20 years of age) (*9*). Two notable findings in our study are that approximately two thirds of medical trainees had EPTB diagnosed and that approximately one third of those infections were microbiologically confirmed; these findings have also been observed in previous studies of HCWs in India (*4*,*5*).

Our study provides critical evidence that medical trainees in India are at risk for drug-susceptible and drug-resistant TB, including TB with single-drug resistance to isoniazid and MDR TB. MDR TB cases are probably underreported, and only 1 case of extremely drug-resistant TB in a HCW has been reported in India (*10*). With increasing MDR TB prevalence and the frequent need for hospitalization of these patients, the incidence of MDR TB among medical trainees in India may increase over time unless awareness is increased and improved infection control measures are rapidly implemented (*1*).

The consequences of active TB among medical trainees may have a substantial public health impact. First, medical trainees may be reluctant to seek early medical care because of stigma, fear of losing training time, poor

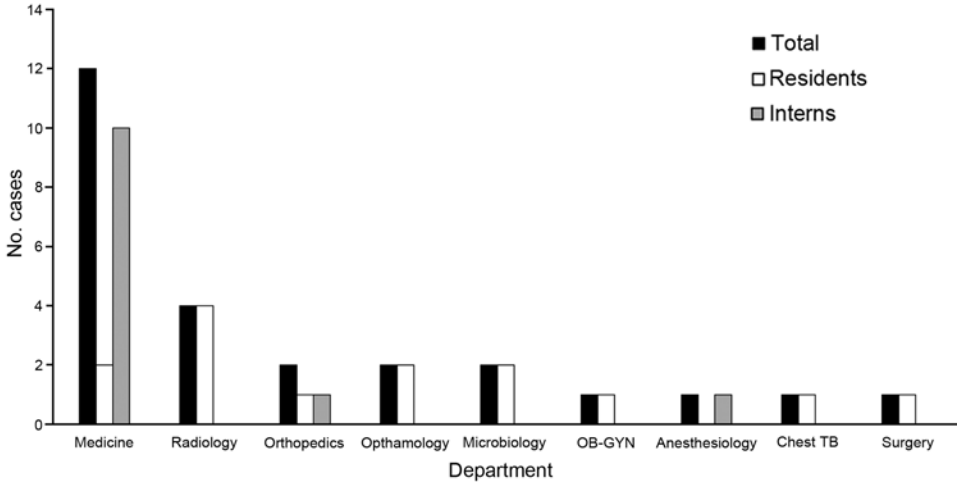

Figure. Number of TB cases among medical trainees, by department, at Byramjee-Jeejeebhoy Government Medical College–Sassoon General Hospital, Pune, India, June 2011–December 2013. TB, tuberculosis; OB-GYN, obstetrics–gynecology.

knowledge, or a perceived lack of vulnerability to TB (*6*,*11*). Thus, trainees can secondarily transmit TB to peers in overcrowded hostels (most trainees reside in hostels close to teaching hospitals in India), patients in clinics and wards, and family members (*6*,*12*). Second, as observed in our study and others, a higher risk for adverse events exists among medical trainees (*4*,*5*). Trainees in whom major adverse drug reactions develop may choose to leave the profession permanently, as we observed with the trainee in whom acute fulminant hepatic failure developed during treatment for TB. Last, although routine treatment of TB and MDR TB is available to medical trainees, while not observed in our cohort, less than two thirds of patients with MDR TB experience successful treatment (*1*).

Our study has some limitations. We may have underestimated TB incidence because some medical trainees may not have reported incident TB because of stigma and unwillingness to participate in a study. In addition, we may have had some recall bias because of the retrospective nature of the study. Despite these limitations, our study underscores the immediate need to for education and implementation of infection control measures to safeguard medical trainees from TB (*6*,*7*). Additional large prospective studies are needed to evaluate the risk for latent TB infection and TB disease among medical trainees and the effectiveness of infection control measures.

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Dr. Basavaraj is associate professor of general medicine at Byramjee-Jeejeebhoy Medical College–Sassoon General Hospital, a public, tertiary-care, teaching hospital in Pune, Maharashtra, India. Her primary research interests are TB/HIV infections, occupational health, and geriatric illnesses.

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Address for correspondence: Anita Basavaraj, Department of Medicine, Byramjee-Jeejeebhoy Medical College–Sassoon General Hospital, Jai Prakash Narayan Rd, Pune, Maharashtra 411001, India; email: anita.basavaraj@rediffmail.com

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. All letters should contain material not previously published and include a word count.

Human Lymphadenopathy Caused by Ratborne *Bartonella*, Tbilisi, Georgia

George Kandelaki,[1] Lile Malania,[1] Ying Bai,[1] Neli Chakvetadze, Guram Katsitadze, Paata Imnadze, Christina Nelson, Shimon Harrus, Michael Kosoy

Lymphadenopathy and fever that developed in a woman in Tbilisi, Georgia, most likely were caused by a ratborne *Bartonella* strain related *B. tribocorum* and *B. elizabethae*. The finding suggests that this *Bartonella* strain could be spread by infected rats and represents a potential human risk.

Cat scratch disease caused by *Bartonella henselae* is a major cause of unilateral reginal lymphadenitis in children and adults (*1*). We report a case of lymphadenopathy and fever in a woman in Tbilisi, Georgia, that most likely was caused by a ratborne *Bartonella* strain.

The Study

In 2012, an 18-year-old woman with no major medical history sought care at an outpatient infectious diseases clinic in Tbilisi with a 2-week history of weakness, malaise, fever ≥38°C for the previous 10 days, enlarging right neck mass, and occasional night sweats. She lived in a residential building in an urban area within Tbilisi. She denied recent travel outside the city, contacts with sick persons, exposure to farm animals, or having pets at home. Physical examination indicated right cervical lymphadenopathy with multiple enlarged, soft, tender lymph nodes, 1 of which was fluctuant on palpation. Ultrasound showed 4 enlarged lymph nodes: 2 in the anterior cervical region (14 mm and 17 mm) and 2 in the posterior cervical region (29 and 38 mm). The largest lymph node had central attenuation with a hypoechoic area suggestive of pus. Laboratory test results were as follows: leukocyte count 9.2 cells/μL (reference 4.0–11.0 cells/μL) with 6.7% (reference 2.5%–7.5%) neutrophils; platelets 358,000/μL (reference 150,000–450,000/μL); hemoglobin 15.4 g/dL (reference 14.0–17.5 g/dL); C-reactive protein 16 mg/L (reference 0–10 mg/L); and erythrocyte sedimentation rate 56 mm/h (reference <30 mm/h). Chest radiograph showed no abnormalities. Serum was negative for antibodies against cytomegalovirus, Epstein-Barr virus, *Toxoplasma*, and HIV. Tuberculin skin test result of 4-mm induration was considered negative.

Ultrasound-guided aspiration of the largest lymph node yielded 2 mL of cloudy yellow fluid. Gram stain, acid-fast stain, bacterial culture, and fungal culture of the aspirate were all negative. Histopathologic examination demonstrated a nonspecific inflammatory response without evidence of granulomas or malignant cells.

Cat scratch disease was presumptively diagnosed on the basis of lymphadenopathy and clinical characteristics, and *B. henselae* was suspected as the etiologic agent, although the patient denied any contact with cats. The lymph node aspirate was submitted to the National Center for Disease Control & Public Health (Tbilisi) for molecular diagnostic testing for *Bartonella*. Genomic DNA was extracted from the lymph node aspirate by using a QIAamp tissue kit (QIAGEN, Valencia, CA, USA) and was analyzed by using conventional PCR targeting a 338-bp fragment of the *glt*A gene (*1*), a molecular target routinely used for detecting *Bartonella* DNA. The test resulted in amplification of the specific target, which suggested a potential *Bartonella* species. Before the PCR result was available, the patient was empirically prescribed amoxicillin/clavulanic acid treatment. After receiving the PCR results suggesting *Bartonella* DNA in the aspirate sample, the drug regimen was switched to azithromycin 500 mg every 8 hours on the first day, then 250 mg every 8 hours daily for 4 additional days. Fever resolved in 2 weeks, and lymphadenopathy gradually improved during the next 4–5 weeks. Weakness and malaise resolved within 2 months.

The DNA was forwarded to the Bartonella and Rodent-Borne Diseases Laboratory of the US Centers for Disease Control and Prevention's Division of Vector-Borne Diseases (Fort Collins, CO, USA) for further characterization. Seven targets (*glt*A, *nuo*G, *rib*C, *rpo*B, *fts*Z, *ssr*A, and internal transcribed spacer [ITS]), all which have been previously used for *Bartonella* descriptions (*2*), were amplified. All positive PCR products were purified by using QIAquick PCR Purification Kit (QIAGEN) and sequenced in both directions by using an Applied Biosystems Model 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The sequences obtained were aligned by each

Author affiliations: Central University Hospital, Tbilisi (G. Kandelaki); National Center for Disease Control & Public Health, Tbilisi, Georgia (G. Kandelaki, L. Malania, N. Chakvetadze, G. Katsitadze, P. Imnadze); Centers for Disease Control and Prevention, Fort Collins, Colorado; USA (Y. Bai, C. Nelson, M. Kosoy); Hebrew University of Jerusalem, Rehovot, Israel (S. Harrus)

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[1]These authors contributed equally to this article.

locus and compared with all known *Bartonella* species by using the ClustalW program within DNASTAR Lasergene package (DNASTAR, Madison, WI, USA). The neighbor-joining method by Kimura 2-parameter distance method was used (1,000 bootstrap replicates).

Sequence analyses of all 7 molecular targets demonstrated that the bacterial DNA belongs to a *Bartonella* species within the *B. elizabethae* species complex, which represents an assemblage of species and strains associated with rats of the genus *Rattus* (*3*). By individual locus, the DNA was closer to *B. tribocorum* than to any other *Bartonella* species by 2 markers (*glt*A [96.1%] and *rpo*B [100%]) and was closer to *B. elizabethae* by all other markers (*nuo*G [99.9%], *rib*C [99.2%], *fts*Z [98.5%], and *ssr*A [99.4%]) and by ITS (99.4%). Comparison of concatenated sequences of all 7 loci indicated the identified genotype had a divergence of 3.4% with *B. elizabethae* and 5.6% with *B. tribocorum*. Additional sequence queries resulted in identification of the Tel Aviv (TA) strain of *Bartonella*, which was prevalent and the only identified strain among black rats (*Rattus rattus*) captured in Tel Aviv, Israel (*4*). For any of the 4 markers used in both studies (*rib*C, *rpo*B, *glt*A, and ITS), the genotype identified in the patient was indistinguishable from the TA strain. The *glt*A sequence from the patient's aspirate also was indistinguishable by the *glt*A from *Bartonella* genotypes identified in a rat from Porto Santo Island, Portugal (*5*), and in 4 rats from Dhaka, Bangladesh (*6*).

Conclusions

The invasion of rats into urban ecosystems and their establishment in such areas can have major implications for human health (*7*). *Bartonella* species and genotypes detected in *Rattus* rats are clustered into a defined phylogenetic lineage that can be subdivided into several subclusters (*3*,*6*). *B. elizabethae* and related species of *Bartonella* have not been detected in rodent hosts except for rats of genera *Rattus* and *Bandicota* (*3*,*6*,*7*). A recent genetic analysis of *Bartonella* strains obtained from rats from 17 countries demonstrated that this bacterial complex evolved and diversified in Southeast Asia before being disseminated by *R. rattus* and *R. norvegicus* to other parts of the globe (*8*).

B. elizabethae was first isolated from a US patient with endocarditis in 1993 (*9*) and subsequently was found in rats from many countries (*2*,*4*). Investigation of febrile human patients from Thailand demonstrated that 8 of the 14 *Bartonella* genotypes identified in patients were similar or identical to homologous sequences identified in rats and were closely related to *B. elizabethae*, *B. rattimassiliensis*, or *B. tribocorum* (*10*).

The identification of bacteria that share genes specific for rat-associated *Bartonella* species in a lymph node aspirate suggests that the finding could be associated with commensal rats occupying residential areas of Tbilisi. The patient did not recall rats in the building but had noticed them in waste containers outside the building. The most striking finding was the identity of this genotype with TA strain. Of 21 *Bartonella* isolates cultured from blood from 62 commensal rats captured in Tel Aviv, 10 isolates were genetically characterized by 6 markers, and all the isolates were identical to each other and closely related to both *B. tribocorum* and *B. elizabethae* (*3*). Identification of the identical strains in urban rats from Portugal and Bangladesh suggests much wider distribution of this strain.

The clinical picture for the patient we report was typical for clinical manifestations of cat scratch disease, which is commonly caused by *B. henselae* (*11*). Nevertheless, evidence is increasing that rodentborne *Bartonella* species can cause diverse clinical signs and symptoms, including fever, myocarditis, endocarditis, neuroretinitis, and lymphadenitis (*12*). *Bartonella* species between rodents appear to be transmitted mainly by fleas (*13*). The Oriental rat flea (*Xenopsylla cheopis*) commonly infests commensal rats within cities and can readily bite humans without being noticed. The detection of a rat-associated *Bartonella* species in the capital of Georgia raises public health concerns and highlights the need to further explore its zoonotic potential and pathogenic characteristics.

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Dr. Kandelaki is an infectious diseases specialist at Central University Hospital, Tbilisi, Georgia. His research interests include clinical diagnosis and treatment of infectious diseases.

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Address for correspondence: Michael Kosoy, Centers for Disease Control and Prevention, 3156 Rampart Rd, Mailstop P02, Fort Collins, CO 80521, USA; email: mck3@cdc.gov

Tuberculosis, Fiji, 2002–2013

Lorenzo Pezzoli, Shakti Gounder, Talatoka Tamani, Mary Raori Daulako, Frank Underwood, Sakiusa Mainawalala, Vasiti Nawadra-Taylor, Eric Rafai, Laura Gillini

During 2002–2013, a total of 1,890 tuberculosis cases were recorded in Fiji. Notification rates per 100,000 population increased from 17.4 cases in 2002 to 28.4 in 2013. Older persons were most affected, but tuberculosis also increased sharply in persons 25–44 years of age.

Tuberculosis (TB) remains a major cause of illness and death globally (*1*,*2*). Fiji, which comprises 332 islands and a total land area of 18,333 km^2, is located in the center of the South Pacific. In 2013, Fiji's Ministry of Health estimated a population of 882,860 persons. The public health system is organized into 4 divisions, 20 subdivisions, and 80 medical areas. The National Tuberculosis Programme (NTP) was established in 1951 and in 1997 adopted the directly observed treatment strategy (DOTS). The 3 DOTS centers are P.J. Twomey, covering Central and Eastern Divisions; Labasa, covering Northern Division; and Lautoka, covering Western Division.

In 2012, per 100,000 population, reported TB incidence in Fiji was 24 (95% CI 21–27) cases, prevalence was 30 (95% CI 10–61), and the case-fatality rate was 1.7 (*3*,*4*). To assess the status of TB epidemiology in Fiji and identify areas of intervention, we used surveillance data to retrospectively analyze trends in TB cases reported by the NTP from 2002 through 2013.

The Study

Case notification data for 2002–2013 were obtained from the 3 NTP TB registers (1 per DOTS center). Data on geographic location (up to medical area level) of TB cases were available electronically from 2005 and on age groups from 2010.

We defined a TB case based on ≥1 of the following diagnostic criteria: sputum or body fluid and tissue that was smear-positive for acid-fast bacilli (AFB), culture-positive for *Mycobacterium tuberculosis* complex, or both; or clinical appearance, radiographic appearance, or both consistent with TB. Cases caused by documented nontuberculous mycobacteria were excluded. We defined a smear-positive pulmonary TB (PTB+) case as ≥2 initial sputum smear examinations (direct smear microscopy) AFB-positive; or 1 sputum examination AFB-positive plus radiographic abnormalities, symptoms, or both consistent with active pulmonary TB. We defined a smear-negative pulmonary TB (PTB–) case as symptoms suggestive of TB, with ≥3 initial smear examinations negative for AFB but with no response to a treatment course with broad-spectrum antimicrobial drugs (excluding quinolones), and/or with radiologic abnormalities consistent with pulmonary TB, followed by a clinician's decision to treat for TB. We defined an extrapulmonary TB (EPTB) case as TB only of organs other than the lungs (e.g., pleura, lymph nodes, abdomen, genitourinary tract, skin, joints and bones, meninges), diagnosed on the basis of 1 culture-positive specimen, or histologic or strong clinical evidence consistent with active extrapulmonary disease, followed by a clinician's decision to treat with a full course of anti-TB chemotherapy.

We described cases by type, patient age, and location. Case notification rates (CNRs) were measured respectively as TB cases or deaths during a given year divided by the population estimate for that year. Age-standard CNRs were calculated by dividing the number of TB cases in each age group by the total number of population estimated in that age group. We analyzed TB case notifications for trends using simple linear regression models.

During 2002–2013, a total of 1,890 new cases of TB were reported. Since 2002, the only significant linear upward trends were for all cases (coefficient 7.86, $p = 0.046$) and PTB+ cases (coefficient 4.02, $p = 0.004$). Since 2007, the model showed significant linear trends for all cases (coefficient 26.82, $p<0.001$), PTB+ cases (coefficient 9.00, $p = 0.002$), PTB– cases (coefficient 12.14, $p = 0.001$), and EPTB cases (coefficient 5.68, $p = 0.037$); the proportion of PTB– cases (coefficient 0.04, $p = 0.004$); and the CNR (coefficient 2.97, $p<0.001$).

In Fiji's 4 divisions during the study period, the highest average CNR was reported from the Central Division (22.5 cases/100,000 population) and the lowest from the Eastern Division (10.9 cases/100,000 population) (Figure; Table 1). The 25–34-year age group had the most cases (198 [22%]); the fewest cases were reported in the 0–4-year group (28 [3%]). The CNR for persons ≥55 years of age consistently exceeded 40 cases/100,000 population. In 2013, the CNR for persons ≥65 years of age increased to 75.7 cases/100,000 population. In persons 25–34 years of age, the CNR increased from 25.8 cases/100,000 population in 2012 to 42.2 cases/100,000 population in 2013 (Table 2).

Author affiliations: Epidemiology Consultant, Suva, Fiji (L. Pezzoli); Ministry of Health, Suva (S. Gounder, T. Tamani, M.R. Daulako, F. Underwood, S. Mainawalala, E. Rafai, V. Nawadra-Taylor); World Health Organization, Suva (L. Gillini)

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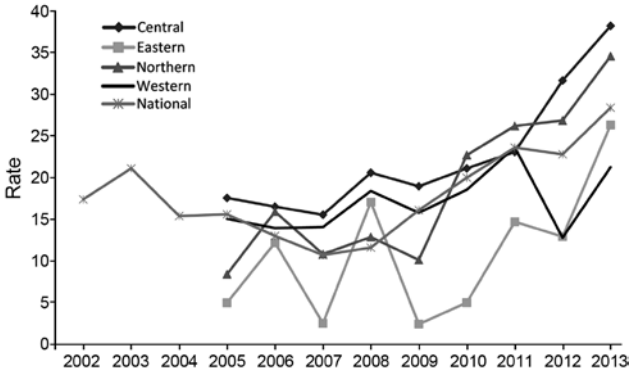

Figure. National tuberculosis case notification rates (2002–2013) and by division (2005–2013), Fiji. Rates are number of cases per 100,000 population.

Table 1. New tuberculosis cases, by year and type, Fiji, 2002–2012*

Year	No. (%) EPTB	PTB–	PTB+	Total no., N = 1,890
2002	38 (25.3)	38 (25.3)	74 (49.3)	150
2003	53 (29.6)	48 (26.8)	78 (43.6)	179
2004	31 (23.7)	38 (29.0)	62 (47.3)	131
2005	40 (30.3)	29 (22.0)	63 (47.7)	132
2006	18 (15.9)	22 (19.5)	73 (64.6)	113
2007	34 (36.6)	7 (7.5)	52 (55.9)	93
2008	19 (18.6)	5 (4.9)	78 (76.5)	102
2009	38 (26.8)	21 (14.8)	83 (58.5)	142
2010	45 (25.1)	45 (25.1)	89 (49.7)	179
2011	44 (20.7)	62 (29.1)	107 (50.2)	213
2012	40 (19.5)	54 (26.3)	111 (54.1)	205
2013	71 (28.3)	74 (29.5)	106 (42.2)	251

*EPTB, extrapulmonary tuberculosis; PTB, pulmonary tuberculosis; –, smear negative; +, smear positive.

Conclusions

Our comprehensive evaluation of TB trends in Fiji shows a steady increase in CNRs since 2007. Older age groups were disproportionally affected. This finding is not surprising because the elderly strata of the population are more likely to have been infected and are more prone to reactivation of dormant mycobacteria (*5*). TB in these persons is of concern because they are more likely to die or have poorer outcomes because of old age and concurrent conditions. The sudden increase in CNR in the 25–34 and 35–44 age groups during 2012–2013 is also of major concern. This finding might indicate increased transmission within the community because most cases in these younger adults are presumably due to recent infections.

The relative decrease in PTB+ cases below the programmatic aim of 50% smear-positive cases contrasts with results from a previous analysis (*4*) and reflects a concurrent increase in PTB– cases diagnosed rather than an increase in EPTB cases. The observation of increased PTB– cases might imply greater awareness and sensitivity by medical practitioners to diagnosing clinical TB syndromes or lower specificity in the diagnosis (i.e., a proportion of PTB– cases might be attributable to other diseases). The 106 PTB+ cases detected in 2013 remain a relatively high number because these patients are more infectious and should be detected as early as possible.

Our assessment is subject to several limitations. First, this is a review of programmatic data, so it is not possible to know whether the trends represent a change in the disease incidence or a change in programmatic functioning. Second, TB data were collated at different sources and were mostly paper based and not easily accessible. Finally, although some major hospitals (e.g., private practices or the Colonial War Memorial Hospital, Suva, Fiji) participate in active case detection, they are not completely involved in TB treatment of patients and might underreport cases.

The increase in CNRs during the study period can be attributed to several factors. First, during 2008–2011, program reviews led to a strengthened TB surveillance system (*6*). The increased CNR also might be attributed to enhanced program support by the Global Fund. Fiji first received money from the Global Fund to Fight AIDS, Tuberculosis and Malaria (http://www.theglobalfund.org) for TB in 2010 and had received US $7,570,339 through 2013. These enhanced program activities are likely to have contributed to the increase in notifications in recent years. However, they cannot fully explain the rise in CNRs. In the 2014 Global Tuberculosis Report, the World Health Organization estimated TB incidence in Fiji at 37 (95% CI 33–42) cases/100,000 population in 2013, which is a considerable increase over the 24 (95% CI 21–27)

Table 2. Tuberculosis cases, by age group and CNR, Fiji, 2002–2012*

Age group, y	No. (CNR) 2010	2011	2012	2013	Overall, no. (mean CNR)
0–4	4 (4.5)	2 (2.3)	11 (12.4)	11 (12.6)	28 (7.9)
5–14	16 (9.4)	12 (7.0)	10 (5.8)	15 (8.9)	53 (7.7)
15–24	29 (17.0)	44 (25.6)	42 (24.5)	47 (27.9)	162 (23.7)
25–34	44 (30.1)	55 (37.3)	38 (25.8)	61 (**42.2**)	198 (33.9)
35–44	27 (22.4)	30 (24.7)	28 (23.1)	45 (37.8)	130 (27.0)
45–54	22 (22.8)	27 (27.8)	34 (35.0)	33 (34.7)	116 (30.0)
55–64	31 (**52.6**)	25 (**42.0**)	26 (**43.8**)	26 (**44.6**)	108 (**45.7**)
≥65	18 (**43.5**)	18 (**43.1**)	29 (**69.5**)	31 (**75.7**)	96 (**58.0**)
All	191 (21.4)	213 (23.6)	218 (24.2)	269 (30.5)	891 (24.9)

*Bold indicates CNR >40 cases/100,000 population. CNR, case notification rate.

cases/100,000 population in 2012 (*1*,*7*) and suggests that the program still needs to optimize its case-finding potential. Prevention and control activities should be intensified in younger adults to reduce the number of new infections. Finally, analyzing trends is only 1 aspect of the evaluation of DOTS, and further research on measuring outcomes should be explored (*8*).

Dr. Pezzoli is an epidemiologist. His research interests include monitoring and evaluation of disease control programs, especially surveillance systems and immunization programs.

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Address for correspondence: Lorenzo Pezzoli, World Health Organization, Department of Pandemic and Epidemic Diseases, Avenue Appia, Geneva, 1211, Switzerland; email: pezzolil@who.int

Borrelia miyamotoi and *Candidatus* Neoehrlichia mikurensis in *Ixodes ricinus* Ticks, Romania

Zsuzsa Kalmár, Hein Sprong, Andrei D. Mihalca, Călin M. Gherman, Mirabela O. Dumitrache, Elena C. Coipan, Manoj Fonville, Vasile Cozma

Author affiliations: University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania (Z. Kalmár, A.D. Mihalca, C.M. Gherman, M.O. Dumitrache, V. Cozma); National Institute of Public Health and Environment, Bilthoven, the Netherlands (H. Sprong, E.C. Coipan, M. Fonville)

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To the Editor: *Ixodes* spp. ticks are vectors for human and animal pathogens. *Ix. ricinus* ticks are widely distributed, frequently reported to feed on humans, and the main vector for a large variety of tickborne pathogens (*1*). The effect of ticks and tickborne diseases on public health, animal health and welfare, and animal production appears to be an increasing global problem, which will lead to considerable economic costs (*2*).

Borrelia miyamotoi is a spirochete that belongs to the relapsing fever group and causes symptoms similar to those of other relapsing fever group pathogens and Lyme borreliosis, including erythema migrans–like skin lesions (*3*). The geographic distribution of *B. miyamotoi* is sporadic; it has been detected in *Ixodes* spp. ticks in many countries in Europe and in North America and Asia. In Russia, the United States, and recently in the Netherlands, *B. miyamotoi* was detected in humans and confirmed to cause disease (*4*,*5*). In Romania, pathogens that cause Lyme borreliosis and reptile-associated borreliae were identified in different tick populations (*6*,*7*). However, no information is available on the presence of relapsing fever group borreliae in this country.

Candidatus Neoehrlichia mikurensis and *Anaplasma phagocytophilum* are obligate, intracellular, tickborne pathogens of the family *Anaplasmataceae*; both are emerging zoonotic agents. *Candidatus* N. mikurensis causes monocytotropic ehrlichiosis in canids and humans and granulocytic anaplasmosis in humans and domestic animals (*8*). These 2 pathogens are found throughout Europe in *Ix. ricinus* ticks (*8*). *A. phagocytophilum* has been reported in questing *Ix. ricinus* ticks, dogs, wild boars, hedgehogs, and tortoises in Romania (*9*). Recently, *Candidatus* N. mikurensis was detected in an *Ix. ricinus* tick that had bitten a human in Romania (*10*). This recently discovered tickborne agent was shown to be a risk for disease in humans and has been detected in questing *Ix. ricinus* ticks throughout Europe and in animal tissue samples and human patients (*8*).

Relapsing fever spirochetes and potential public health risks associated with tickborne pathogens are a serious medical problem. Thus, we assessed the presence of *B. miyamotoi*, *A. phagocytophilum*, and *Candidatus* N. mikurensis in questing *Ix. ricinus* ticks in Romania.

Questing *Ix. ricinus* ticks were available from previous studies conducted by our research group. A random sampling approach was used as described (*7*). To detect potentially pathogenic bacteria, 468 questing *Ix. ricinus* ticks were collected from 4 regions from Romania, randomly selected, and analyzed.

Detection of pathogens was performed by using multiplex quantitative PCRs (qPCRs) specific for the *flaB* and *ospA* genes of *B. miyamotoi*, the *msp2* gene of *A. phagocytophilum*, and the g*roEL* gene of *Candidatus* N. mikurensis. We used IQ Multiplex Powermix (Bio-Rad, Carlsbad, CA, USA) and a final reaction volume of 20 mL (*8*). For detection of *A. phagocytophilum* and *Candidatus* N. mikurensis, we also performed multiplex qPCR as described (*8*). For detection of *B. miyamotoi*, a specific region of the

Table. Prevalence of 3 bacterial species in questing *Ixodes ricinus* in 14 localities, Romania

Locality (county)	No. ticks tested (nymphs, males, females)	No. (%) ticks positive, by bacterial species		
		Borrelia miyamotoi	*Anaplasma phagocytophilum*	*Candidatus* Neoehrlichia mikurensis
Cugir (Alba)	19 (8, 4, 7)	0	2 (10.53)	1 (5.26)
Vladimirescu (Arad)	17 (0, 5, 12)	0	2 (11.76)	0
Bicaci (Bihor)	23 (12, 5, 6)	0	4 (17.4)	0
Bistrița (Bistriţa-Năsăud)	30 (0, 10, 20)	0	0	0
Poiana Mărului (Braşov)	66 (0,10, 56)	2 (3.03)	0	0
Vultureni (Cluj)	44 (3, 10, 31)	1 (2.27)	3 (6.82)	2 (4.55)
Micești (Cluj)	62 (0, 15, 47)	1 (1.62)	0	2 (3.23)
Reșița (Caraș-Severin)	21 (0, 10, 11)	0	0	0
Corund (Harghita)	59 (7, 17, 37)	0	1 (1.7)	4 (6.78)
Bistra (Maramureș)	26 (1, 10, 15)	0	0	0
Icland (Mureș)	37 (6, 5, 26)	2 (5.41)	4 (10.81)	8 (21.62)
Mediaș (Sibiu)	12 (2, 4, 6)	1 (8.33)	0	2 (16.67)
Rătești (Satu Mare)	22 (7, 10, 15)	0	0	1 (4.55)
Lugoj (Timiș)	30 (0, 11, 19)	0	0	5 (16.67)
Total	468 (46, 126, 296)	7 (1.5)	16 (3.42)	25 (5.34)

flab gene was targeted by using multiplex qPCR according to a previous described protocol (*1*). For quality control of qPCRs, we included positive and negative controls. Sequences of qPCR products were analyzed and compared with sequences available in GenBank.

B. miyamotoi was detected in 7 ticks: 2 (1.59%) of 126 males, 2 (0.68%) of 296 females, and 3 (6.52%) of 46 nymphs. *A. phagocytophilum* was detected in 16 ticks: 1 (0.79%) of 126 males, 11 (3.72%) of 296 females, and 4 (8.70%) of 46 nymphs. *Candidatus* N. mikurensis was detected in 25 ticks: 5 (3.97%) of 126 males, 18 (6.08%) of 296 females, and 2 (4.35%) of 46 nymphs. Overall prevalences were 1.50% for *B. miyamotoi*, 3.42% for *A. phagocytophilum*, and 5.34% for *Candidatus* N. mikurensis. Prevalences of each pathogen in specific varied by locality (Table). No co-infections were detected.

We analyzed *flab*, *msp2*, and *groEL* gene sequences obtained by qPCR. These sequences showed 99%–100% identities with gene sequences of *B. miyamotoi* (GenBank accession no. KJ847050), *A. phagocytophilum* (accession no. KP164415), and *Candidatus* N. mikurensis (accession no. FJ966365).

In Romania, the density of *Ix. ricinus* ticks is high and their host diversity is extensive (*7*). However, data for effects of tickborne pathogens on public health are scarce in this country. In this study, we detected *B. miyamotoi*, *A. phagocytophilum*, and *Candidatus* N. mikurensis in questing *Ix. ricinus* ticks in Romania, which confirms the emerging trend of these pathogens in Europe. Because of the scarcity of information on human infections with these pathogens in Romania, serologic and molecular investigations and their implementation are needed for diagnosis, which might help in assessing the effect of these pathogens on public health.

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Address for correspondence: Zsuzsa Kalmár, University of Agricultural Sciences and Veterinary Medicine, Calea Mănăștur 3–5, Cluj-Napoca 400372, Romania; email: zsuzsa_kalmar@yahoo.com

Suspected Rabies in Humans and Animals, Laikipia County, Kenya

Mark Obonyo, James M. Akoko, Austine B. Orinde, Eric Osoro, Waqo Gufu Boru, Ian Njeru, Eric M. Fèvre

Author affiliations: Ministry of Agriculture, Livestock and Fisheries, Nairobi, Kenya (M. Obonyo, A.B. Orinde); Field Epidemiology and Laboratory Training Program, Nairobi (M. Obonyo, W.G. Boru); International Livestock Research Institute, Nairobi (J.M. Akoko, E.M. Fèvre); Kenya Zoonotic Disease Unit, Nairobi (A.B. Orinde, E. Osoro); Ministry of Health, Kenya (W.G. Boru, I. Njeru); University of Liverpool Institute of Infection and Global Health, Liverpool, UK (E.M. Fèvre)

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To the Editor: Dog bites are a serious public health problem because of the associated risk for rabies virus exposure in countries to which the virus is endemic (*1*,*2*).

Human rabies can be prevented by administration of postexposure prophylaxis (PEP). However, PEP rabies vaccine may be unavailable or prohibitively expensive (*3*). Delay in or failure to receive PEP after possible rabies virus exposure contributes to increased incidence of human rabies deaths (*3*).

We performed a retrospective investigation of animal bites and postbite treatment in Laikipia North sub-county, Kenya, during January 2013–February 2014. Laikipia North is 1 of 3 sub-counties in Laikipia County and has a population of 32,726 (*4*). Our investigation was instigated by 3 suspected human rabies deaths that were informally reported to the Kenya Government Zoonotic Disease Unit (ZDU) during early 2014. We reviewed animal bite records from sub-county health facilities and veterinary offices and administered a structured household questionnaire to determine outcomes, knowledge of rabies, bite management, healthcare-seeking behavior, and economic costs. This public health response was government coordinated and approved; no personal identifiers were retained.

During January 1, 2013–February 10, 2014, a total of 106 bites were recorded by 6 government-run health facilities in Laikipia North. Median reported bite incidence per month was 24 bites/100,000 persons (range 6–45 bites/100,000 persons). The median age of bite victims was 13 years (range 1–81 years); 61 (58%) bites occurred in males. Of all bites recorded, 94 (88%) were by dogs, 8 (8%) by scorpions, and 4 (4%) by humans.

The deaths of 3 humans reported to the ZDU occurred in November and December 2013. To assess whether these cases were part of an exposure cluster, we followed up on bite cases during November 1–December 31, 2013. During this period, 17 additional animal bite cases were recorded. Of these 20 bite cases, we successfully traced the households of 11 (55%) case-patients, including 2 of the 3 who died from rabies. Bites were predominantly received from owned pets (82%), and most bites (82%) were reported to be unprovoked. All bites were inflicted on extremities, and almost all (91%) were single-bite injuries (Table 1).

Of 11 animals that bit case-patients, 7 had unknown histories of rabies vaccination and 4 were not vaccinated (Table 1). Four of the 11 animals were suspected to be rabid, including 1 cat and 3 dogs. All the suspected rabid animals were reported to exhibit aggressive or abnormal behavior, drooling or salivation, vocalization, and roaming tendencies (*5*; online Technical Appendix Table 1, http://wwwnc.cdc.gov/EID/article/22/3/15-1118-Techapp1.pdf). Three of the animals reportedly died; status was unknown for 1.

Of the 11 traced bite case-patients, 9 washed their wound before going to a healthcare facility and 8 were prescribed PEP. The median time from bite to reporting to a health facility was 1 day (range 0–3 days). Four respondents delayed in starting PEP: 3 after 3 days, and 1 after 2 days. Reasons given for delay included the high cost of PEP by 3 (including 1 who died); a health facility being too far away by 1, who died; and vaccine unavailable at nearest health facility by 2, 1 of whom died. Of 8 respondents who received PEP, 7 traveled >10 km to reach the nearest health facility. PEP availability was inconsistent at the sub-county hospital and local dispensaries; 6 of 8 respondents seeking PEP visited multiple facilities to receive PEP, including a county referral facility that was >100 km away. The World Health Organization's 5-dose PEP regimen is recommended in Kenya (*1*). However, only 3 case-patients were prescribed and

Table. Responses to questionnaire interview of 11 animal bite victims assessed for rabies, Laikipia County, Kenya, 2014*

Variables/categories	No. (%) case-patients
Time of bite	
Evening	6 (55)
Morning	4 (36)
Afternoon	1 (9)
Part of body bitten	
Legs	8 (73)
Arms	3 (27)
Circumstances of bite	
Unprovoked	9 (82)
Animal provoked	2 (18)
Type of animal	
Dog	10 (91)
Cat	1 (9)
Ownership of biting animal	
Owned	9 (82)
Stray	2 (18)
Rabies vaccination history of biting animal	
Unknown	7 (64)
Not vaccinated	4 (36)
Outcome of biting animal	
Alive and normal	7 (64)
Deceased	4 (36)
Wound washed after bite	
Yes	9 (82)
No	2 (18)
Treatment at healthcare facility	
Anti-tetanus	9 (82)
PEP rabies vaccination	8 (72)
Pain killers	5 (46)
Distance from nearest PEP facility, km	
>10	7 (64)
5–10	3 (27)
0–5	1 (9)
Source of PEP	
Government facility	5 (63)
Chemist	2 (25)
Private hospital	1 (13)
Costs of PEP, US$†	
No. doses of PEP administered	23
Cost categories	Average (range)
Cost/dose of PEP	≈8 (2–15)
Total cost of PEP doses	≈23 (8–50)
Direct medical cost	≈65 (2–500)
Indirect medical cost	≈34 (4–100)
Average cost for obtaining 1 dose of PEP	≈45 (8–120)

*PEP, postexposure prophylaxis.
†Average annual exchange rate during 2013 was 1 Kenya shilling/$0.011586 US.

received 5 doses. Five respondents were prescribed 3, 4, or 6 doses (online Technical Appendix Table 2). This finding indicates large inconsistencies in the PEP prescribing practices in this region of Kenya, a pattern that is similar in other parts of East Africa (*6*).

Respondents bore all medical costs without subsidy. Direct medical costs were ≈$2 $500 (US) per bite victim, and indirect medical costs were ≈$4 $100. The average cost of obtaining a single dose of PEP ranged from $8 to $120 (Table; online Technical Appendix Table 2).

All respondents had heard of rabies. Nine (82%) knew it was transmitted to humans through a bite from a rabid dog, and 4 (36%) knew that rabies among dogs could be prevented through vaccination.

During 2014, at least 3 suspected human rabies deaths and 4 domestic animal deaths were associated with this cluster. Postbite care, including PEP, is a heavy economic burden on this community, moreso because rabies vaccine is not always locally accessible. Dog vaccination rates are low in this region and rabies in suspected animals is rarely definitively diagnosed, increasing risks for human rabies virus exposures and the economic burden of PEP administration. We recommend implementation of regular and comprehensive mass dog vaccination campaigns, in line with Kenya's National Rabies Elimination Strategy (*7*), and further detailed studies on the epidemiology of rabies in this ecosystem, which supports human, wildlife, and domestic dog populations.

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Address for correspondence: Mark O. Obonyo, Ministry of Agriculture, Livestock and Fisheries, Cathedral Road, Nairobi, PO Box 34188-00100, Kenya; email: mobonyo@feltp.or.ke

Generalized Cowpox Virus Infection in a Patient with HIV, Germany, 2012

Philipp Fassbender, Sabine Zange, Sofi Ibrahim, Gudrun Zoeller, Frank Herbstreit, Hermann Meyer

Author affiliations: Universitaet Duisburg-Essen, Essen, Germany (P. Fassbender, F. Herbstreit); Bundeswehr Institute of Microbiology, Munich, Germany (S. Zange, S. Ibrahim, G. Zoeller, H. Meyer); Edgewood Chemical Biological Center, Aberdeen Proving Ground, Maryland, USA (S. Ibrahim)

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To the Editor: In October 2012, a 35-year-old man with clinical category C HIV infection was admitted to the intensive care unit at the University of Duisburg–Essen, Essen, Germany. The man had severe respiratory distress syndrome with septic shock, and he was infected with hepatitis B and C viruses and Epstein-Barr virus. Standard infection-control procedures were followed: the patient was placed in a single room; healthcare providers wore personal protective equipment (gown, face shield, mask, and gloves); and a closed system was used for endotracheal suctioning.

Physical examination of the patient revealed multiple skin lesions on his right forearm and right leg. In the following days, more skin lesions appeared on his abdomen and head. The skin lesions were inflamed macules with central livid, hemorrhagic ulceration (1–2 cm in diameter) and raised edges. Kaposi sarcoma was suspected initially, but on hospital day 5, a skin biopsy showed large intracellular eosinophilic inclusion bodies pathognomonic for infection with cowpox virus (family *Poxviridae*, genus *Orthopoxvirus*). To confirm the diagnosis of cowpox virus infection, we conducted biopsies of 3 skin lesions on hospital day 7. Despite antimicrobial drug and supportive therapy, the patient died that day from septic shock.

The 3 biopsy samples obtained on hospital day 7 were cultured on African green monkey kidney (MA104) cells, and within 2 days, many plaques were observed. DNA extracted from homogenates and virus isolated from the biopsy material were tested by orthopoxvirus real-time PCR (*1*); results were positive for all 6 samples. We confirmed the presence of cowpox virus DNA in all samples by sequencing the hemagglutinin gene.

Serum obtained from the patient on day 2 after admission, when the first lesions were noted, was also positive for

orthopoxvirus DNA by real-time PCR (*1*); approximately 50 genome copies were detected, corresponding to a cycle threshold of 29.7. No orthopoxvirus-specific IgG was detected by immunofluorescence assay; this lack of detection is in agreement with observations that orthopoxvirus antibodies can first be detected in the pustular stage of disease but not as early as the macular stage (http://www.bt.cdc.gov/agent/smallpox/smallpox-biological-weapon-abstract.asp). The patient was born after the cessation of mandatory smallpox vaccination, so vaccine-induced IgG is unlikely.

Generalized cowpox virus infection in humans is atypical; the disease usually manifests as a single painful, ulcerated vesiculopustular lesion, which subsequently forms a scar, accompanied by malaise, fever, and long-lasting, painful local lymphadenopathy. However, in immunocompromised persons and persons with eczema, a generalized (and lethal, in at least 1 case) smallpox-like infection can develop (*2*–*7*). Phylogenetic analysis, based on the hemagglutinin gene, of the cowpox isolate from this study (GenBank accession no. KT182068) (Figure) revealed a close relationship with cowpox viruses isolated from humans in Germany during 1990 and 2009 (*8*). This group of viruses forms a distinct clade that is closer to taterapox, camelpox, and variola (smallpox) viruses than to another clade that contains other strains of cowpox virus and vaccinia and monkeypox viruses. Previous reports have postulated the existence of at least 2 species of cowpox virus (*8*).

Cattle were initially incorrectly presumed to be cowpox virus reservoirs; today, wild rodents are considered to be the true reservoirs. Cowpox virus is transmitted to humans by direct contact with infected animals, mainly cats, which become infected when hunting small rodents. The incubation period is typically 7–12 days. The source of infection for the patient in our study remains unclear; interviews with his family revealed no previous contact with pet animals.

Vaccinia virus, another orthopoxvirus, is known to have induced a generalized infection in a 19-year-old military recruit after smallpox vaccination; the recruit had HIV infection, but this was not known before vaccination (*9*). Satellite ulcers at the site of inoculation and a widespread, disseminated pustular rash resulted in disseminated

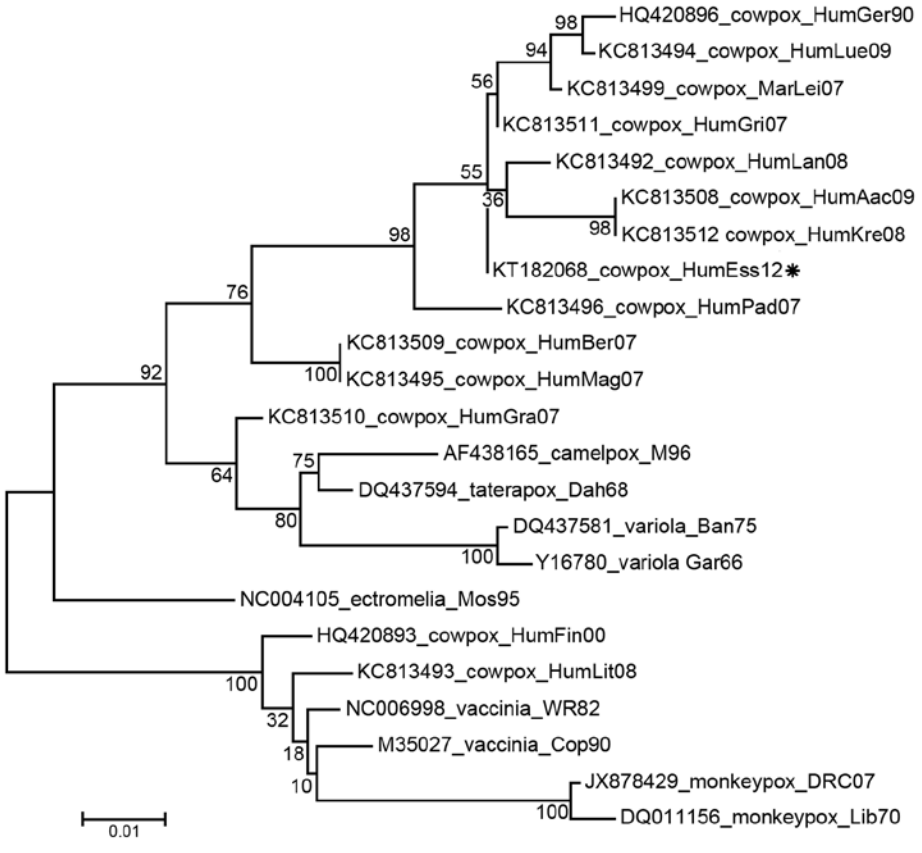

Figure. Evolutionary relationships of cowpox virus isolated from a 35-year-old man with HIV infection treated in the intensive care unit at the University of Duisburg–Essen, Essen, Germany (KT182068_HumEss12, asterisk), other human isolates of cowpox virus, and other orthopoxviruses. The evolutionary history was inferred by using the maximum-likelihood method. The percentage of trees in which the associated taxa clustered together is shown next to the branch nodes. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 23 nt sequences (GenBank accession numbers are indicated). All positions containing gaps and missing data were eliminated; the final dataset had 772 positions. Evolutionary analyses were conducted in MEGA6 (http://www.megasoftware.net). Aac, Aachen; Ban, Bangladesh; Ber, Berlin; Cop, Copenhagen; Dah, Dahomey; DRC, Democratic Republic of Congo; Ess, Essen; Fin, Finland; Gar, Garcia; Ger, Germering; Gra, Graz; Gri, Grimmen; Hum, human; Kre, Krefeld; Lan, Landau; Lei, Leipzig; Lib, Liberia; Lit, Lithuania; Lue, Luedenscheid; Mag, Magdeburg; Mar, Patagonian mara (*Dolichotis patagonum*); Mos, Moscow; Pad, Paderborn; WR, Western Reserve. Scale bar indicates the number of nucleotide changes per site.

vaccinia and AIDS-associated complications that culminated in death of the recruit 18 months after vaccination.

In patients without underlying disease, cowpox infections manifest as self-healing diseases. However, in the absence of vaccination and among a population with increased numbers of immunocompromised persons, the risk for human poxvirus infections is increasing. Early diagnosis is essential for differentiating cowpox from illnesses and skin reactions with similar signs and symptoms, such as smallpox, monkeypox, generalized vaccinia virus infection, disseminated herpes zoster and herpes simplex virus infections, drug-associated eruptions, erythema multiforme, enterovirus infections, secondary syphilis, scabies, insect bites, impetigo, and molluscum contagiosum. The oral drug tecovirimat (previously known as ST-246), as well as cidofovir, CMX–001 (an antiviral substance), and vaccinia immune globulin, should be considered for use as postexposure therapeutic treatment for orthopoxvirus disease (*10*).

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Address for correspondence: Hermann Meyer, Neuherbergstrasse 11, 80937 Munich, Germany; email: hermann1meyer@bundeswehr.org

Absence of Middle East Respiratory Syndrome Coronavirus in Camelids, Kazakhstan, 2015

Eve Miguel,[1] Ranawaka A.P.M. Perera,[1] Almagul Baubekova, Véronique Chevalier, Bernard Faye, Nurlan Akhmetsadykov, Chun Yin Ng, François Roger, Malik Peiris

Author affiliations: Centre de Coopération Internationale en Recherche Agronomique pour le Développement, CIRAD, AGIRs, Montpellier, France (E. Miguel, V. Chevalier, B. Faye, F. Roger); The University of Hong Kong, Hong Kong, China (R.A.P.M. Perera, C.Y. Ng, M. Peiris); LLP Antigen Research and Production Enterprise Antigen, Almaty, Kazakhstan (A. Baubekova, N. Akhmetsadykov); Food and Agriculture Organization of the United Nations Camel Project, AlKharj, Saudi Arabia (B. Faye)

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To the Editor: Middle East respiratory syndrome coronavirus (MERS-CoV) acquired from animals causes severe pneumonia in humans, with some chains of human-to-human transmission, leading to large outbreaks. MERS-CoV is a cause of concern for global public health. The only natural host of MERS-CoV identified so far is the dromedary camel (*Camel dromedarius*) (*1*,*2*), and transmission from camels to humans has been documented (*3*). The geographic distribution of MERS-CoV in dromedaries extends beyond the Arabian Peninsula (where human cases have been reported) to North and East Africa (where human cases have not been reported) (*2*,*4*). However, MERS-CoV from a camel in Egypt and MERS-CoV from a human were phenotypically similar in tropism and replication competence in ex vivo cultures of the human respiratory tract (*5*).

Our previous study demonstrated no evidence of MERS-CoV infection in Bactrian camels in Mongolia (*6*). The question whether MERS-CoV is endemic in camelids in Central Asia remains unanswered. MERS-CoV RNA was detected in swab samples from camels in Iran, which had been imported from Pakistan; however, where the infection was acquired is unclear (*7*).

In Asia, Kazakhstan is of particular interest because large populations of 2 major camelid species overlap: 90% Bactrian (Kazakh breed including 3 ecotypes) and ≈10% dromedary (Arvana breed from Turkmenistan) and their hybrids (*8*). To determine whether MERS-CoV is present in camelids in Kazakhstan, we conducted a seroepidemiologic survey.

During February–March 2015, blood was collected from 550 female camels (455 dromedary, 95 Bactrian)

[1]These authors contributed equally to this article.

(Figure) in 2 regions, Almaty and Shymkent, which differ in camelid density (0.034 and 0.20 camels/km^2, respectively; http://www.stat.gov.kz). Dromedaries were sampled in the cities/villages of Kyzylorda (105 animals from 2 herds), Zanakorgan (35 animals from 1 herd), Sholakkorgan (110 animals from 2 herds), and Akshiy (205 animals from 4 herds). Bactrian camels were sampled in Sholakkorgan (40 animals from 1 herd) and Kanshengel (55 animals from 1 herd) (Figure). For dromedary camels, mean age was 6.1 years (SD 3–7 years) and mean herd size was 53.6 animals (SD 31–70); for Bactrian camels, mean age was 6.5 years (SD 5–8 years) and mean herd size was 48.6 animals (SD 40–55). Serum samples were tested for MERS-CoV antibodies at a screening dilution of 1:20 by using a validated MERS-CoV (strain EMC) spike pseudoparticle neutralization test (*9*). Positive and negative controls were included in each run. Absence of positivity for any sample indicated a lack of recent or past MERS-CoV infection.

Two randomly selected samples each from dromedaries from Kyzlorda, Zanakorgan, and Akshiy and Bactrians from Sholakkorgan and Kanshenegel were tested for neutralizing antibody to bovine coronavirus (*9*). All 10 samples were seropositive, as has been reported for Bactrian camels in Mongolia and the Middle East (*6,9*).

Given the uniformly high seroprevalence of MERS-CoV infection among dromedaries in Africa and the Arabian Peninsula, the lack of infection in dromedaries in southern Kazakhstan was surprising. Because genetically diverse MERS-CoV from Africa remains antigenically conserved with viruses from the Arabian Peninsula, the lack of antibodies is probably not explained by antigenically divergent strains (*9*). Feral dromedaries in Australia, which originated from animals imported from Afghanistan or Pakistan during 1840–1907, are also seronegative for MERS-CoV (*10*). In contrast, bovine-like coronavirus seems to be present in dromedaries everywhere (including Kazakhstan and Australia).

Our study was limited by sample size and by geographic coverage. Of the ≈180,000 camels in Kazakhstan, we studied camelids from only 2 of the 13 provinces. No samples were collected from the western part of the country near Turkmenistan, where dromedaries are also common.

Dromedaries are clearly a natural host of MERS-CoV. However, the finding that MERS-CoV is not endemic in dromedaries in all geographic regions suggests the

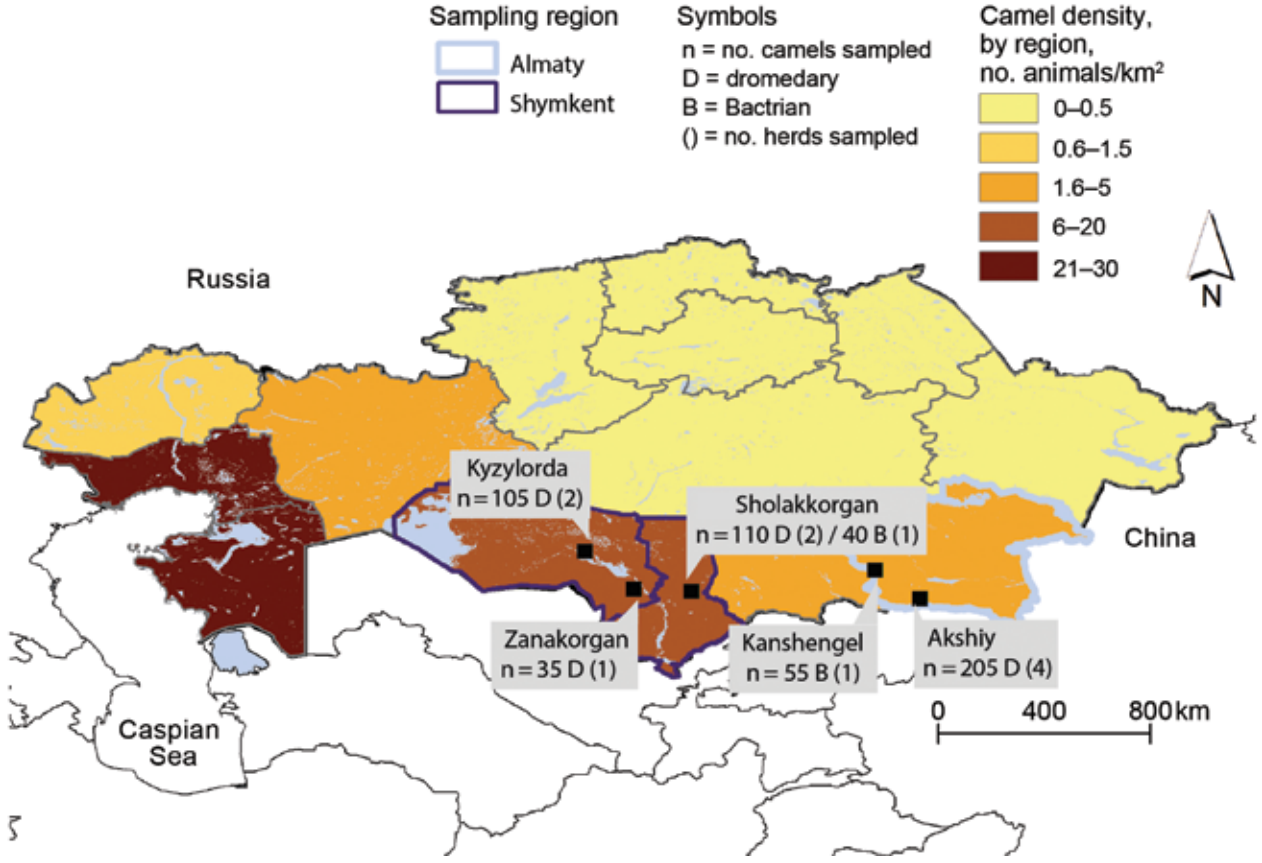

Figure. Density of camelids in Kazakhstan (extracted from the Ministry of National Economy of the Republic of Kazakhstan Committee on Statistics, Department of Statistics; http://www.stat.gov.kz) and specimen collection for detection of Middle East respiratory syndrome virus, by species and region, 2015.

possibility that dromedaries may not be the ultimate natural reservoir (i.e., the long-term host of a pathogen of an infectious disease). Topography (i.e., mountain chains) may limit camel movements from the Middle East or Africa to Central Asia, although such interchange certainly occurred centuries ago as a consequence of the silk-trade routes through southern Kazakhstan. The only known recent imports to Kazakhstan are dromedaries (Arvana breed), brought from Turkmenistan for cross-breeding with Bactrians to improve milk production (*8*). The findings that MERS-CoV is not universally endemic in dromedaries raises the hypothesis that certain species of bats or some other animal, the environment, or both, may constitute a maintenance community and be the true natural reservoir of MERS-CoV and that the virus spills over to camels and is maintained within camels for varying periods of time. Further studies on the epidemiology of MERS-CoV infection among camelids from central Asia are warranted.

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Address for correspondence: Eve Miguel, CIRAD, AGIRs, Avenue Agropolis, 34398 Montpellier CEDEX 5, France; email: eve.miguel@cirad.fr

Novel Reassortant Avian Influenza A(H5N1) Virus in Human, Southern Vietnam, 2014

Ikuyo Takayama,[1] Nguyen Trung Hieu,[1] Masayuki Shirakura, Mina Nakauchi, Seiichiro Fujisaki, Hitoshi Takahashi, Shiho Nagata, Nguyen Thanh Long, Takato Odagiri, Masato Tashiro, Tsutomu Kageyama

Author affiliations: National Institute of Infectious Diseases, Tokyo, Japan (I. Takayama, M. Shirakura, M. Nakauchi, S. Fujisaki, H. Takahashi, S. Nagata, T. Odagiri, M. Tashiro, T. Kageyama); Pasteur Institute, Ho Chi Minh City, Vietnam (N.T. Hieu, N.T. Long)

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To the Editor: The first case of human infection with highly pathogenic avian influenza A(H5N1) virus in Vietnam was reported in December 2003 (*1*), and >120 human cases were confirmed through 2013, with a high case-fatality rate (*2*). In 2013, clade 2.3.2.1a/c H5N1 viruses circulated widely in poultry across the country, although clade 1.1.1/1.1.2 H5N1 viruses predominated in poultry from the Mekong Delta region to central Vietnam (*3*,*4*).

In 2014, two cases of human infection with A(H5N1) virus were identified in southern Vietnam. One case was associated with a clade 1.1.2 reassortant virus, A/Vietnam/14012902/2014 (Global Initiative on Sharing

[1]These first authors contributed equally to this article.

All Influenza Data [GISAID; http://www.gisaid.org] accession nos. EPI624919–EPI624926), which had been previously detected in Cambodia and Vietnam (*5*,*6*). We isolated the virus from the other case, performed phylogenetic analysis to identify the clade of this virus, and identified a novel virus that had undergone gene reassortment.

The case-patient was a 52-year-old man who lived in Binh Phuoc Province (140 km northeast of Ho Chi Minh City). On January 11, 2014, he experienced mild fever and general fatigue; high fever developed on January 13. He was hospitalized with dyspnea on January 16 and died 2 days later. He was not given antiviral drug treatment. Dead poultry infected with H5N1 viruses were found scattered near his house during January 1–16, and he buried his 2 dead chickens on January 5. H5N1 virus infection was detected in the patient's throat swab specimen by real-time reverse transcription PCR at the Pasteur Institute in Ho Chi Minh City. Virus was isolated by inoculating the throat swab specimens into 10-day-old embryonated chicken eggs; the resulting isolate, A/Vietnam/14011801/2014 (GISAID accession nos. EPI624911–EPI624918), then underwent gene sequencing. The 8 viral genes were amplified with SuperScript III Reverse Transcriptase Kit (Fisher Scientific, Pittsburg, PA, USA) and Phusion High-Fidelity DNA Polymerase (New England BioLabs, Ipswich, MA, USA) with specific paired primers, according to the manufacturer's instructions, and sequenced on an ABI 3730 automated sequencer with Big-Dye Terminator Cycle Sequencing reagents (Applied Biosystems, Foster City, CA, USA). Whole genome sequence was determined.

By gene sequencing analysis, A/Vietnam/14011801/2014 was found to have the multibasic cleavage site of hemagglutinin (HA) protein, which indicates highly pathogenic avian influenza A(H5N1) viruses, and was shown to predict binding specificity to an avian α2,3 sialic acid receptor. The neuraminidase gene possessed no amino acid substitutions associated with decreased antiviral activity, nor did the virus have amino acid substitutions associated with increased adaptation, virulence, infectivity, or transmissibility in mammalian hosts, including the E627K and D701N mutations in polymerase basic protein 2 (*7*).

Phylogenetic analyses of the 8 viral genes of A/Vietnam/14011801/2014 were performed by using databases (GISAID and the Influenza Virus Resource, National Center for Biotechnology Information, Bethesda, MD, USA; http://www.ncbi.nlm.nih.gov/genomes/FLU/FLU.html) that contained complete sequences of viral genomes belonging to clades 1.1.1, 1.1.2, and 2.3.2.1 a/b/c, most of which were collected in Vietnam, particularly after 2012. Neighbor-joining and Kimura 2-parameter methods were implemented by using MEGA version 5.0 software (http://www.megasoftware.net). Reliability of the phylogenetic analysis was tested by using 1,000 bootstrap replications. Lineages of the HA gene were defined by using previously described criteria (*8*). Lineages of the other 7 genes were defined by using criteria and nomenclature of Nguyen et al. (*9*).

The HA of A/Vietnam/14011801/2014 belonged to clade 2.3.2.1c (online Technical Appendix Figure, panel A, http://wwwnc.cdc.gov/EID/article/23/3/15-1360-Techapp.pdf). The neuraminidase, polymerase basic proteins 1 and 2, and polymerase acid protein genes of this virus were also derived from respective lineages of ancestor clade 2.3.2.1c (online Technical Appendix Figure, panels B–E). However, nucleoprotein, matrix, and nonstructural genes were classified as lineages of ancestor clade 2.3.2.1a (online Technical Appendix Figure, panels F–H) and differed from the gene lineages of almost all clade 2.3.2.1c viruses isolated from poultry in Vietnam. As reported in the Influenza Virus Resource, 2 viruses collected in Vietnam in December 2013 (A/muscovy duck/Long An/43/2013 and A/muscovy duck/Long An/46/2013) were similar reassortant viruses of clade 2.3.2.1c (Figure). However, the ancestor of the nonstructural gene lineage of these 2 viruses is clade 2.3.2.1c, which differs from A/Vietnam/14011801/2014.

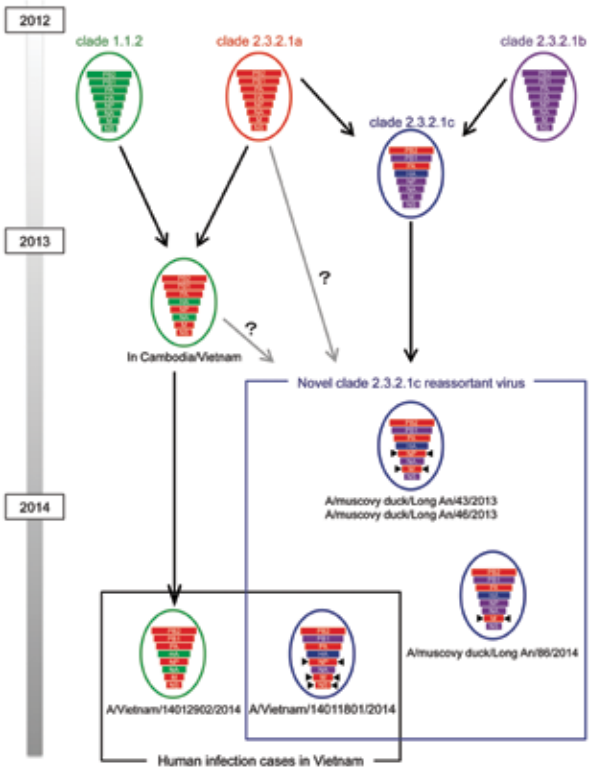

Figure. Novel reassortant virus (A/Vietnam/14011801/2014) identified in a human case of influenza A(H5N1) virus infection in Vietnam, 2014. Ancestry of genes is denoted in the hemagglutinin clades. Arrows indicate genes that differ from the gene lineages of original clade 2.3.2.1c viruses.

The differences indicate that A/Vietnam/14011801/2014 is a novel reassortant virus between clades 2.3.2.1a and 2.3.2.1c, between clades 1.1.2 and 2.3.2.1c, or both (Figure). This novel reassortant virus has not been reported in poultry in Vietnam, although novel reassortants between clade 1.1.2 and clade 2.3.2.1a viruses have been detected in Vietnam since 2013 (i.e., A/Vietnam/VP13-28H/2013, GISAID accession nos. EPI624927–EPI624934; and A/Vietnam/14012902/2014) (*6*). These novel reassortment viruses were first identified in human, animal, and environmental samples in Cambodia in 2013 (*5*). Other novel gene reassortments in clade 2.3.2.1 viruses have been previously reported (*10*), and new clade 2.3.4.4 viruses have been observed in Vietnam since 2014.

As multiple clade viruses co-circulate, reassortment events occur frequently in Vietnam. Continuous surveillance of avian influenza A(H5N1) viruses, not only in humans but also in poultry and wild birds, is needed for infection control measures during epidemics of these viruses.

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Address for correspondence: Tsutomu Kageyama, Influenza Virus Research Center, National Institute of Infectious Diseases, 4-7-1 Gakuen, Musashimurayama-shi, Tokyo 208-0011, Japan; email: tkage@nih.go.jp

Mycobacterium arupense as an Emerging Cause of Tenosynovitis

Fiorella Krapp Lopez, Madeline Miley, Babafemi Taiwo

Author affiliations: Northwestern University, Chicago, Illinois, USA (F. Krapp Lopez, B. Taiwo); Northwestern Memorial Hospital, Chicago (M. Miley)

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To the Editor: *Mycobacterium arupense* was identified in 2006 as a novel species within the *M. terrae* complex with close similarity to *M. nonchromogenicum* (*1*). Since then, 8 cases describing clinically notable disease have been published (*2–8*), including 5 cases of tenosynovitis. We report *M. arupense* tenosynovitis in an immunocompromised person who received the selective interleukin (IL) 1 β-inhibitor canakinumab.

In July 2014, a 62-year-old man sought treatment at the emergency department, Northwestern Memorial Hospital (Chicago, Illinois, USA), after 1 week of pain and swelling in the right hand. During the previous 5 years, he had received multiple immunomodulatory drugs for treatment of natural killer cell deficiency, hyper–IL-6 syndrome, recurrent polychondritis, and Sweet syndrome. His medications were prednisone (42.5 mg/d), intravenous immunoglobulin (400 mg/kg monthly), and subcutaneous canakinumab (180 mg every 8 weeks, which began 3 weeks before onset of symptoms).

His first symptom was a tender red nodule on the right palm that increased in size and became extremely tender over the following week (Figure, panels A, B). He did not recall any trauma and denied fever or chills. No improvement was seen after he received oral linezolid for 5 days. A

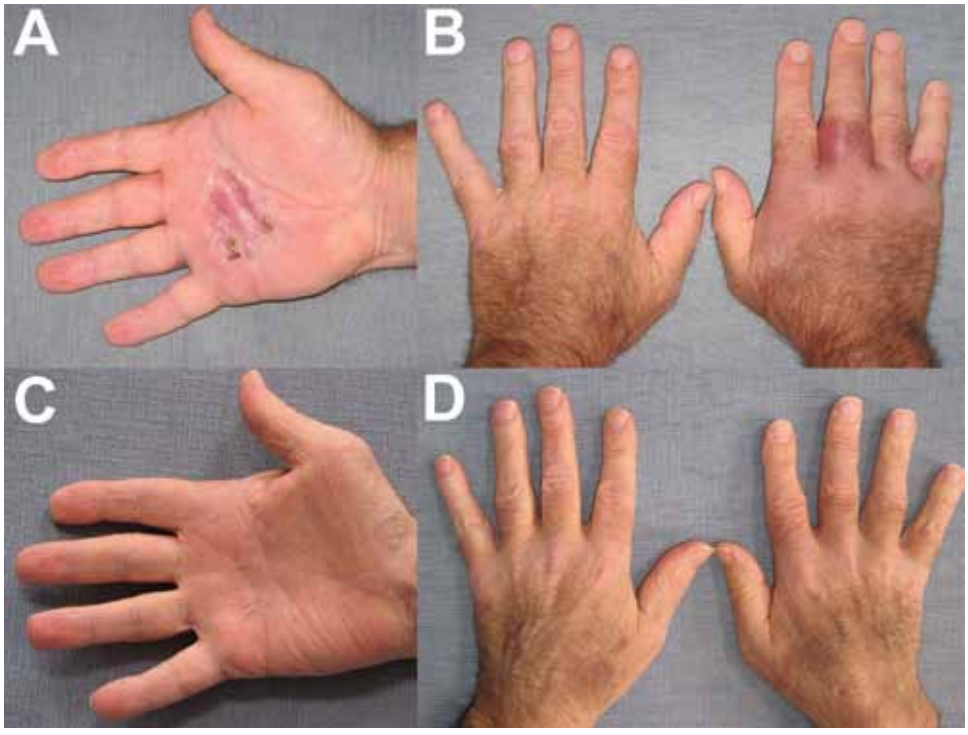

Figure. Hands of a 62-year-old man in Chicago, Illinois, USA, who had *Mycobacterium arupense* tenosynovitis, at the time treatment was sought (panels A, B) and after 6 months of treatment (panels C, D).

skin punch biopsy specimen showed a neutrophilic interstitial infiltrate with no granulomas; results of microbiological stains, including acid-fast bacilli, were negative, . His prednisone dosage was increased to 60 mg/d for suspected Sweet syndrome and, subsequently, to 80 mg/d when no improvement was observed after 2 weeks. A second dose of canakinumab was administered 8 weeks after the first. Shortly after, he was readmitted to the hospital with progression of edema and pain and signs consistent with carpal tunnel syndrome and trigger finger syndrome of the right index finger. Magnetic resonance imaging showed extensive tenosynovitis of the carpal tunnel flexor tendons and no bone erosions. Surgical release and tenosynovectomy of the carpal tunnel was performed; pathologic features demonstrated chronic inflammation of the synovium and absence of granulomas. Results of microbiological stains were negative.

M. arupense grew on Löwenstein-Jensen culture from the skin biopsy specimen after 35 days and from a synovium specimen after 22 days. No growth was observed on liquid culture media. Empiric treatment was started immediately after the first positive culture: clarithromycin (500 mg 2×/d), ethambutol (1,200 mg/d), and rifabutin (300 mg/d). Prednisone was decreased to 45 mg/d, and canakinumab was discontinued. Susceptibility testing confirmed the *M. arupense* strain's susceptibility to clarithromycin, ethambutol, and rifabutin (MICs <4.0, <1.25, and <0.12, respectively); intermediate resistance to rifampin and amikacin (MIC 4.0); and resistance to moxifloxacin and ciprofloxacin (MIC >4.0) and to kanamycin (MIC >8.0). Clinical improvement occurred after 8 weeks of treatment; the condition resolved after 6 months (Figure, panels C, D). Treatment was continued for 12 months.

Five other cases of *M. arupense* tenosynovitis have been reported (*2*,*4*,*5*,*7*,*8*); all patients were immunocompetent or minimally immunocompromised (i.e., diabetes mellitus) (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/22/3/15-1749-Techapp1.pdf). The hand was the site of infection in all cases, and 4 of 5 patients reported prior trauma to the affected area, which suggests that inoculation was the infection mechanism. In the case we describe, the disease appeared to progress much faster than in the immunocompetent patients (weeks vs. months to years). Acid-fast bacilli stain was negative in all of the cases where it was performed (*2,7,8*; this study), and growth on solid Löwenstein-Jensen stain or Middlebrook media was seen after a prolonged incubation time, ranging from 27 days to 2 months. Liquid culture media appears to be unreliable for the growth of *M. arupense* (*8*; this study).

A combination of tenosynovectomy and prolonged antimycobacterial treatment, guided by in vitro strain susceptibility, was used in all the reported cases; a positive outcome was achieved in 6–14 months. The strain susceptibility results we found are comparable with those in the previous cases, showing consistent susceptibility to clarithromycin, ethambutol, and rifabutin; variable susceptibility to linezolid, streptomycin, and amikacin; and resistance to rifampin and quinolones.

Two cases of *M. arupense* infection have been reported in immunosuppressed persons, both in HIV/AIDS patients (manifesting as pulmonary infection in 1 patient and disseminated disease in the other) (*6*). In our study, the immunocompromised patient with *M. arupense* tenosynovitis received canakinumab, a relatively new biologic agent with a prolonged selective IL-1 β-blockade. Even though the contribution of canakinumab in this case is confounded by concomitant immune deficiencies (natural killer cell deficiency, high-dose corticosteroids), the temporal association between initiation of canakinumab and the onset of symptoms raises concern of a possible association. Animal studies have shown that IL-1 plays a key role in host resistance to mycobacterial infections by regulating Th1/Th2 immune responses and inducing granuloma formation (*9*). Clinical trials and systematic reviews assessing the safety of IL-1 inhibitors, including anakinra, rilonacept, and canakinumab, have not shown that these drugs lead to an increased risk of tuberculosis or other mycobacterial infections (*10*). Nonetheless, our report provides increased evidence that *M. arupense* is an emerging cause of tenosynovitis and that it is potentially associated with immunosuppression.

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Address for correspondence: Fiorella Krapp Lopez, Division of Infectious Diseases, Department of Medicine, Northwestern University, 645 N Michigan Ave, Ste 900, Chicago, IL 60611, USA; email: fiorella.krapp@northwestern.edu

Candida haemulonii Complex Species, Brazil, January 2010–March 2015

João Nobrega de Almeida, Jr., João Guilherme Pontes Lima Assy, Anna S. Levin, Gilda M.B. Del Negro, Mauro C. Giudice, Marcela Pullice Tringoni, Danilo Yamamoto Thomaz, Adriana Lopes Motta, Edson Abdala, Ligia Camara Pierroti, Tania Strabelli, Ana Lucia Munhoz, Flávia Rossi, Gil Benard

Author affiliation: Universidade de São Paulo, São Paulo, Brazil

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To the Editor: The epidemiology of yeast infections is evolving, and species in the *Candida haemulonii* complex have been identified as a cause of candidiasis (*1*). In 2012, *C. haemulonii* complex was reclassified as 2 species and 1 variety: *C. haemulonii* (former group I), *C. duobushaemulonii* (former group II) and *C. haemulonii* var. *vulnera* (*1*).

Despite the growing knowledge about the biology and clinical relevance of these pathogens, species-specific data comparing clinical and microbiological aspects are lacking. We describe the clinical and microbiological characteristics of patients from 5 hospitals in São Paulo, Brazil, whose cultures were positive for the *C. haemulonii* complex species.

During January 2010–March 2015, samples from case-patients in 5 hospitals affiliated with the University of São Paulo were cultured; samples positive for *C. haemulonii* were further analyzed. Clinical and epidemiologic data were retrospectively collected. Species identification of the first isolate from each patient was made by sequencing the internal transcribed spacer region of the rRNA gene (*2*). Sequence similarity searches were done by using BLAST (http://www.ncbi.nlm.nih.gov/blast). Antifungal susceptibility testing was performed by using the Clinical

and Laboratory Standards Institute reference method for susceptibility testing of yeasts (*3*) for amphotericin B (AMB), fluconazole, voriconazole, caspofungin (all from Sigma, St. Louis, MO, USA), and anidulafungin (Pfizer, New York, NY, USA).

Among the 14,642 specimens that showed positive yeast cultures, 40 (0.3%) isolates from 31 patients belonged to the *C. haemulonii* complex. Most sample sources were bone and soft tissue samples from lower extremity chronic wounds (n = 17, 42%) and blood cultures (n = 11, 32%). Other positive sources were central venous catheter (CVC) tips (n = 3), toenail scrapings (n = 3), vaginal discharge (n = 2), bile (n = 1), peritoneal fluid (n = 1), pleural effusion (n = 1), and purulent fluid from the mediastinum (n = 1).

Molecular identification characterized 14 isolates as *C. haemulonii* (2 alleles), 8 as *C. haemulonii* var. *vulnera,* and 9 as *C. duobushaemulonii* (online Technical Appendix Table 1, Figure, http://wwwnc.cdc.gov/EID/article/22/3/15-1610-Techapp1.pdf). Clinical and microbiological features of the 31 patients who tested positive are summarized in the Table. Diabetes mellitus was found substantially more frequently among patients with *C. duobushaemulonii* (66% vs. 25%–28% for the other 2 species), but rates for other underlying conditions were similar for all 3 species.

Susceptibility testing results varied by drug and species (Table). *C. duobushaemulonii* showed higher MICs for AMB than *C. haemulonii* and *C. haemulonii* var. *vulnera*, but all isolates showed high MICs for fluconazole and voriconazole. Conversely, MICs were low for caspofungin and anidulafungin. However, 1 isolate of *C. duobushaemulonii* showed high MICs of 8 μg/mL for caspofungin and 0.5 μg/mL for anidulafungin.

Of the 31 patients investigated, 11 had chronically infected wounds of lower extremities with positive surgically collected bone or soft tissue cultures, or both (Table). Samples for 4 of those patients had positive cultures for *C. haemulonii*, 3 for *C. haemulonii var. vulnera*, and 4 for *C. duobushaemulonii.* In most patients (n = 9, 82%), samples showed polymicrobial growth; *Staphylococcus* spp. (n = 7) were the most common concomitant microorganisms. All patients were treated by surgical debridement.

Samples from 8 (25%) of the 31 patients were positive for candidemia; 7 had *C. haemulonii* (3 var. *vulvera*) and 1 *C. duobushaemulonii* (online Technical Appendix Table 2).

Table. Demographic, clinical, and microbiological features of patients whose cultures were positive for *C. haemulonii*, var. *Vulnera*, and *C. duobushaemulonii*, January 2010–March 2015, Brazil*

Characteristic	*Candida haemulonii*, n = 14	*Candida haemulonii* var. *vulnera*, n = *8*	*Candida duobushaemulonii*, n = 9
Mean age, y (range)	42 (0–85)	46 (16–78)	49 (0–85)
Sex, F/M	8/6	6/2	4/5
Mean hospitalization, d (range)	20 (0–140)	28 (0–78)	26 (0–67)
ICU-acquired, %	2 (14)	1 (12)	4 (44)
Polymicrobial culture, %	4 (28)	5 (62)	4 (44)
Underlying conditions, %			
Malignancy†	3 (21)	3 (37)	2 (22)
Solid organ transplant	2 (14)	ND	ND
Systemic lupus erythematosus	ND	1 (12)	ND
Diabetes mellitus	4 (28)	2 (25)	6 (66)
Vascular diseases	3 (21)	3 (37)	4 (44)
Risk factors			
Previous antimicrobial drug therapy	12 (85)	6 (75)	8 (89)
Previous antifungal drug therapy	6 (42)	2 (25)	3 (33)
Chronic lower-extremity infected wounds	3 (21)	4 (50)	4 (44)
Candidemia	4 (28)	3 (37)	1 (11)
Antifungal susceptibility testing			
Amphotericin B			
GM, μg/mL (range)	1.56 (1–4)	1 (0.5–2)	4 (2–8)
MIC_{90}	4	2	8
Fluconazole			
GM, μg/mL (range)	8.4 (1–64)	17.4 (2–>64)	10.07 (0.25–>64)
MIC_{90}	64	64	64
Voriconazole			
GM, μg/mL (range)	1.9 (0.25–>16)	1.53 (0.125–>16)	1.07 (0.125–>16)
MIC_{90}	16	16	16
Caspofungin			
GM, μg/mL (range)	0.12 (0.125–0.5)	0.26 (0.125–0.5)	0.22 (0.06–16)
MIC_{90}	0.25	0.5	16
Anidulafungin			
GM, μg/mL (range)	0.015 (<0.015–0.015)	0.016 (<0.015–0.03)	0.06 (<0.015–0.5)
MIC_{90}	0.015	0.03	0.5

*Values are no. (%) patients except as indicated. ND, no data; GM, geometric mean; MIC_{90}, concentration that inhibits 90% of isolates.
†Solid tumors (n = 5) and hematologic malignancies (n = 3).

Five (62%) patients had received antimicrobial drugs before the infection. Drug therapy failed in 5 (62%) that had positive cultures during deoxycholate AMB (n = 4) or fluconazole (n = 1) therapy. Among the 7 patients with CVC-associated candidemia, 4 had the CVC removed; 3 of those survived. The 30-day all-cause mortality rate was 50%.

Our study showed a prevalence of 0.3% *C. haemulonii* among yeast isolates, which was much higher than previously reported (*4*). Older commercial methods are unable to correctly identify *C. haemulonii* species, contributing to this underestimation (*4*). More closely related species such as *C. auris*, mainly found in South Africa, Asia, and the Middle East, have been misidentified as *C. haemulonii* and *C. famata* by using older systems. Thus, matrix-assisted laser desorption/ionization–time of flight mass spectrometry and internal transcribed spacer rRNA sequencing are necessary to provide the correct identification (*5*–*7*).

The data we document suggest that patients with diabetes mellitus are more likely to have positive cultures for *C. duobushaemulonii* than for the 2 *C. haemulonii* species. Moreover, *C. duobushaemulonii* isolates have higher AMB MICs than the *C. haemulonii* species. As previously reported (*8*), echinocandins showed better in vitro activity than azole compounds.

In summary, we demonstrated that *C. haemulonii* species complex are critical pathogens of chronic lower extremity wounds and that fungemia by such species remains a rare event. The 30–day all-cause mortality rate among patients with candidemia was 50%, lower than previously reported in our institution (*9*) and other centers in Brazil (*10*). We believe that in cases of candidemia by *C. haemulonii* spp. that 1) empirical use of AMB or azole compounds should be avoided; 2) removal of CVC should be performed; and 3) antifungal susceptibility testing should be done to guide antifungal therapy.

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Address for correspondence: João Nobrega de Almeida, Jr., Laboratorio de Microbiologia, DLC, PAMB, Instituto Central. Av. Dr. Enéas de Carvalho Aguiar, 255–Cerqueira César, 05403-000 São Paulo, Brazil; e-mail: jnaj99@gmail.com

Review of Cases and a Patient Report of Myiasis with Tracheostomy, Peru

Virgilio E. Failoc-Rojas, Heber Silva-Díaz

Author affiliations: Universidad Nacional Pedro Ruiz Gallo, Lambayeque, Peru (V.E. Failoc-Rojas, H. Silva-Díaz); Hospital Regional Lambayeque, Lambayeque (H. Silva-Díaz)

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To the Editor: Myiasis is the infestation in humans of larvae of flies (order Diptera). These larvae can infect

skin, necrotic tissues, and natural cavities of living persons. Myiasis can be primary if it infects intact skin or secondary if it infects a previous injury. Depending on the degree of parasitism, myiasis may be obligatory (requiring a live host for parasite survival), facultative (developing in live or dead organic matter), or accidental (developing accidentally in an inappropriate host) (*1*). In South America, the species that most frequently cause myiasis are *Dermatobia hominis* and *Cochliomyia hominivorax*.

Factors contributing to development of myiasis are low socioeconomic status, unhealthy environments, advanced age, alcoholism, neurologic diseases, and lack of personal hygiene (*1,2*). Myiasis may occur different tissues, but reports of myiasis of the tracheal stoma are rare. We searched PubMed, MedLine, Lilacs, Scopus, and Google Scholar databases for scientific articles published in English or Spanish languages during 1990–2015 by using the search term "myiasis and tracheostomy." We found reports of 10 patients (Table).

We also report a case of tracheostomal myiasis in a 67-year-old man from Tύcume, Peru. The patient had a history of esophageal tumor lesion with considerable airway stenosis related to upper esophageal cancer (stage III). Six months before onset of myiasis, he had respiratory difficulty caused by obstructed airway and underwent a tracheostomy and gastrostomy. When the patient was admitted to the emergency department of a hospital in Lambayeque, located ≈35 km from the patient's home, mobile larvae were present at the tracheostomy site, which also contained brown secretions with traces of blood and obvious signs of inflammation. A cervical abscess surrounded by necrotic tissue was visible, which, according to family members, developed after the larval infection. We manually removed the larvae and began treatment with ivermectin orally (1 dose, 200 μg/kg), ceftriaxone orally (2 g/d), and metronidazole intravenously (500 mg every 8 h). Three days after the patient started treatment, the tracheostomy tube was surgically removed for changing, and a large number of dead larvae were then observed and removed. The patient showed no signs of septicemia. He had slight relative eosinophilia (6%), but his hemoglobin and leukocyte levels were within reference ranges; the larvae would be unable to penetrate cells at these levels. The patient improved with no clinical symptoms of cervical abscess or evidence of phlogosis. He was discharged 9 days after admission, with a postdischarge treatment of oral metronidazole (500 mg every 12 h for 3 d).

Three specimens of larvae were sent to the hospital's parasitology laboratory, which identified the larvae as *C. hominivorax* stage L-3 (infection began with fly oviposition ≈6 days before admission; L-1, L-2, and L-3 are stages of larval development from hatching until pupation, requiring ≈7 days). The larvae were 10 mm × 3 mm and had a cylindrical, pale yellow body segmented with pigmented tracheal trunks visible in the last 4 posterior segments. Microscopic examination showed that the anterior end had a prominent jaw and segments with small bands of cuticular spines; the rear end had exposed spiracles, each with 3 straight grooves and open peritrematic membranes (reference *13* in the online Technical Appendix, http://wwwnc.cdc.gov/EID/article/22/3/15-1631-Techapp1.pdf).

The life cycle of *C. hominivorax* is similar to any other species in the Diptera order. Open wounds and body orifices (e.g., a tracheostomy) emitting odors from natural secretions are conducive for oviposition by flies and development of myiasis. A study from Brazil mentions that open wounds are the leading cause of development of the *C. hominivorax* parasite (*2*). Chronic extensive wounds are often infested by *C. hominivorax* (*2,5*).

Myiasis infection is concerning because it can lead to secondary infections such as *Escherichia coli*, *Serratia marcescens*, and *Enterococcus faecalis* (*6*). The infection is most dangerous when patients have concurrent conditions such as immunosuppression.

Table. Reports in the literature about myiasis associated with tracheostomy, by date of publication*

Country	Patient age, y/sex	Associated conditions	Fly species	Year of publication	Reference†
Canada	85/F	Comatose state for 2 mo	Unidentified	1993	(*3*)
Italy	57/M	Persistent vegetative state	*Lucilia caesar*	2006	(*4*)
Brazil	49/M	Neck carcinoma	*Cochliomyia hominivorax*	2011	(*5*)
India	78/M	Tracheostomy by car accident	*Chrysomya bezziana*	2011	(*6*)
Argentina	8/NA	Cerebral palsy	Unidentified	2012	(*7*)
India	52/M	Laryngeal cancer	*Musca domestica* (housefly)	2013	(*8*)
India	73/NA	Carcinoma supraglottis and diabetes	*Chrysomya bezziana*	2013	(*9*)
Turkey	86/F	Poor hygienic condition and tetraplegia	*Lucilia caesar*	2014	(*10*)
India	57/M	Proliferative ulcer on vocal cords and glottic stenosis	*Chrysomya bezziana*	2015	(*11*)
Italy	5/M	Werdnig-Hoffmann disease	*Sarcophaga argyrostoma*	2015	(*12*)
Peru	67/M	Esophageal cancer	*Cochliomyia hominivorax*		This study

*NA, not available.
†Sources: PubMed, Medline, Scopus, LILACS, and Google Scholar. References *11,12* are in the online Technical Appendix (http://wwwnc.cdc.gov/EID/article/22/3/15-1631-Techapp.pdf).

Treatment of myiasis involves manual removal of larvae and surgical debridement, in conjunction with ivermectin and systemic broad-spectrum antimicrobial drugs to prevent secondary infections (*1,2*). Treatment with ivermectin can kill the larvae (*1*; references *14,15* in the online Technical Appendix) and result in considerable reduction of larvae in infested wounds. Ivermectin has a broad antiparasitic spectrum that causes immobilization of parasites by inducing tonic paralysis of the parasite's muscles, mainly at the pharyngeal level, resulting in the death of the parasites by suffocation and starvation.

For the patient in this report, the single oral dose (0.2 mg/kg) of ivermectin was an effective treatment for myiasis. However, to control the underlying disease and prevent recurrences, ivermectin should be used with oral antimicrobial drugs and wound care when the wound has a high number of larvae, which are associated with bacterial infections (*4,5*).

For bedridden patients, patients with superficial wounds who live in myiasis-endemic areas, or patients who undergo a tracheostomy or have open wounds, health workers and caregivers should consider preventive care of wounds, which are risk factors for myiasis infection. This care consists of suitable wound dressing and proper personal and environmental hygiene.

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Address for correspondence: Virgilio E. Failoc-Rojas, Av Manuel Seoane 1343-La Victoria, Chiclayo, Peru; email: virgiliofr@gmail.com

Trends in Liver Transplantation in Hepatitis C Virus–Infected Persons, United States

Ryan B. Perumpail, Robert J. Wong, Andy Liu, Channa R. Jayasekera, Douglas T. Dieterich, Zobair M. Younossi, Aijaz Ahmed

Author affiliations: Stanford University School of Medicine, Stanford, California, USA (R.B. Perumpail, A. Ahmed); Highland Hospital, Oakland, California, USA (R.J. Wong); Albert Einstein School of Medicine, Bronx, New York, USA (A. Liu); California Pacific Medical Center, San Francisco, California, USA (C.R. Jayasekera); Icahn School of Medicine at Mount Sinai, New York, New York, USA (D.T. Dieterich); Inova Fairfax Hospital Center for Liver Diseases, Falls Church, Virginia, USA (Z.M. Younossi); Inova Health System Betty and Guy Beatty Center for Integrated Research, Falls Church (Z.M. Younossi)

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To the Editor: The Centers for Disease Control and Prevention and US Preventive Services Task Force recommend a one-time screening for hepatitis C virus (HCV) infection in adults born during 1945–1965 (birth cohort), a demographic group with a disproportionately high prevalence of HCV infection (*1*,*2*). However, some experts have warned against routine HCV screening of persons in the birth cohort, stating that this recommendation is based on unproven assumptions about the benefit of screening in reducing HCV-related mortality, given that only a minority of infected persons develop end-stage liver disease (ESLD) (*3*). To determine the relative effect of the birth cohort on HCV-related ESLD incidence in the United States, we analyzed trends in liver transplantation (LT) waitlist registrations and LT surgeries during 1995–2012. Using data from the United Network for Organ Sharing national registry, we evaluated birth cohort–specific (birth cohort vs. non–birth cohort) and etiology-specific (HCV vs. non-HCV) trends in LT waitlist registrations and LT surgeries performed in the United States during that 18-year period.

The proportion of HCV-infected persons born during 1945–1965 among all persons with LT waitlist registrations in the United States increased from 17.8% in 1995 to 35.2% in 2012 (Table). The highest proportion of LT

Table. Liver transplant waitlist additions and liver transplant recipients, United States*

Transplant status	1995	2001	2006	2012
New waitlist additions				
Birth cohort† total	3,227 (47.4)	6,329 (62.3)	7,378 (71.9)	8,476 (77.0)
HCV	1,212 (17.8)	2,960 (29.1)	3,312 (32.3)	3,872 (35.2)
Non-HCV	2,015 (29.6)	3,369 (33.2)	4,066 (39.6)	4,604 (41.8)
Non–birth cohort total	3,583 (52.6)	3,830 (37.7)	2,878 (28.1)	2,350 (23.0)
HCV	767 (11.3)	818 (8.1)	505 (4.9)	408 (3.7)
Non-HCV	2,816 (41.3)	3,012 (29.6)	2,373 (23.2)	2,122 (19.3)
Total	6,810 (100.0)	10,159 (100.0)	10,256 (100.0)	11,006 (100.0)
Liver transplant recipients				
Birth cohort total	1,677 (48.9)	2,926 (63.7)	4,324 (71.2)	4,475 (78.1)
HCV	598 (17.4)	1,416 (30.8)	2,004 (33.0)	2,029 (35.4)
Non-HCV	1,079 (31.5)	1,510 (32.9)	2,320 (38.2)	2,446 (42.7)
Non–birth cohort total	1,751 (51.1)	1,667 (36.3)	1,747 (28.8)	1,256 (21.9)
HCV	396 (11.6)	393 (8.6)	309 (5.1)	208 (3.6)
Non-HCV	1,355 (39.5)	1,274 (27.7)	1,438 (23.7)	1,048 (18.3)
Total	3,428 (100.0)	4,593 (100.0)	6,071 (100.0)	5,731 (100.0)

*HCV, hepatitis C virus.
†US adults born during 1945–1965.

waitlist registrations for HCV-related ESLD was for persons in the birth cohort and increased incrementally from 61.2% in 1995 to 90.5% in 2012. The proportion of LT waitlist registrations for HCV-related ESLD among persons younger than the birth cohort was 1.0% in 1995 and 3.6% in 2012; among persons older than the birth cohort, the proportion was 37.8% in 1995 and 5.9% in 2012.

Similarly, among LT recipients, the proportion of HCV-infected persons born during 1945–1965 doubled from 17.4% in 1995 to 35.4% in 2012 (Table). The proportion of LT surgeries performed for HCV-related ESLD among persons in the birth cohort increased from 60.2% in 1995 to 90.7% in 2012. Among persons younger than the birth cohort, the proportion of LT surgeries performed for HCV-related ESLD was 0.7% in 1995 and 5.0% in 2012; among persons older than the birth cohort, the proportion was 39.1% in 1995 and 4.3% in 2012.

During 1995–2012, the ratio of new LT waitlist registrations to LT surgeries performed for HCV-infected persons in the birth cohort remained unchanged at 1.9:2.0 despite the aging of this birth cohort. Overall trends in HCV-related LT waitlist registrations and LT surgeries stabilized during 2001–2012; the proportion of HCV-infected persons in the birth cohort increased, and the proportion of HCV-infected persons not in the birth cohort decreased.

To exclude the possibility that HCV-related ESLD has always simply affected persons 50–70 years of age, we performed a subanalysis examining the proportion of LT waitlist registrations and LT surgeries for persons 50–70 years of age in each year from 1995 through 2012. During this 18-year period, among persons 50–70 years of age, new HCV-related LT waitlist registrations increased from 43.9% to 93.0%, and LT surgeries performed increased from 47.1% to 86.2%. This finding suggests that persons born during 1945–1965 are a distinct birth cohort that is increasingly affected by HCV-related ESLD.

Although persons born during 1945–1965 make up an estimated 27% of the US population, they account for ≈75% of all HCV infections and 73% of HCV-associated deaths in the United (*1*). Our findings are consistent with those of an earlier modeling study by Davis et al. (*4*), which suggested that the age of persons with HCV-related cirrhosis and its complications will continue to increase.

Limitations of our study include inherent limitations of retrospective design and registry data. The designation of HCV infection and birth cohort status is based entirely on data entered into the database, which are not necessarily subject to cross-checking confirmatory measures. However, any errors in data entry that may have occurred are probably nondifferential. Despite these limitations, our analysis demonstrates that >90% of HCV-infected persons registered for LT or undergoing LT surgeries in 2012 were in the birth cohort.

Earlier diagnosis and preemptive cure of HCV infection with highly effective and safe direct-acting antiviral drugs may delay or reduce the need for LT among persons in the birth cohort (*5*). Testing and linkage to care for HCV-infected persons, particularly persons in the birth cohort, can be expected to reduce HCV-related illness and death (*1*,*2*). In response to the approval of higher efficacy antiviral drugs and rapidly rising liver failure–related death among this cohort (*6*,*7*), the use of HCV-infected donors has increased, resulting in truncated wait times for HCV-infected LT recipients in many regions (*8*), whereas HCV-uninfected persons are generally waiting considerably longer, often years, for HCV-uninfected donors (*9*). This phenomenon is another index of the extent of HCV-related ESLD in the United States.

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Address for correspondence: Ryan B. Perumpail, Division of Gastroenterology and Hepatology, Stanford University School of Medicine, 750 Welch Rd, Ste 210, Stanford, CA 94304, USA; email: rperumpail@gmail.com

Wohlfahrtiimonas chitiniclastica Infections in 2 Elderly Patients, Hawaii, USA

Masayuki Nogi, Matthew J. Bankowski, Francis D. Pien

Author affiliations: University of Hawaii at Manoa John A. Burns School of Medicine, Honolulu, Hawaii, USA (M. Nogi, M.J. Bankowski, F.D. Pien); University of Hawaii at Manoa, Honolulu (M. Nogi, M.J. Bankowski, F.D. Pien); Diagnostic Laboratory Services, Inc., Aiea, Hawaii, USA (M.J. Bankowski)

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To the Editor: We describe 2 cases of *Wohlfahrtiimonas chitiniclastica* sepsis and skin and soft tissue infections in 2 elderly patients; 1 case was fatal. Both patients lived in poor hygienic conditions in Hawaii, USA.

The first case occurred in a 72-year-old man with history of stroke and deafness. After being unattended for 3 days, he was found unconscious on the floor of his home. He was hypotensive, bradycardic, and hypothermic. Maggots were crawling out of an umbilical wound and were present in a 2 × 3 cm laceration on his right dorsal foot. His leukocyte count was 2.4×10^3 cells/μL with 42% band cells, creatinine level was 2.5 mg/dL, and lactic acid level was 1.8 mg/dL. Aerobic and anaerobic cultures of blood collected at hospital admission grew *Escherichia coli* and *W. chitiniclastica* within 12 hours. Initial treatment consisted of intravenous piperacillin/tazobactam (4.5 g every 6 h), intravenous clindamycin (900 mg every 8 h), and intravenous vancomycin (1,000 mg every 12 h). The patient died from septic shock on his second hospital day. *W. chitiniclastica* was identified by using 16S rRNA sequencing (MicroSeq 500 16S rDNA Bacterial Identification Kit; Applied Biosystems, Foster City, CA, USA) and analyzed by using RipSeq mixed DNA interpretation software (iSentio Ltd., Bergen, Norway). A 100% match with *W. chitiniclastica* type strain H100 (GenBank accession no. HQ407275) was observed. Antimicrobial drug–susceptibility testing was performed by using a microdilution method (MicroScan Dried Overnight Gram-Negative Panel; Siemens Medical Solutions, Malvern, PA, USA). The isolate was sensitive to all drugs tested, including classes of penicillin, cephalosporin, fluoroquinolone, carbapenem, tetracycline, and aminoglycoside.

The second case occurred in a 69-year-old homeless woman with a history of right hemiparesis from a ruptured cerebral aneurysm. She reported having had sacral pain and painful urination during the week before admission. Physical examination revealed stable vital signs, disheveled appearance, and multiple purulent decubitus ulcers in her sacral area. Her leukocyte count was high (20.9×10^3 cells/μL). Urinalysis revealed pyuria, positive nitrates, and moderate leukocyte esterase, indicative of a urinary tract infection. Two blood cultures and urine culture were obtained at admission. She was given intravenous ceftaroline fosamil (600 mg every 12 h) to treat the urinary tract and decubitus ulcer infections. She then underwent surgical debridement of her decubitus ulcers, where tissue from a deep wound was obtained for aerobic and anaerobic culture. The deep wound culture grew polymicrobial flora that included *W. chitiniclastica*, *Staphylococcus aureus*, *Aeromonas* spp., *S. simulans*, and *Bacteroides fragilis*. The anaerobic bottle from both blood cultures grew a gram-negative anaerobic bacillus, *Anaerobiospirilum succinicproducens.* In addition, *Proteus mirabilis* was isolated from a urine culture. These culture results prompted a change in the patient's antimicrobial drug regimen to intravenous meropenem (1 g every 8 h), which the patient received for 12 days. She responded well and was discharged and prescribed oral amoxicillin/clavulanate (875 mg/125 mg every 12 h) for

3 weeks, for what would amount to a 34-day course of antimicrobial treatment since her hospital admission. *W. chitiniclastica* was identified by using 16S rRNA sequencing (MicroSeq 500 16S rDNA Bacterial Identification Kit). A 100% match with *W. chitiniclastica* type strain H100 (GenBank accession no. HQ407275) was observed. Antimicrobial drug–susceptibility testing results were the same as those observed for the previously described patient.

W. chitiniclastica is a short, gram-negative, facultative anaerobic, and motile gammaproteobacterium with strong chitinase activity. It was isolated from the homogenated third-stage larvae of the *W. magnifica* fly (*1*) (Figure). This fly has been reported as the cause of myiasis in live vertebrates in Spain, France, Hungary, Turkey, Egypt, Iran, and Korea (*2*); its distribution is known to be progressively expanding, in part because of its broad adaptation capacities. Reported cases of human bacteremia have been mainly from Europe and South America; patients included a 60-year-old homeless woman in southeastern France (*3*), a 70-year-old homeless man with alcoholism in Argentina (*4*), and an 82-year-old woman in the United Kingdom (*5*). A skin and soft tissue infection was reported in a child with orofacial gangrene (noma) in Niger (*6*), and an osteomyelitis case was reported in India (*7*). The northernmost region from which a case has been reported is Estonia, where a 64-year-old man with chronic foot gangrene was coinfected with *W. chitiniclastica* and *Myroides odoratimimus* (*8*). Cases from the United States include septicemia in a deer in Michigan (*9*) and a leg wound infection in a 26-year-old man in New York (*10*).

For the 2 patients in Hawaii, maggots were not collected, and we could not identify the specific fly species. In the first patient, *W. chitiniclastica* was clinically relevant because it was isolated from blood culture and maggots were observed in his wound. However, the coexisting *E. coli* infection may have played a critical role in the patient's death. The second case was nonfatal, and we cannot determine the clinical relevance of *W. chitiniclastica* because it was isolated from a polymicrobial wound in which no maggots were observed and because *A. succinicproducens* was isolated from blood culture. Even so, these reports should help increase awareness of this specific type of infection related to myiasis in homeless and hygiene-deficient patients in the United States.

Figure. Adult *Wohlfahrtiimonas magnifica* fly. Image courtesy of Joaquim Alves Gaspar, Wikimedia Commons.

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Address for correspondence: Masayuki Nogi, Department of Internal Medicine, John A. Burns School of Medicine, University of Hawaii at Manoa, 1301 Punchbowl St, Honolulu, HI 96813, USA; email: msyknog@me.com

Mycobacterium microti Infection in Dairy Goats, France

Lorraine Michelet, Krystel de Cruz, Yohann Phalente, Claudine Karoui, Sylvie Hénault, Marina Beral, María L. Boschiroli

Author affiliations: Agence Nationale de Sécurité Sanitaire de l'Alimentation, de l'Environnement et du Travail, Maisons-Alfort, France (L. Michelet, K. de Cruz, Y. Phalente, C. Karoui, S. Hénault, M.L. Boschiroli); Direction Régionale de l'Agriculture et de la Forêt, Bourgogne, Dijon, France (M. Beral)

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To the Editor: *Mycobacterium microti* is a member of the *Mycobacterium tuberculosis* complex (MTBC). This complex also includes *M. tuberculosis*, which causes human tuberculosis, and *M. bovis* and *M. caprae*, which cause bovine tuberculosis. *M. microti* was initially described as a pathogen of small rodents and also frequently affects domestic animals, especially cats, and has also been described in wildlife, especially wild boars and badgers (*1*). This mycobacterium has also been involved in human pulmonary tuberculosis cases, which highlights its potential zoonotic risk (*2*). We report a case of *M. microti* infection in a dairy goat herd, which underlines the risk for confounding bovine tuberculosis diagnosis and potential consequences for livestock management.

France has been considered officially free of bovine tuberculosis by the European Union since 2001. Surveillance of this disease is based on antemortem testing with tuberculin skin tests and on systematic postmortem inspection at abattoirs through sanitary inspection of carcasses to detect bovine tuberculosis–like lesions. However, because bovine tuberculosis evolves in an insidious manner and antemortem or postmortem diagnostic tests are not efficient for detecting latently infected animals, an infected herd may remain unidentified for long periods and can be responsible for contamination of other herds by animal movement or contact with animals of neighboring herds. For this reason, to detect other potentially associated cases, investigations in herds epidemiologically linked to the index outbreak are also performed, either through skin testing or through diagnostic culling of those animals introduced from the infected herd or any other animal with a skin test–positive result.

This case of bovine tuberculosis in a goat was reported in a region of the Alps Mountains in France. The herd was composed of 140 dairy goats, which were used for raw milk cheese production. Goats were semi-extensively bred and kept in pastures ≥6 months per year. Investigations conducted after identification of a case of bovine tuberculosis in a neighboring cattle herd infected with *M. bovis*, in which the index case was identified at an abattoir, highlighted the epidemiologic link with the goat herd because animals in both populations shared the same pastures. Thus, the goat herd was subjected to single intradermal tuberculin tests.

Three adult goats (goats A, B, and C) showed positive results and were culled for direct diagnosis. Goats A and B showed no lesion at abattoir inspections, but goat C had bovine tuberculosis–like lesions on the retropharyngeal and mediastinal lymph nodes. Retropharyngeal, tracheobronchial, and mediastinal lymph nodes were sampled from the 3 goats. Retromammary lymph nodes were also sampled from goats B and C (both females). Lesions from goat C were examined by histopathologic analysis and showed a profile suggestive of bovine tuberculosis with necrosis, Langhans giant cells, and few acid–alcohol-resistant bacilli by Ziehl-Neelsen staining (Figure). All samples were subjected to bacterial culture and molecular diagnosis (*3*).

Although after 3 months culture results were negative for all samples, DNA extracted from the retropharyngeal lymph node of goat C showed a positive PCR result for MTBC DNA by the LSI VetMAX *Mycobacterium tuberculosis* Complex Real-Time PCR Kit (Thermo Fisher Scientific, Villebon sur Yvette, France). Further characterization of this DNA was performed by using molecular analysis specific for the regions of difference, which enables differentiation of MTBC members (*4*), and spoligotyping (*5*).

The infectious agent from goat C was identified as *M. microti* spoligotype SB0118. Moreover, a bovine tuberculosis investigation in wildlife (*6*) identified *M. microti* spoligotype SB0118 infection case in a dead badger found 8 km from the goat farm. Thus, the long time during which

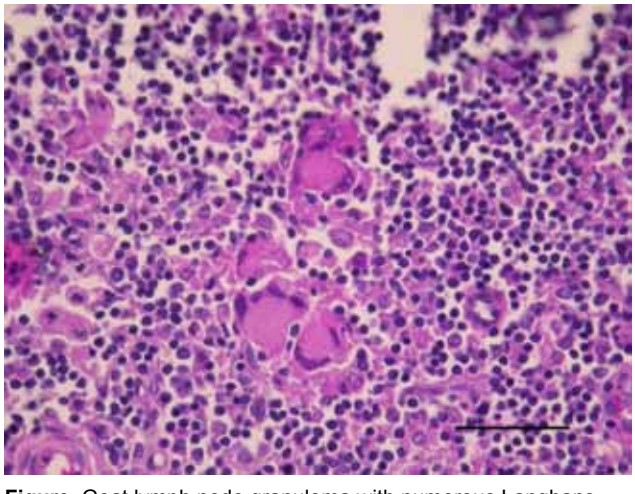

Figure. Goat lymph node granuloma with numerous Langhans-type multinucleated giant cells from a goat in France infected with *Mycobacterium microti* (hematoxylin and eosin stain). Scale bar indicates 50 μm.

goats remain in pastures might have favored environmental contamination by interaction with wildlife. Furthermore, an additional case of *M. microti* infection in a cat reported in 2011 in the same region also had the SB0118 spoligotype (*7*), which demonstrated that this bacillus is actively circulating in animals from this area.

M. microti was previously isolated on the basis of a skin test–positive result for cattle in the United Kingdom (*8*), which demonstrated the risk for infection in livestock. These findings raise concern on reliability of diagnostic tests used for bovine tuberculosis surveillance. *M. microti*, which is phylogenetically similar to *M. bovis* or *M. caprae* and widely disseminated in the environment, could be responsible for misleading diagnostic results, as demonstrated in this study.

Highly specific tests are needed to accurately identify *M. bovis* (or *M. caprae*) infection at antemortem examination through use of specific antigens, such as ESAT 6 and CFP10, which are absent in *M. microti* and are currently used in the interferon-g test in France (*9*). In addition, at postmortem diagnosis, use of specific molecular tools capable of rapidly distinguishing members of the MTBC should be considered. Histopathologic analysis lacks specificity, and obtaining results for bacterial culture takes too much time for these particularly slow-growing and fastidious mycobacteria.

M. microti has already been reported to cause tuberculosis in immunocompromised and immunocompetent patients in France (*10*). Thus, potential risk for infection of humans by consumption of raw goat milk cheese cannot be ruled out.

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Address for correspondence: Maria L. Boschiroli, Agence Nationale de Sécurité Sanitaire de l'Alimentation, de l'Environnement et du Travail, 14 Rue Pierre et Marie Curie, Maison-Alfort 94700, France; email: maria-laura.boschiroli@anses.fr

Mycobacterium orygis–Associated Tuberculosis in Free-Ranging Rhinoceros, Nepal, 2015

Jeewan Thapa,[1] Sarad Paudel,[1] Amir Sadaula, Yogendra Shah, Bhagwan Maharjan, Gretchen E. Kaufman, Deborah McCauley, Kamal P. Gairhe, Toshio Tsubota, Yasuhiko Suzuki, Chie Nakajima

Author affiliations: Hokkaido University, Sapporo, Japan (J. Thapa, S. Paudel, Y. Shah, T. Tsubota, Y. Suzuki, C. Nakajima); National Trust for Nature Conservation, Biodiversity Conservation Center, Chitwan, Nepal (A. Sadaula); German Nepal Tuberculosis Project, Kathmandu, Nepal (B. Maharjan); Veterinary Initiative for Endangered Wildlife, Bozeman, Montana, USA (G.E. Kaufman, D. McCauley); Chitwan National Park Department of National Parks and Wildlife Conservation, Chitwan (K.P. Gairhe)

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[1]These first authors contributed equally to this article.

To the Editor: *Mycobacterium orygis*, previously described as oryx bacilli, has recently been categorized as a member of *M. tuberculosis* complex and has been reported to cause tuberculosis (TB) in a variety of animals and in humans. Most reported isolates were of South Asian origin (*1*). In a previous study (*2*), we isolated and molecularly characterized *M. orygis* isolates from wild animals living in a captive facility in Kathmandu, Nepal.

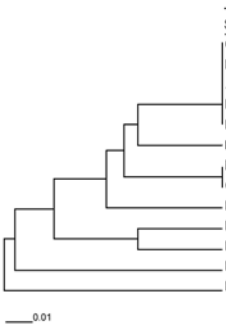
The greater one-horned rhinoceros (*Rhinoceros unicornis*), or Indian rhinoceros, is the largest species of rhinoceros. It is listed in Appendix I of the Convention on International Trade in Endangered Species (https://cites.org/eng/app/appendices.php), designated as vulnerable by the International Union for Conservation of Nature Red List (http://www.iucnredlist.org/search), and designated as a protected species by the Government of Nepal (*3*). Because of successful conservation efforts, the current wild population of greater one-horned rhinoceros in Nepal and India has increased from 600 in 1975 to 3,555 in mid-2015 (*4*). As of 2015, the population of these rhinoceros in Nepal was 645, including 605 animals living in Chitwan National Park (CNP) (*5*).

On February 16, 2015, CNP officials observed a sick female rhinoceros in the buffer zone of the western sector of the park near Amaltari. The rhinoceros was dull, depressed, and not feeding. The following day, the animal was found dead in the same area (online Technical Appendix Figure 1, http://wwwnc.cdc.gov/EID/article/22/3/15-1929-Techapp1.pdf). Superficial maggot-infested wounds were on both sides of the vulva, indicating that the rhinoceros was not able to naturally remove the maggots and suggesting that the animal was sick for some time. During the necropsy, several granulomatous lesions were observed in the lungs and considered to be compatible with TB infection. The lesions were extensively distributed and well encapsulated and contained caseous necrotic material (online Technical Appendix Figure 2). No other pathologic changes were observed in any of the organs examined, leading to the conclusion that the rhinoceros died from TB.

A lung tissue sample positive for TB by acid-fast staining was cultured on Lowenstein-Jensen media. We performed spoligotyping and mycobacterial interspersed repetitive units–variable-number tandem-repeat (MIRU-VNTR) procedure on the isolate as previously described (*6*,*7*). Spoligotyping analysis, performed as previously described (*2*), showed that the isolate had a spoligo–international type 587 pattern, indicating it was *M. orygis*. We also performed multilocus sequence typing on various genes (*2*), and confirmed that the isolate was *M. orygis*. We then constructed a dendrogram by comparing the MIRU-VNTR result from rhinoceros isolate with published *M. orygis* MIRU-VNTR types (Figure) (*1*,*2*,*8*). The rhinoceros *M. orygis* isolate fell in a unique position in the dendrogram; we identified a difference in only 1 locus (MIRU 424) when we compared the isolate with the largest cluster of reported *M. orygis* isolates, including those previously reported from Nepal.

In our earlier study (*2*), we isolated *M. orygis* from chital deer (*Axis axis*) and blue bull (*Boselaphus tragocamelus*) from a captive wild-animal facility and postulated that the origin of the infection might be from infected animals in CNP, where the deer and blue bull originated. This new finding of a different strain type of *M. orygis* in a free-ranging rhinoceros in CNP provides evidence for our hypothesis. Other reports of *M. orygis* in captive wild animals in Nepal (*2*), cattle and a rhesus monkey in Bangladesh (*1*), humans in South Asia (*1*), and an immigrant from India in New Zealand (*9*) further support this bacterium's potential widespread distribution in South Asia and attests to the One Health significance of this organism.

In a demographic study of rhinoceros in Nepal (*10*), the animals were found to be living in a narrow area of



Isolates																						
Source	Geographic origin	154	424	577	580	802	960	1644	1955	2059	2163b	2165	2401	2461	2531	2687	2996	3007	3192	3690	4052	4156	4348
Oryx*	The Netherlands	2	2	5	3	2	7	4	3	2	×	×	4	2	4	1	4	3	4	4	2	3	2
Human*	South Asia	2	2	5	3	2	7	4	3	2	×	×	4	2	4	1	4	3	4	4	2	3	2
African buffalo†	South Africa	2	2	5	3	2	7	4	3	2	×	2	4	2	4	1	4	3	4	4	2	3	2
Deer‡	Nepal	2	2	5	3	2	7	4	3	2	×	×	4	2	4	1	4	3	4	4	2	3	2
Blue bull‡	Nepal	2	2	5	3	2	7	4	3	2	×	×	4	2	4	1	4	3	4	4	2	3	2
Rhinoceros§	Nepal	2	**1**	5	3	2	7	4	3	2	×	×	4	2	4	1	4	3	4	4	2	3	2
Monkey*	Bangladesh	2	2	5	3	2	6	4	3	2	×	×	4	2	4	1	4	3	4	4	2	3	2
Cow*	Bangladesh	2	2	5	3	2	6	4	3	2	×	×	4	2	4	1	4	3	4	4	2	3	2
Human*	South Asia	2	2	5	3	3	7	4	3	2	×	×	4	2	4	1	4	3	4	4	2	3	2
Human*	Southeast Asia	2	2	5	3	2	7	4	3	1	×	3	4	2	4	1	4	3	3	4	2	3	2
Human*	South Asia	2	2	5	3	2	7	4	3	2	×	×	4	2	4	1	4	3	3	4	2	3	2
Human*	South Asia	2	2	5	3	2	7	4	3	1	×	×	5	2	4	1	4	3	4	4	2	3	2
Human*	South Asia	2	2	6	3	2	9	4	3	2	×	×	4	2	4	1	4	3	4	4	2	3	2

Figure. Phylogeny of *Mycobacterium orygis* isolates as determine on the basis of mycobacterial interspersed repetitive units–variable-number tandem-repeat (MIRU-VNTR) results of 22 loci. The unweighted pair group method with arithmetic mean dendrogram was drawn by using MIRU-VNTR*plus* software (http://www.miru-vntrplus.org). The order of MIRU-VNTR is as follows, left to right: 154, 424, 577, 580, 802, 960, 1644, 1955, 2059, 2163b, 2165, 2401, 2461, 2531, 2687, 2996, 3007, 3192, 3690, 4052, 4156 and 4348. *Isolates from (*1*), †isolate from (*8*), ‡isolates from (*2*), §isolate from this study. Bold MIRU-VNTR copy number of locus 424 in rhinoceros isolate indicates a single locus difference in MIRU-VNTR type from the largest cluster. X, unamplifiable. Scale bar indicates genetic distance.

riverine grassland in CNP. A chronic and devastating disease like TB in this vulnerable and isolated population, which is already threatened from habitat destruction and poaching, is a matter of great conservation concern for the animal's long-term survivability. Also, CNP is listed by the United Nations Educational, Scientific and Cultural Organization as a World Heritage site because of its rich biodiversity and as an important habitat for endangered animals, including Bengal tigers (*Panthera tigris*) and Asian elephants (*Elephas maximus*). Thus, *M. orygis*–associated TB in rhinoceros in CNP may also indicate a threat to other animals, including some that are endangered. There is a strong possibility of unknown maintenance hosts of *M. orygis* in and around the national park. Our findings support the need for further investigation to understand the ecology and epidemiology of *M. orygis* and provide justification for active surveillance of this bacterium in animals in the national park and in livestock and humans in the buffer-zone areas. Furthermore, the increasing evidence for widespread distribution of *M. orygis* in South Asia provides a new picture of TB and may lead to a new understanding of *M. tuberculosis* complex.

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Address for correspondence: Chie Nakajima, Division of Bioresources, Hokkaido University Research Center for Zoonosis Control, Kita 20 Nishi 10, Kita-ku, Sapporo 001-0020, Japan; email: cnakajim@czc.hokudai.ac.jp

Another Dimension

Emerging Infectious Diseases accepts 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.

Cristóbal Rojas (1857–1890), *La muerte de Girardot en Bárbula* (*The death of Girardot in Bárbula*), 1883. Oil on canvas, 113.9 in × 85.4 in/287 cm × 217 cm. Public domain digital image (copyright expired)

Depictions of Heroism in Battle and Anguish from Tuberculosis

Terence Chorba and Byron Breedlove

Venezuelan artist Cristóbal Rojas was born in Cúa in the Valles de Tuy, a town that was war-torn throughout his early childhood. His grandfather, José Luis Rojas, taught the child how to draw, but when Cristóbal was 13, his father died, and he began work in a tobacco factory to help support his family. In 1878, an earthquake destroyed much of the Valles del Tuy region, and Rojas moved to Caracas, where he continued his artistic development under José Manuel Maucó at the Universidad Central de Venezuela. From 1880 through 1882, Rojas developed an interest in oil painting, and he captured memories of the impact of the 1878 earthquake in some of his early paintings. In Caracas, he also served as the assistant to the now comparably well-known artist Antonio Herrera Toro and worked with him on painting the interior of the Caracas Cathedral.

In 1883, Cristóbal Rojas exhibited one of his signature works, *La muerte de Girardot en Bárbula* (*The death of Girardot in Bárbula*), featured as this month's cover

Author affiliation: Centers for Disease Control and Prevention, Atlanta, Georgia, USA

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image. He entered this painting into competition in the Salón del Centenario in Caracas, to commemorate the centennial of the birth of Simon Bolivar on whose side Rojas' great grandfather had fought in the War for Independence; the painting remains his only heroic or patriotic work. This dramatic painting depicts an event in 1813 when the Colombian hero, Anastasio Girardot, a close confidant and supporter of Bolivar, was defeated by Spanish forces during the battle of Bárbula. Rojas shows the mortally wounded colonel falling backward as the flag curls down around him dominates the scene. Flanked by his fallen comrades, the stricken Girardot collapses, struck by a fatal shot as he attempts to raise the flag of the Republic. The painting was immediately purchased by the Government of Venezuela, and its execution won Rojas a medal and a scholarship from the government to study in France.

Rojas moved to Paris in late 1883. There he lived in Montmartre, spent much of his time studying in the Louvre, and began experimenting with different stylistic approaches and elements, from neo-Classical to Romanticism to Impressionism. Among his greatest works are renowned melancholic masterpieces that reflect suffering and early death, often from tuberculosis (TB), which was more common in that period. These works include *La miseria* (Misery, 1886, a desolate scene of a young husband sitting next to his supine wife who has just died in an impoverished setting) and *La primera y última comunión* (The first and last communion, 1888, a haunting scene of a priest administering the sacrament for the first time to a young girl who is dying in her mother's arms). Art historian Vivian Barclay has remarked that whereas Rojas' "Venezuelan audience wanted to see heroism and patriotism ... his Parisian audience wanted sadness and melodrama." Having TB himself, Rojas returned to Venezuela from France, and cognizant of his own impending death, he completed his final work, *El purgatorio*, a depiction of purgatory. He began the painting while in France but completed it in Venezuela in 1890. At the end of that year, when Rojas died of TB at age 32, nearly one third (30%) of all deaths in Paris were attributed to TB.

In France and Venezuela, the incidence of TB was much higher in the 19th century than it is today. However, actual incidence numbers are crude estimates, as it was not until 24 March 1882—a year before the exhibition of *La muerte de Girardot en Bárbula*—that Robert Koch identified the tubercle bacillus as the etiologic agent. In Venezuela today, the average national incidence of TB is moderate, more than 27 cases per 100,000 persons. Despite recent advances in TB care and control that have helped lower TB incidence in Venezuela, great disparities exist in the incidence of TB across different segments of the population, revealing inequities that are prominent in most Latin American countries. Disability and death due to TB continue to have major economic and social implications for areas of high endemicity, where economic and disease issues take on a chicken-and-egg type of relationship.

Although TB was romanticized in the literature and music of the 19th century, a few studies from the pre-antimicrobial era enable us to estimate that the lifetime case-fatality rate of smear-positive pulmonary cases was more than 70%. Today, globally, TB is responsible for more than 9 million new cases of active disease and 1.5 million deaths annually, or a death rate of more than 16%. However, since the 1990s, the emergence of multidrug resistance (MDR) in *Mycobacterium tuberculosis*, principally in the developing world and in the former Soviet Union, is reflected now in an estimated 480,000 incident MDR TB cases per year, of which only about a quarter are detected and reported. Case-fatality rates among the growing population of patients with MDR TB approximate those of TB in general seen in the pre-antimicrobial era (the time of Cristóbal Rojas' death). If drug resistance increases substantially, TB elimination will become more difficult to achieve. To regain the lost ground, it is important that health professionals engage in informed and timely approaches to diagnosis, treatment, and prevention and that there be expansion of testing for TB drug susceptibility and HIV, provision of antiretroviral treatment, reevaluation of existing drugs for their anti-TB potential, and development of a greater number and variety of antimicrobials targeting TB.

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Address for correspondence: Byron Breedlove, EID Journal, Centers for Disease Control and Prevention, 1600 Clifton Rd NE, Mailstop C19, Atlanta, GA 30329-4027, USA; email: wbb1@cdc.gov

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- Microevolution of Monophasic *Salmonella* Typhimurium during Epidemic, United Kingdom
- Domestically Transmitted Shiga Toxin 1–producing *Shigella sonnei*, California, United States, 2014–2015
- Exportations of Symptomatic Cases of MERS-CoV Infection to Countries outside the Middle East C. Carias et al.
- Definitive Hosts of *Versteria* Species (Cestoda: Taeniidae) Causing Fatal Infection in North America
- Severe Infections with Adenovirus 7d in 2 Adults in Family, Illinois, USA, 2014
- Deletion Variants of Middle East Respiratory Syndrome Coronavirus from Humans, Jordan, 2015
- Arenavirus Diversity among Phylogroups of *Mastomys natalensis* Rodents, Nigeria
- *Neisseria meningitidis* Serogroup X in sub-Saharan Africa
- Hypervirulent *emm*59 Clone Identified in Invasive Group A *Streptococcus* Outbreak, Southwestern United States
- Morbillivirus and Pilot Whale Deaths, Canary Islands, Spain, 2015
- Porcine Deltacoronavirus, Thailand, 2015
- Ebola Virus in Breast Milk in an Ebola Virus–Positive Mother with Twin Babies, Guinea, 2015
- Follow-up of Ebola Patient, 2014–2015
- New Delhi Metallo-β-Lactamase-1–Producing *Klebsiella pneumoniae*, Florida, USA
- Sustained Elevated Cytokine Levels during Recovery Phase of Mayaro Virus Infection
- High Hepatitis E Virus Seroprevalence in Blood Donor Population, Ouagadougou, Burkina Faso

Complete list of articles in the April issue at http://www.cdc.gov/eid/upcoming.htm

Upcoming Infectious Disease Activities

March 2–5, 2016
ISID
17th International Congress on Infectious Diseases
Hyderabad, India
http://www.isid.org/icid/

April 18–20, 2016
19th Annual Conference on Vaccine Research
Baltimore, MD, USA
http://www.cvent.com/events/19th-annual-conference-on-vaccine-research/event-summary-9c2a6b5301a64921afbd9c07a4cffa14.aspx?refid=spcoc

May 18–21, 2016
The Society for Healthcare Epidemiology of America
Atlanta, GA, USA
http://www.shea-online.org/Education/SHEASpring2016Conference.aspx

June 16–20, 2016
American Society for Microbiology
Boston, MA, USA
http://www.asmmicrobe.org/

July 18–22, 2016
21st International AIDS Conference
Durban, South Africa
http://www.aids2016.org/

October 29–November 2, 2016
American Public Health Association
Denver, Colorado, USA
https://www.apha.org/events-and-meetings/annual/past-and-future-annual-meetings

December 3–8, 2016
ASLM
African Society for Laboratory Medicine
Cape Town, South Africa
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Article Title

Patient Report and Review of Rapidly Growing Mycobacterial Infection after Cardiac Device Implantation

CME Questions

1. Your patient is a 65-year-old man recently implanted with a cardiac implantable electronic device (CIED) in whom mycobacterial infection is suspected. According to the case report and review by Phadke and colleagues, which of the following statements about the clinical and etiologic considerations regarding mycobacterial CIED infections is correct?

A. Most mycobacterial CIED infections are slow growing
B. CIED infections are not usually evident until at least 1 year after last manipulation
C. Device-related endocarditis has not been reported
D. Of 33 cases, 23 (70%) were due to rapidly growing mycobacteria (RGM) species including *Mycobacterium fortuitum*, 8 from *M. tuberculosis* complex, and 2 from *M. avium* complex

2. According to the case report and review by Phadke and colleagues, which of the following statements about antibiotic resistance of CIED infections due to RGM is correct?

A. Extended incubation of routine cultures or dedicated mycobacterial culture is unnecessary
B. The Clinical and Laboratory Standards Institute recommends routine broth microdilution susceptibility testing of all RGM isolates against amikacin, cefoxitin, ciprofloxacin, clarithromycin, doxycycline, imipenem, and sulfamethoxazole
C. Current guidelines recommend therapy with only 1 drug for a period of several weeks for optimal results
D. The *M. fortuitum* group is usually thought to be the most antibiotic resistant

3. According to the case report and review by Phadke and colleagues, which of the following statements about management of CIED infections due to RGM is correct?

A. Macrolide-based regimens are the recommended therapy of choice for serious *M. fortuitum* infection
B. Device removal is unnecessary
C. Clinicians should be aware of the arrhythmogenicity of the antimicrobials traditionally recommended for infections arising from these organisms
D. Newer antimicrobials have even greater direct arrhythmogenic effects

Activity Evaluation

1. The activity supported the learning objectives.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
2. The material was organized clearly for learning to occur.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
3. The content learned from this activity will impact my practice.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
4. The activity was presented objectively and free of commercial bias.				
Strongly Disagree				Strongly Agree
1	2	3	4	5

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Article Title

Epidemiology of Histoplasmosis Outbreaks, United States, 1938–2013

CME Questions

1. You are advising a state health department regarding the potential for a histoplasmosis outbreak. According to the literature review by Benedict and Mody, which of the following statements about epidemiologic features of reported US histoplasmosis outbreaks during 1938–2013 is correct?

A. During 1938–2013, there were 105 reported US outbreaks involving a total of 2,850 cases
B. Outbreaks were reported in all 50 states and in Puerto Rico
C. No outbreaks occurred in Florida, which has a low level of endemicity
D. The findings are not likely to underestimate the number of histoplasmosis outbreaks

2. According to the literature review by Benedict and Mody, which of the following statements about risk factors associated with reported US histoplasmosis outbreaks during 1938–2013 is correct?

A. Workplace exposures accounted for the majority of outbreaks
B. Only major environmental disturbances, such as excavation or clearing foliage, were associated with outbreaks
C. Cases were not reported among persons who did not participate directly in the outbreak-initiating activities
D. Birds, bats, or their droppings were reported to be present in 77% of outbreak settings

3. According to the literature review by Benedict and Mody, which of the following statements about clinical features and outcomes in reported US histoplasmosis outbreaks during 1938–2013 is correct?

A. Respiratory tract symptoms always predominate
B. Nonspecific symptoms of fever, cough, headache, fatigue, and chest pain can persist for weeks to months
C. Histoplasma infection is easy to diagnose and outbreaks are clearly apparent
D. Approximately one-quarter of acute cases are fatal

Activity Evaluation

1. The activity supported the learning objectives.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
2. The material was organized clearly for learning to occur.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
3. The content learned from this activity will impact my practice.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
4. The activity was presented objectively and free of commercial bias.				
Strongly Disagree				Strongly Agree
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Article Title

Tuberculosis Caused by *Mycobacterium africanum*, United States, 2004–2013

CME Questions

1. You are evaluating a 40-year-old man with a severe cough and weight loss for 3 months. Results on chest x-ray suggest that he has tuberculosis. What is the percentage of cases of clinical tuberculosis caused by *Mycobacterium africanum* in the current study of patients in the United States?

A. 0.4%
B. 2.9%
C. 6.5%
D. 12.0%

2. What should you consider regarding the epidemiology and phylogenetics of *M. africanum* infection in the current study?

A. Nearly all cases were L5, and only a small percentage were L6
B. Approximately 30% of patients affected were born outside of the United States
C. Most cases were reported among farm workers in rural areas
D. Most cases were not part of clusters

3. After full adjustment for patient factors, which of the following descriptions was a clinical characteristic of patients with *M. africanum* infection compared with those with *M. tuberculosis* in the current study?

A. *M. africanum* was more common among patients with HIV infection
B. *M. africanum* was more commonly associated with extrapulmonary disease only
C. *M. africanum* was less likely to produce cavitary lesions on chest x-ray
D. *M. africanum* and *M. tuberculosis* had similar clinical presentations

4. Which of the following variables was a risk factor for *M. africanum* vs. *M. tuberculosis* infection in the current study?

A. Age 65 years or older
B. Homelessness
C. Excessive alcohol use
D. Black race

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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- New infections resulting from changes or evolution of existing organisms.
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Why an "Emerging" Infectious Diseases journal?

The Centers for Disease Control and Prevention (CDC), the agency of the U.S. Public Health Service charged with disease prevention and health promotion, leads efforts against emerging infections, from AIDS, hantavirus pulmonary syndrome, and avian flu, to tuberculosis and West Nile virus infection. CDC's efforts encompass improvements in disease surveillance, the public health infrastructure, and epidemiologic and laboratory training.

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   - Reports laboratory and epidemiologic findings within a broader public health perspective.
   - Provides swift updates of infectious disease trends and research: new methods of detecting, characterizing, or subtyping pathogens; developments in antimicrobial drugs, vaccines, and prevention or elimination programs; case reports.

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   - Encourages insightful analysis and commentary, stimulating global interest in and discussion of emerging infectious disease issues.
   - Harnesses electronic technology to expedite and enhance global dissemination of emerging infectious disease information.

Emerging Infectious Diseases is a peer-reviewed journal established expressly to promote the recognition of new and reemerging infectious diseases around the world and improve the understanding of factors involved in disease emergence, prevention, and elimination.

The journal is intended for professionals in infectious diseases and related sciences. We welcome contributions from infectious disease specialists in academia, industry, clinical practice, and public health, as well as from specialists in economics, social sciences, and other disciplines. Manuscripts in all categories should explain the contents in public health terms. For information on manuscript categories and suitability of proposed articles, see below and visit http://wwwnc.cdc.gov/eid/pages/author-resource-center.htm.

Summary of Authors' Instructions

Author's Instructions. For a complete list of EID's manuscript guidelines, see the author resource page: http://wwwnc.cdc.gov/eid/page/author-resource-center.

Manuscript Submission. To submit a manuscript, access Manuscript Central from the Emerging Infectious Diseases web page (www.cdc.gov/eid). Include a cover letter indicating the proposed category of the article (e.g., Research, Dispatch), verifying the word and reference counts, and confirming that the final manuscript has been seen and approved by all authors. Complete provided Authors Checklist.

Manuscript Preparation. For word processing, use MS Word. Set the document to show continuous line numbers. List the following information in this order: title page, article summary line, keywords, abstract, text, acknowledgments, biographical sketch, references, tables, and figure legends. Appendix materials and figures should be in separate files.

Title Page. Give complete information about each author (i.e., full name, graduate degree(s), affiliation, and the name of the institution in which the work was done). Clearly identify the corresponding author and provide that author's mailing address (include phone number, fax number, and email address). Include separate word counts for abstract and text.

Keywords. Use terms as listed in the National Library of Medicine Medical Subject Headings index (www.ncbi.nlm.nih.gov/mesh).

Text. Double-space everything, including the title page, abstract, references, tables, and figure legends. Indent paragraphs; leave no extra space between paragraphs. After a period, leave only one space before beginning the next sentence. Use 12-point Times New Roman font and format with ragged right margins (left align). Italicize (rather than underline) scientific names when needed.

Biographical Sketch. Include a short biographical sketch of the first author—both authors if only two. Include affiliations and the author's primary research interests.

References. Follow Uniform Requirements (www.icmje.org/index.html). Do not use endnotes for references. Place reference numbers in parentheses, not superscripts. Number citations in order of appearance (including in text, figures, and tables). Cite personal communications, unpublished data, and manuscripts in preparation or submitted for publication in parentheses in text. Consult List of Journals Indexed in Index Medicus for accepted journal abbreviations; if a journal is not listed, spell out the journal title. List the first six authors followed by "et al." Do not cite references in the abstract.

Tables. Provide tables within the manuscript file, not as separate files. Use the MS Word table tool, no columns, tabs, spaces, or other programs. Footnote any use of boldface. Tables should be no wider than 17 cm. Condense or divide larger tables. Extensive tables may be made available online only.

Figures. Submit editable figures as separate files (e.g., Microsoft Excel, PowerPoint). Photographs should be submitted as high-resolution (600 dpi) .tif or .jpeg files. Do not embed figures in the manuscript file. Use Arial 10 pt. or 12 pt. font for lettering so that figures, symbols, lettering, and numbering can remain legible when reduced to print size. Place figure keys within the figure. Figure legends should be placed at the end of the manuscript file.

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Types of Articles

Perspectives. Articles should not exceed 3,500 words and 40 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. Articles should provide insightful analysis and commentary about new and reemerging infectious diseases and related issues. Perspectives may address factors known to influence the emergence of diseases, including microbial adaptation and change, human demographics and behavior, technology and industry, economic development and land use, international travel and commerce, and the breakdown of public health measures.

Synopses. Articles should not exceed 3,500 words in the main body of the text or include more than 40 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (not to exceed 150 words), a 1-line summary of the conclusions, and a brief biographical sketch of first author or of both authors if only 2 authors. This section comprises case series papers and 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. If detailed methods are included, a separate section on experimental procedures should immediately follow the body of the text.

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.

In This Issue

Perspective

Synopses

Research

Dispatches

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