Synopses

Surveillance of Leprosy in Kiribati, 1935–2017
S.T. Chambers et al. 833

Biphasic Outbreak of Invasive Group A Streptococcus Disease in Eldercare Facility, New Zealand
K.A. Worthing et al. 841

Epidemiology of Tick-Borne Relapsing Fever in Endemic Area, Spain
M.C. Domínguez et al. 849

Food Safety and Invasive Cronobacter Infections during Early Infancy, 1961–2018
J. Strysko et al. 857

Contaminated powdered infant formula from opened containers is the most commonly identified transmission vehicle.

Clinical Outcomes of Patients Treated for Candida auris Infections in a Multisite Health System, Illinois, USA
K. Arensman et al. 866

Mosquito Control Activities During Local Transmission of Zika Virus, Miami-Dade County, Florida, USA, 2016
J.C. McAllister et al. 872

Research

Blastomycosis in Minnesota, USA, 1999–2018
M. Ireland et al. 881
Providers should consider a blastomycosis diagnosis for pneumonia patients who have been in disease-endemic areas.

Effectiveness of Live Poultry Market Interventions on Human Infection with Avian Influenza A(H7N9) Virus, China
W. Wang et al. 891

E. Rogier et al. 902

Systematic Review and Meta-Analysis of Sex Differences in Social Contact Patterns with Implications for Tuberculosis Transmission and Control
K.C. Horton et al. 910

Effects of Air Pollution and Other Environmental Exposures on Estimates of Severe Influenza Illness, Washington, USA
R. Somayaji et al. 920

Epidemiologic and Clinical Progression of Lobomycosis among Kaiabi Indians, Brazil, 1965–2019
M.C. Florian et al. 930

Rhizopus microsporus Infections Associated with Surgical Procedures, Argentina, 2006–2014
J.R. Bowers et al. 937
Zika Virus Circulation in Mali
I. Diarra et al. 945

Possible Transmission Mechanisms of Mixed Mycobacterium tuberculosis Infection in High HIV Prevalence Country, Botswana
Y. Baik et al. 953

Policy Reviews

Nonpharmaceutical Measures for Pandemic Influenza in Nonhealthcare Settings—International Travel–Related Measures
S. Ryu et al. 961

Nonpharmaceutical Measures for Pandemic Influenza in Nonhealthcare Settings—Personal Protective and Environmental Measures
J. Xiao et al. 967

Nonpharmaceutical Measures for Pandemic Influenza in Nonhealthcare Settings—Social Distancing Measures
M.W. Fong et al. 976

Dispatches

Candidatus Rickettsia xinyangensis as Cause of Spotted Fever Group Rickettsiosis, Xinyang, China, 2015
H. Li et al. 985

Pretreatment Out-of-Pocket Expenses for Presumptive Multidrug-Resistant Tuberculosis Patients, India, 2016–2017
P. Rathi et al. 989

Capybara and Brush Cutter Involvement in Q Fever Outbreak in Remote Area of Amazon Rain Forest, French Guiana, 2014
J.R. Christen et al. 993

Research Letters

Crimean-Congo Hemorrhagic Fever Virus Endemicity in United Arab Emirates, 2019
J.V. Camp et al. 1019

Zika Inquiries Made to the CDC-INFO System, December 2015–September 2017
T.K. Sell et al. 1022
Serologic Detection of Middle East Respiratory Syndrome Coronavirus Functional Antibodies
N.M.A. Okba et al. 1024

Novel Ehrlichia Strain Infecting Cattle Tick Amblyomma neumanni, Argentina, 2018
L. Fargnoli et al. 1027

Multidrug-Resistant Salmonella Serotype Anatum in Travelers and Seafood Imported from Asia, United States
B.E. Karp et al. 1030

Fatal Rodentborne Leptospirosis in Prison Inmates, South Africa, 2015
K. Naidoo et al. 1033

Diplorickettsia Bacteria in an Ixodes scapularis Tick, Vermont, USA
C. Merenstein et al. 1036

Case of Babesia crassa-Like Infection, Slovenia, 2014
K.S. Smrdel et al. 1038

Hepatitis A Hospitalization Costs, United States, 2016
M.G. Hofmeister et al. 1040

S. Grech-Angelini et al. 1041

Rise in Murine Typhus in Galveston County, Texas, USA, 2018
K. Ruiz et al. 1044

Human Adenovirus 7d Strains Associated with Influenza-Like Illness, New York, USA, 2017–2019
D.M. Lamson et al. 1047

Risk for Transportation of Coronavirus Disease from Wuhan to Other Cities in China
Z. Du et al. 1049

Potential Presymptomatic Transmission of SARS-CoV-2, Zhejiang Province, China, January, 2020
Z.-D. Tong et al. 1052

Book Review
Superbugs: The Race to Stop an Epidemic
A.F. Read 1055

About the Cover
Auspicious Symbols of Rank and Status
B. Breedlove, I.C.-H. Fung 1056

Etymologia
Coronavirus
R. Henry 1027
Yellow Book 2020 includes important travel medicine updates

- The latest information on emerging infectious disease threats, such as Zika, Ebola, and henipaviruses
- Considerations for treating infectious diseases in the face of increasing antimicrobial resistance
- Legal issues facing clinicians who provide travel health care
- Special considerations for unique types of travel, such as wilderness expeditions, work-related travel, and study abroad

The fully revised and updated CDC Yellow Book 2020: Health Information for International Travel codifies the US government’s most current health guidelines and information for clinicians advising international travelers, including pretravel vaccine recommendations, destination-specific health advice, and easy-to-reference maps, tables, and charts.
Leprosy (also called Hansen’s disease) was well established in Kiribati by the early 20th century, possibly as a result of contact with Western and Chinese traders (1,2). Colonial records indicate that there were 28 known cases in 1925, when the population was ≈31,000. Kiribati, formerly the Gilbert Islands, is a country of 33 atolls, 21 of which are inhabited, spread over >1 million square miles of ocean. The country covers an area on both sides of the International Date Line and north and south of the equator. The islands became a British colony in 1916, were occupied by Japan during 1942–1943, and became an independent country in 1979. The population in the 2015 census was 110,136, with the main population located in South Tarawa (39,058 persons) and Betio (17,330 persons) (3). Betio, an islet with a deepwater port, is connected to South Tarawa by a causeway.

Leprosy, caused by the bacterium *Mycobacterium leprae*, is a chronic disease with an indolent onset, resulting in a long period between the manifestation of the disease and the person seeking healthcare (4). Its 2 clinical forms, paucibacillary disease (PB) and multibacillary disease (MB), may have long-term consequences if untreated and can result in peripheral nerve damage, chronic ulceration, blindness, and facial disfigurement, as well as social isolation and family discord. Complications are more common in MB leprosy (5,6).

Humans are the main reservoir of *M. leprae*. The primary mode of transmission is understood to be person to person by the respiratory route, but this route has not been proven conclusively (7). Patients with MB disease excrete *M. leprae* from their nasal mucosa and skin. Persons most at risk are close household contacts of those with MB, but social contacts are also at risk. Social and economic factors play a role in transmission (8). Poverty, undernutrition, crowding, and rapid uncontrolled internal migration have been associated with high rates of leprosy (9). Higher rates of leprosy were found in households of >7 persons than those with <4 persons and in homes in which >2 shared a bedroom (10,11).

The First International Leprosy Conference, held in Berlin in 1897, adopted segregation as the global response to the threat of leprosy; it was commonly used by colonial governments (12). Newly diagnosed patients with leprosy were initially isolated in Kiribati before they were transported to the leprosy isolation island, Makogai, in the Fijian archipelago. Patients from Kiribati were first admitted to Makogai in 1937, although the isolation facility began accepting patients by 1911. Dapsone, the first effective drug to
treat leprosy, became available in 1945 and enabled patients on Makogai to be treated and repatriated. The leprosy isolation facility was closed in 1969. Multidrug therapy (MDT) consisting of rifampin, dapsone, and clofazamine was successfully introduced to Kiribati by 1990 (13).

Optimism about the efficacy of MDT and evidence of leprosy control led the 44th World Health Assembly to adopt resolution WHA44.9 in May 1991 to eliminate leprosy as a public health problem by the year 2000 (14). The elimination target was defined as a prevalence of <1 case/10,000 population. For calculating prevalence, cases were defined as patients registered for MDT treatment, which reflected the burden of disease. Concern that Kiribati would not meet the elimination goal was raised in 1996 when leprosy was found to be highly endemic to Kiribati, and mass screening of the whole population was conducted in 1997 and repeated in 1998, supported by the World Health Organization (15). Chemoprophylaxis (single-dose rifampicin, ofloxacin, and minocycline) was administered to the population of South Tarawa, including Betio, and Christmas Island (15). Reported cases fell following this intervention, and Kiribati reached the elimination goal in 2000 (prevalence 0.94 cases/10,000 population). However, prevalence has since rebounded above elimination levels, with high numbers of new cases among children, a marker of transmission (16). Leprosy has also been identified among Kiribati nationals who have moved to neighboring countries including the Solomon Islands, Fiji, and New Zealand (4). Leprosy control is recognized as a priority by the Government of Kiribati (17). We describe the rates of new cases of leprosy from historic and recent medical records to document emergence and transmission of leprosy in Kiribati.

Methods
We aimed to use the most reliable surveillance data sources available for this study. One source was the records of patients admitted to Makogai isolation island in Fiji. Patients with presumed leprosy were identified in Kiribati and sent to Makogai, where a leprologist examined them and validated the diagnosis. Those with leprosy were then interned on Makogai. After the isolation facility was closed, the case records of all patients were transported to the Patrick Twomey Memorial Leprosy Hospital in Suva, Fiji, where they were stored and later entered into an electronic database.

A second source of information was the medical records of the National Leprosy Unit of Kiribati, which is located in the only secondary medical facility in Kiribati. All cases of leprosy in Kiribati are referred to this center, which is responsible for validating cases, reviewing complex cases, and ensuring medication is sent to patients across the country and which maintains clinical records.

Population Statistics

Case Definition
A case of leprosy is defined as illness in a person who has ≥1 features and who has not completed a full course of treatment. The features are the following: hypopigmented or reddish lesions with definite loss of skin sensation; involvement of peripheral nerves; as demonstrated by definite thickening with definite loss of skin sensation; and detection of acid-fast bacilli in the skin by biopsy or slit skin smear (19). Cases were classified into PB disease and MB disease in accordance with WHO criteria. Grade 2 disability was defined as visible deformity or damage present in the hands or feet or severe visual impairment (vision worse than 6/60; inability to count fingers at 6 m; lagophthalmos, iridocyclitis, or corneal opacities) (19,20).

Record Sources
We (J.B.) compiled, checked, and locked an electronic database from the medical records of case-patients from Kiribati admitted to Makogai (1935–1964). The records have been held at the Twomey Leprosy Hospital in Suva, Fiji, by the Pacific Leprosy Foundation (PLF), a nonprofit organization that supports leprosy prevention, treatment, and patient welfare work around the Pacific, working in partnership with the Ministries of Health under a memorandum of understanding.

We obtained the case registers from the National Leprosy Unit (NLU) at the Nawerewere hospital in Kiribati and entered information on cases from 1988–2010 into a separate database. We (S.T.C.) checked the accuracy of data entry (90%). In 2010, staff at the NLU began entering data prospectively. We backed up this database to cloud-based storage weekly, and the PLF checked for completeness, double entries, and other errors. In addition, PLF staff review the data on regular site visits. These records are the source documents for WHO reports.
To diagnose leprosy, the medical and nursing staff of the NLU perform clinical examination of patients in North and South Tarawa in person, and by radio consultation with medical assistants and nurses on the outer islands. In 1997–1998, all cases were seen and validated by leprologists involved in a nationwide screening program that covered 92.2% of the population (15). In 2007–2017, a consultant leprologist supported by the PLF validated cases during regular visits.

**Intensification of Case Finding**

Because of concern that the number of new cases was increasing, we intensified the control program beginning in 2008. A consultant leprologist (A.C.) visited Kiribati every 3–4 months and conducted regular educational workshops for medical assistants, nurses, and staff in the NLU.

We introduced active case finding for 1 year in 2010, followed by ongoing publicity campaigns that included visits to village meeting houses by drama groups, radio advertising, and a song recorded by a local musician. Since 2015, health promotion activities have been intensified; we held free dermatology clinics in areas of apparent high leprosy case load in South Tarawa and Betio. Active screening of household contacts of leprosy patients began in 2016.

**Statistical Methods**

We performed statistical analyses using Microsoft Excel (Microsoft, https://www.microsoft.com) and R statistical software (21). We summarized cases by year, age, and clinical form. We calculated crude rates with 95% Wilson binomial CIs by dividing counts by the population estimates obtained from the World Bank. We standardized rates by age, using 5-year categories, to the WHO’s world 2000–2025 population. We estimated incidence rate ratios comparing age and sex, stratified by clinical form and adjusting for year, using Poisson regression models.

**Ethics Considerations**

The Ministry of Health and Medical Services in Kiribati approved the study design. The ethics committee of the University of Otago, Christchurch, New Zealand, approved the study.

**Results**

**Population of Kiribati**

Kiribati has been experiencing rapid population growth and increasing concentration of people in South Tarawa and Betio, where the percentage of the population rose from 5% in 1947 to 51% in 2015 (Table 1) (3,18). We observed a corresponding increase in population density, but numbers per household were relatively stable over time (Table 2). The number of occupants per household was higher in South Tarawa and Betio than the outer islands of Kiribati. GDP per capita increased 300% between 1990 and 2015, but from a very low base (from US $550 to US $1,648; current dollars, World Bank data) (18).

**Cases from Makogai Case Register**

The database recorded 236 patients admitted to Makogai with leprosy. Of these, 87 were admitted during 1935–1940; another 5 during 1942–1946, during and immediately after World War II; and 141 during 1947–1954. The last 4 patients were admitted during 1956–1964, before admissions were stopped. Of the 236 cases, 67 were classified as cutaneous/tuberculoid, 121 as lepromatous, 47 as neural leprosy, and 1 as indeterminate. The Kiribati population was relatively stable from 1931–1940; another 5 during 1942–1946, during and immediately after World War II; and 141 during 1947–1954. The last 4 patients were admitted during 1956–1964, before admissions were stopped. Of the 236 cases, 67 were classified as cutaneous/tuberculoid, 121 as lepromatous, 47 as neural leprosy, and 1 as indeterminate. The Kiribati population was relatively stable from 1931–1947 at ≈30,000 persons, giving an annual new case rate of 3.9/10,000 population.

**Cases from the National Leprosy Unit Case Register, 1988–2017**

No case records were archived before 1988. During 1988–2017, a total of 2,287 new cases were reported in Kiribati, 1,242 (54%) of which were in male patients. A total of 757 cases (33%) were MB, and 750 (33%) were in patients <15 years of age. Of the MB case-patients, 63% were male. Grade 2 disability was reported in 46 (3%) of the 1,338 cases reported from 2009–2017; the data are inconsistent before 2009.

The large number of new cases reported in 1997 was because of the nationwide screening and treatment campaign (92.2% of the population), precipitated by the rise in cases seen in 1996 and the adoption of the WHO elimination target of a prevalence of <1/10,000 population by 2000 (Figure 1). South

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**Table 1. Population of Kiribati and the population centers of South Tarawa and Betio, 1931–2015**

<table>
<thead>
<tr>
<th>Census year</th>
<th>Kiribati population</th>
<th>South Tarawa and Betio population (% total population)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1931</td>
<td>29,751</td>
<td>3,013 (10)</td>
</tr>
<tr>
<td>1947</td>
<td>31,513</td>
<td>1,617 (5)</td>
</tr>
<tr>
<td>1963</td>
<td>43,336</td>
<td>6,109 (14)</td>
</tr>
<tr>
<td>1968</td>
<td>47,735</td>
<td>10,616 (22)</td>
</tr>
<tr>
<td>1973</td>
<td>51,926</td>
<td>14,861 (29)</td>
</tr>
<tr>
<td>1978</td>
<td>56,213</td>
<td>17,921 (32)</td>
</tr>
<tr>
<td>1985</td>
<td>63,883</td>
<td>21,393 (33)</td>
</tr>
<tr>
<td>1990</td>
<td>72,335</td>
<td>25,380 (35)</td>
</tr>
<tr>
<td>1995</td>
<td>77,658</td>
<td>28,350 (37)</td>
</tr>
<tr>
<td>2000</td>
<td>84,494</td>
<td>36,717 (43)</td>
</tr>
<tr>
<td>2005</td>
<td>92,533</td>
<td>40,331 (44)</td>
</tr>
<tr>
<td>2010</td>
<td>103,058</td>
<td>50,182 (49)</td>
</tr>
<tr>
<td>2015</td>
<td>110,110</td>
<td>56,324 (51)</td>
</tr>
</tbody>
</table>
Tarawa and Betio were screened again in 1998, covering 90.3% of the population. These efforts identified a large number of cases in South Tarawa and Betio rather than in the Outer Islands, which had previously been the site of most cases. The spike in reported cases in 2010 was from active case finding over that year. During 2009–2017, Betio and South Tarawa together contributed 786 (76%) of reported cases. The overall rate of both PB and MB leprosy rose with time, and the percentage of PB diagnosed increased in times of active surveillance and fell when surveillance was stopped.

The age distribution of MB and PB patients has remained relatively stable over time. We pooled the data to demonstrate the mean percentage of cases by age at diagnosis for MB and PB. Our findings showed...
that PB was diagnosed more frequently than MB in children <10 years of age and MB more frequently in patients 15–24 years of age (Figure 2). Changes in age-specific rates over time demonstrate that the rates of leprosy have been increasing in all age groups (Figure 3). Estimates from Poisson regression models suggested that incidence rates of MB in those 15–65 years of age were twice as high in male patients as in female patients (incidence rate ratio 2.1, 95% CI 1.7–2.4; p<0.001). We saw no difference in rates by sex for those with MB disease <15 years of age (incidence rate ratio 1.1, 95% CI 0.79–1.5; p = 0.59) and no difference in incidence rate ratio by sex for PB disease (p = 0.76).

The mean annual number of cases for a 5-year period for Kiribati has risen from 6.9/10,000 population in 1988–1993 to 15.2/10,000 population in 2013–2017 (Table 2). We grouped new diagnoses by location over 5-year periods using the census date as the center point; our analysis was limited because location was inconsistently recorded for the years 2007 and 2008, although age, sex, and classification data were complete. We omitted these 2 years from the location analysis (Table 2). The new case rate increased from 7.6/10,000 population/year in 1988–1992 to 27.0/10,000 population/year in 2013–2018 in Betio, from 7.3/10,000 population/year to 20.6/10,000 population/year in South Tarawa, and from 6.6/10,000 population/year to 7.4/10,000 population/year in the Outer Islands. The increase in case rate occurred at a similar time as the increase in overall population density, whereas the number of persons per household was stable.

**Discussion**

Control of leprosy in Kiribati has never been achieved. Initial control efforts by isolation of known leprosy cases in Kiribati were clearly documented in the medical records from Makogai; these records indicate a new case rate of ≈4/10,000 population/year in Kiribati. The incidence rate is almost certainly an underestimate given the stigma associated with leprosy and the tendency to avoid persons with leprosy, transport them away from their families, and isolate them on an island thousands of miles away from home. Despite the introduction of dapsone, closure of Makogai, and widespread use of MDT, the elimination of leprosy as a public health problem was only reached in 1999 and has not been maintained in Kiribati. Rather, the number of cases has continued to rise since 1999. The speed and scale of the increase in cases demonstrate the potential for case numbers to rebound.

Unsurprisingly, increased case finding efforts have identified more cases in Kiribati. Surveys in India and Brazil have similar findings, which has raised concerns that cases may remain undetected even in areas of apparently low endemicity (22–24). The intensive activity in 1997 of Daulako et al. was a landmark event; they screened >90% of the population of Kiribati (15). Although the number of new cases dropped dramatically after this intervention, temporarily reaching the elimination target, case numbers have steadily climbed since. A combination of factors, such as a belief that leprosy had reached the WHO elimination target and was therefore defeated, a temporary effect of single dose prophylaxis administered...
in 1998, lack of active case finding, and low-resource status, may have contributed to this change.

The increase in annualized rate of new cases beginning around 2009 is most notable in South Tarawa and Betio, but a rise was also reported from the outer islands. The number of cases reported in the outer islands may be an underestimate, given that the increased detection, treatment and control efforts have been focused on South Tarawa and Betio. The data suggest that the conditions for spread persist in the outer islands but may be worsening in South Tarawa and Betio because of internal migration and worsening of socioeconomic conditions (8–10).

The percentage of cases of PB disease and low reported percentage of disability are consistent throughout 1988–2017. The period includes the well-documented whole-population survey in which Daulako observed similar findings (15). The high percentage of PB disease and low percentage of disability found in Kiribati are consistent with reports from other Micronesia countries, such as the Marshall Islands and the Federated States of Micronesia, which share similar demographic and socioeconomic characteristics (16).

In Kiribati, 32% of all new case-patients during 1988–2017 were children. One of the highest reported worldwide, this rate indicates a failure to control transmission. Other reports of high national percentages among children, including those from the Marshall Islands (15/80, 19%), the Federated States of Micronesia (40/169, 24%), Papua New Guinea (89/356, 25%), and Solomon Islands (7/43, 16%), indicate that conditions for transmission are not limited to Kiribati but are widespread in other regions of the Western Pacific region (16).

The high rate of leprosy in Kiribati is probably related to the socioeconomic conditions, but these relationships are not well understood. Household crowding has been associated with high rates of leprosy in both Brazil and Indonesia (10,11). Households of more than 7 persons, which is common in Kiribati and particularly in Betio and South Tarawa, appear to be at risk for leprosy infection. High rates of disease are also reported in isolated populations and those marked by displacement and civil unrest that may increase crowding and poverty, both of which are associated with transmission of M. leprae (25,26). The marked increases in the populations in the urban
and semiurban areas of both South Tarawa and Betio have been driven by increased opportunities for work in Betio, with development of the port and light industry, and in South Tarawa, the location of central government and the international airport. Crowding caused by limited land availability and single-story homes has amplified the risk for spread of leprosy as well as other infections, such as tuberculosis, which is also reported at a high rate in Kiribati (27). These conditions may exacerbate deficiencies in healthcare services such that clinical infection remains unrecognized and untreated for prolonged periods.

Poor nutrition plays a role in susceptibility to leprosy. Case control studies in Brazil, India, and Bangladesh have identified food insecurity, intermittent starvation, and a lack of diversity in the diet as contributors to a high rate of leprosy (28–30). Pediatric undernutrition, maternal obesity, and micronutrient deficiencies are present in Kiribati. Children <5 years of age are particularly at risk; 34% are reported to have stunted growth and 37% to have anemia (31–32). A recent study demonstrated that low dietary diversity and a high prevalence of multiple micronutrient deficiencies were common in Kiribati (33).

Economic conditions in Kiribati, although improving, are rising from a low base; we expect to see substantial pressures on economic resources and land use associated with climate change and sea level rise, as well as population increase. These changes may have substantial effects on living standards and leprosy rates.

The limitations of our analysis include the potential for error in diagnosis, case recording, and data transcription. To mitigate the risk for errors, we have validated cases by a leprologist, recorded data prospectively, and checked the entered data against the case registers. Underdiagnosis of cases is likely but will have been reduced with active case finding to identify previously unsuspected cases. Overall, the changes in rates suggest that our observations are sufficiently robust to indicate real changes in the spread of leprosy in Kiribati.

In conclusion, the number of new cases and age-standardized rates of leprosy reported in Kiribati have risen over the past decade, despite the ready availability of MDT. The long incubation period for leprosy implies that it may reemerge and rates increase if conditions such as crowding worsen, if economic development is not achieved, or if leprosy services are poorly resourced. Reaching the WHO-specified elimination goal may be temporary without an ongoing commitment to comprehensive control programs over the long term (34–36). The introduction of postexposure prophylaxis to household contacts or to high-risk populations may offer a new tool to reduce the number of cases, the social consequences of stigma, and disability; this treatment has begun in several poorly resourced countries (37–40).

Acknowledgments
We thank Jill Tomlinson, Lala Gittoes, Teetitia Uriam, Kiteon Kabure, Apisalome Nakolinivalu, and Judy Baker. J.B. compiled the electronic database of medical records from patients isolated on Makogai.

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About the Author
Dr. Chambers is an infectious diseases physician at Christchurch Hospital, Christchurch, New Zealand, a professor of pathology at the University of Otago, Christchurch, and a board member of the Pacific Leprosy Foundation who convenes the Foundation’s medical committee. His primary research interests are community-acquired pneumonia, staphylococcal infections, legionellosis, and methyl group metabolism and leprosy.

References
SYNOPSIS


Address for correspondence: Stephen T. Chambers, University of Otago, Christchurch—Pathology, PO Box 4345, Christchurch 8140, New Zealand; email: steve.chambers@otago.ac.nz
Biphasic Outbreak of Invasive Group A *Streptococcus* Disease in Eldercare Facility, New Zealand

Kate A. Worthing, Anja Werno, Ramon Pink, Liam McIntyre, Glen P. Carter, Deborah A. Williamson, Mark R. Davies

A 3-month outbreak of invasive group A *Streptococcus* disease at an eldercare facility, in which 5 persons died, was biphasic. Although targeted chemoprophylaxis contained the initial outbreak, a second phase of the outbreak occurred after infection control processes ended. To retrospectively investigate the genomic epidemiology of the biphasic outbreak, we used whole-genome sequencing and multiple bioinformatics approaches. Analysis of isolates from the outbreak and isolates prospectively collected during the outbreak response indicated a single *S. pyogenes* *emm*81 clone among residents and staff members. Outbreak isolates differed from nonoutbreak *emm*81 isolates by harboring an integrative conjugative genomic element that contained the macrolide resistance determinant *erm*(TR). This study shows how retrospective high-resolution genomic investigations identified rapid spread of a closed-facility clonal outbreak that was controlled, but not readily cleared, by infection control management procedures.

*Streptococcus pyogenes*, or group A *Streptococcus* (GAS), is a gram-positive, human-adapted opportunistic bacterial pathogen. GAS causes a wide variety of clinical manifestations, from relatively benign self-limiting infections of the nasopharynx or skin to life-threatening invasive infections such as bacteremia, pneumonia, and necrotizing fasciitis (1). The incidence of invasive GAS infections is highest among older adults (2,3), particularly those living in long-term care facilities (2,4). Outbreaks of GAS infections are often linked with distinct epidemiologic markers such as *emm* type (5,6). *emm* typing is a sequence-based method that analyzes heterogeneity in the 5’ end of the ubiquitous *emm* gene that encodes the M-protein (7). Although *emm* typing provides useful information about the potential relatedness of outbreak isolates, whole-genome sequencing enables outbreak investigations to proceed with a far greater level of discrimination than single-gene typing of GAS isolates (8,9). For this study, we used multiple whole-genome–based approaches to examine the genetic relationships and molecular drivers of a biphasic GAS outbreak in an eldercare facility in which 14 persons became ill and 5 died.

**Methods**

**Setting**

During winter 2014, an outbreak of invasive and noninvasive GAS disease occurred in an eldercare facility in South Island, New Zealand. The outbreak was recognized by a senior laboratory scientist who noted the sudden increase in positive blood cultures from the facility. The outbreak occurred in 2 phases. The first phase started in late May 2014 and ended when the last case-patient (a resident) was hospitalized in early June 2014. The initial 3 case-patients were admitted within 24 hours of each other, and GAS was isolated from blood or tissue cultures from all 3 case-patients. During the first phase, 6 case-patients were admitted within 24 hours of each other, and GAS was isolated from blood or tissue cultures from all 3 case-patients. The first phase started in late May 2014 and ended when the last case-patient (a resident) was hospitalized in early June 2014. The initial 3 case-patients were admitted within 24 hours of each other, and GAS was isolated from blood or tissue cultures from all 3 case-patients. The first phase started in late May 2014 and ended when the last case-patient (a resident) was hospitalized in early June 2014. The initial 3 case-patients were admitted within 24 hours of each other, and GAS was isolated from blood or tissue cultures from all 3 case-patients. The first phase started in late May 2014 and ended when the last case-patient (a resident) was hospitalized in early June 2014. The initial 3 case-patients were admitted within 24 hours of each other, and GAS was isolated from blood or tissue cultures from all 3 case-patients.

During the first phase, 6 case-patients with confirmed GAS infection were hospitalized; 5 died of presumed sepsis. Outbreak investigations and control measures were subsequently implemented and included screening staff members and residents by collecting throat swab samples and providing targeted chemoprophylaxis for residents and staff members who were in direct contact with case-patients.

These interventions continued until early July 2014; however, in late July, the outbreak recurred...
and continued until mid August 2014. In this second phase, a staff member was hospitalized with severe pharyngitis, after which 5 residents were hospitalized with soft tissue infections, septicemia, or both.

Case Definitions
According to disk-diffusion testing, all GAS isolates collected during the first phase were susceptible to penicillin but resistant to erythromycin. Accordingly, a suspected case-patient was defined as any resident or staff member from the facility who was unwell from early June 2014 through mid-November 2014, and a confirmed case-patient was any person from the facility from whom erythromycin-resistant GAS was cultured from blood, throat, or skin samples. The definition of a suspected case-patient was kept intentionally broad because of the wide-ranging symptoms among initial case-patients. A carrier was defined as any asymptomatic person from the facility from whom erythromycin-resistant GAS was isolated from a skin or throat swab sample.

Comparison of Outbreak Isolates with Nonoutbreak Isolates
After the initial 3 cases were confirmed, throat swab samples were collected from all residents and from facility staff members who worked in nursing, kitchen, or waste collection. Swab samples were also collected from any skin lesion on staff members or residents. Hospital staff working in the wards where residents had been admitted were also asked to consent to collection of throat swab samples. The outbreak isolates were compared with nonoutbreak isolates for contextual purposes. Nonoutbreak isolates were defined as clinical isolates submitted to the Institute of Environmental Science and Research (ESR) in New Zealand from across New Zealand during 2002–2014. Although including only contemporaneous nonoutbreak isolates would have been ideal (that is, only those collected in 2014), New Zealand’s small population and the fact that GAS infections are not notifiable in New Zealand meant that we had to select nonoutbreak isolates over a broader time frame. All outbreak and nonoutbreak isolates underwent initial _emm_ typing at ESR according to previously described methods (7). We included in our comparative analysis only nonoutbreak isolates that had the same _emm_ type as the outbreak isolates. Data collection was approved by the Medicine, Dentistry and Health Sciences Human Ethics Sub-Committee at the University of Melbourne (ID no. 1853078).

Genome Sequencing and Assembly
We performed genome sequencing and assembly for outbreak and nonoutbreak isolates. All isolates underwent Illumina whole-genome sequencing (https://www.illumina.com). To enable fine-mapping of the outbreak, we completely sequenced a representative _emm_81 outbreak isolate, DMG1800716, by using Pacific Biosciences long-read technology (https://www.pacb.com). To validate the consensus assembly of the reference genome, we used Illumina short reads. We used Prokka (10) with manual curation to annotate the final sequence and SPAdes version 3.9.0 (11) for de novo assembly of raw Illumina reads into draft assemblies. We performed pairwise BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) comparisons of the 55 _emm_81 genomes relative to DMG1800716 by using the BLAST Ring Image Generator (12). We submitted the complete genome sequence of DMG1800716 to GenBank under accession no. CP027771. Short reads of all sequenced isolates are available at the National Center for Biotechnology Information sequence read archive (https://www.ncbi.nlm.nih.gov/sra) under BioProject PRJNA494270.

Phylogenetic Analyses
To determine whether outbreak isolates were genetically related, we mapped the genomes of the outbreak isolates and nonoutbreak isolates from across New Zealand to the newly generated 1,869,673-bp _emm_81 outbreak reference genome, DMG1800716 (Appendix 1, https://wwwnc.cdc.gov/EID/article/26/5/19-0131-App1.pdf). We inferred phylogenetic relationships by both maximum-likelihood and Bayesian assessment of core-genome single-nucleotide polymorphism (SNPs). We used consensus SNP alignments to build a maximum-likelihood tree with RAxML version 8.0.1 (13) and assessed temporal phylogenetic analysis by using BEAST version 2.4.7 (14), and a Hasegawa-Kishino-Yano plus gamma site model with a strict clock model after assessing temporal signal by using TempEst (15).

Results
Clinical Epidemiology of the Outbreak
During the 2 phases of the outbreak, 14 cases of erythromycin-resistant GAS infection were confirmed: 10 in residents and 4 in staff members. Eleven of the confirmed cases (10 in residents, 1 in a staff member) were detected by swabbing of unwell persons with suspected cases, and the other 3 confirmed cases were identified by prospective sampling of all 75 residents and 30 hospital staff members (for each of these staff members, the outbreak strain was isolated from skin
lesions on their hands). Prospective swabbing also identified 1 resident as a carrier (erythromycin-resistant GAS was isolated from the resident’s throat).

The average case-patient age was 79.5 years. Residents exhibited a variety of signs and symptoms (e.g., fever, malaise, suspected septic arthritis, diarrhea and vomiting, abdominal pain, and skin lesions). One staff member was hospitalized with severe pharyngitis; the other staff members were treated at home for minor skin infections.

During the first phase of the outbreak, a characteristic feature was the rapidity with which case-patient conditions deteriorated; 1 died within a few hours of symptom onset. Five confirmed case-patients, all residents, died of streptococcal sepsis during the first phase.

During the second phase of the outbreak, no deaths were reported. Five persons had suspected cases (4 residents, 1 staff member) during the outbreak but were excluded from this analysis because erythromycin-resistant GAS was not isolated.

Outbreak Isolates
During our investigation, we obtained 18 erythromycin-resistant GAS isolates, which were cultured from a variety of body sites including blood, throat, and soft tissue (Table). For 2 residents, identical outbreak strains were isolated from 2 different body sites; the remaining isolates each came from different patients. The phenotypic antimicrobial sensitivity pattern of the outbreak isolates included susceptibility to penicillin, methicillin (oxacillin), amoxicillin, and vancomycin and resistance to erythromycin with inducible resistance to clindamycin. No GAS with this antibiogram was cultured from the 65 screening throat swab samples from external hospital staff (those working in wards where case-patients were admitted).

Outbreak Management Interventions
After the initial 3 cases were confirmed and the outbreak was recognized, public health staff members initiated targeted chemoprophylaxis. A 10-day course of penicillin or amoxicillin was given to all staff members, any resident who was unwell or had been in contact with a case-patient, and any resident from whom GAS was isolated. The 4 staff members with outbreak strain infections stayed away from work until they had completed their course of antimicrobial therapy, their clinical signs of infections had resolved, and a throat swab sample culture was negative. Outbreak control measures initially continued for 1 month after the last case in the first outbreak phase was identified.

When the outbreak recurred, additional surveillance and environmental control measures were initiated and continued for 3 months after the last case of the second phase was identified. Other additional control measures included educating staff and residents about hand hygiene, monitoring the temperature of any resident with a skin lesion, cleaning all furniture and upholstery with diluted bleach where possible, replacing all toothbrushes, using disposable wound dressing trays rather than trolleys, inspecting the hands of staff members for skin lesions daily, and instructing the hospital to collect blood and throat swab samples for culture from any residents admitted from this eldercare facility and to place them in a single room. Items used communally by residents and staff (e.g., salt and pepper shakers, portable telephones) were cleaned with diluted bleach after meals or each use.

Genomic Epidemiology
Molecular analysis of the 18 outbreak GAS strains indicated that they all contained the emm81.0 gene allele. Only 5 contemporaneous nonoutbreak emm81 isolates were collected in New Zealand during 2014; the remaining 32 nonoutbreak isolates were collected during 2002–2013. Core-genome comparisons of the 18 outbreak strains with the 37 nonoutbreak emm81 isolates showed that the outbreak isolates were highly clonal and formed a separate clade in the emm81 phylogeny (Figure, panel A). Although 336 core-genome SNPs were identified among all emm81 isolates studied, no SNPs were identified between 15 of the outbreak isolates and 1 SNP difference was identified in the remaining 3 isolates. One isolate from a staff member differed from the outbreak isolates by a single SNP in the murM locus; the remaining isolates from staff members were indistinguishable from isolates from residents (Appendix 2, https://wwwnc.cdc.gov/EID/article/26/5/19-0131-App2.xlsx). These data suggest spread of the outbreak clone between staff members and residents to which directionality cannot be inferred. A single nonoutbreak isolate, DMG1800755, differed from the outbreak clade by 2 SNPs. The isolate came from an aspirate from a patient in the same southern region of New Zealand in mid-2014, around the temporal midpoint of the outbreak. The remaining nonoutbreak isolates showed a distant evolutionary relationship to the outbreak emm81 lineage.

To understand molecular differences between the outbreak emm81 lineage and the unrelated non-outbreak emm81 isolates, we investigated genomewide heterogeneity of the 55 emm81 genomes. We
screened core genomes for mutations within the key GAS regulatory genes covR/S, ropB, mga, which had previously been linked to increased virulence among GAS isolates (1); we found no differential mutations between outbreak and nonoutbreak isolates. Comparison of the accessory (variable) genome content of the 55 emm81 isolates revealed that all isolates sampled from South Island during the

Table. Details of *Streptococcus pyogenes* emm81 strains in study of biphasic outbreak of invasive group A *Streptococcus* disease in eldercare facility, New Zealand*

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<th>Source</th>
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†Isolates with this symbol are from the same resident.
‡Isolates with this symbol are from the same resident.
§Reference genome.
outbreak period harbored an integrative conjugative element (ICE); all other nonoutbreak isolates sampled during the outbreak period, which were all from other geographic regions, were ICE negative (Figure, panel B). The outbreak ICE element, called ICE- SpDMG1800716, shared 99% nucleotide sequence homology with ICE-Sp1108, previously described for an erythromycin-resistant GAS isolate from Italy (17) (Figure, panel C). Comparative analysis revealed that ICE-SpDMG1800716 contained the inducible macrolide-resistance gene, erm(Tr). ICE-SpDMG1800716 also harbored the abortive infection operon, AbiE operon, which is associated with bacteriophage resistance and stabilization of extrachromosomal elements (17). The ICE was integrated between the 3' end of the 23s tRNA methyltransferase (rum) gene (17) and the 5' end of a phosphorylase gene of a representative nonoutbreak isolate (DMG1800744) (Figure, panel C). Bayesian temporal analysis of the emm81 population indicated that ICE-SpDMG1800716 was acquired during 2007–2013 and that the ICE-positive clade subsequently expanded in 2013 (95% confidence range 2013–2014; Appendix 1 Figure).

Discussion

Through our clinical and genomic epidemiologic analyses, we determined that a fatal GAS outbreak in an eldercare facility was associated with a single emm81 GAS clone that was resistant to erythromycin and exhibited inducible clindamycin resistance. emm81 GAS is one of the most common M-types that causes invasive disease in New Zealand (3). The role of emm81 as a global GAS strain is highlighted by its inclusion in the experimental 30-valent M-protein vaccine (19). Traditional typing methods, such as emm typing, would not have had the discriminatory power to differentiate the outbreak isolates from other emm81 isolates that were already in New Zealand. Along with other recent reports of GAS outbreaks of a single emm type (8,9), our study highlights the utility of whole-genome sequencing as an epidemiologic tool for GAS outbreak investigations.

Comparative analyses of the outbreak clone with 37 nonoutbreak emm81 isolates identified that the outbreak clone had acquired a macrolide resistance determinant within a putative integrative and conjugative element, ICE-SpDMG1800716. Outbreaks of GAS disease have previously been linked to the acquisition of mobile genetic elements, such as an ongoing polyclonal emm12 and emm1 scarlet fever outbreak in Hong Kong and mainland China associated with horizontal acquisition of multidrug resistance and a superantigen-encoding prophage (6). Outbreaks of invasive GAS disease have also been associated with acquisition of, or mutations within, genotypic regulatory systems that result in increased phenotypic virulence (20,21). However, this clonal invasive GAS outbreak differs from previously reported outbreaks (6,20,21) because it was linked primarily to the acquisition of a transposable element with no obvious virulence determinant. Widespread use of macrolides in New Zealand, particularly in elderly patients and during the winter, when this outbreak occurred (22), may well have contributed to the selection and expansion of the macrolide-resistant outbreak clone.

In addition to harboring macrolide-resistance genes, the integrative conjugative element in the outbreak isolates also contained the abortive infection protein AbiE, which may have contributed to the relative fitness of the outbreak isolates. AbiE may confer bacteriophage resistance and has been shown to stabilize extrachromosomal elements such as plasmids (23); thus, its presence may have helped maintain ICE-SpDMG1800716 within the genomes of the outbreak isolates.

GAS carriage among healthcare workers in this and other outbreaks serves as a reminder that staff member sampling is integral to GAS outbreak investigations (9). Such practices, although common in hospital settings, are not universally followed during investigations of outbreaks in long-term care or eldercare facilities (5). In addition to a geographic and temporal link between the outbreak isolates and their closest nonoutbreak relative (both being from southern New Zealand and isolated in 2014), no contact history could be determined between the clonal nonoutbreak isolate and the outbreak facility. We therefore hypothesize that the outbreak probably commenced from an unsampled community source that gained entry to the facility by contact with either a resident or staff member. Although an environmental source is unlikely, environmental sampling was not undertaken; thus, fomites such as communal dinnerware or telephones could have been the common source of infection that resulted in the second phase of the outbreak. In a review of 17 reports of GAS outbreaks in long-term care facilities, fomites were not definitively implicated in outbreak transmission (5); therefore, an environmental source indeed seems less likely as a source of the recurrence of this outbreak and an unsampled human source seems more likely.

As was the case for other reported outbreaks in long-term care facilities (8,9), improved infection control measures and chemoprophylaxis were the cornerstones of outbreak control in this outbreak.
Figure. Comparative genomic analyses of 55 (18 outbreak and 37 nonoutbreak) associated emm81 group A Streptococcus (GAS) isolates from New Zealand, 2014. A) Midpoint-rooted maximum-likelihood phylogenetic analysis of the emm81 GAS population based on alignment of 336 high-quality single-nucleotide polymorphisms. Green branches indicate nonoutbreak isolates and blue branches indicate the clonal outbreak isolates. Outbreak isolates obtained from eldercare residents (blue) and staff members (orange) were indistinguishable at the whole-genome level. Numbers on major internal nodes indicate branch support as a percentage over 100 bootstrap replicates. The tree was created by using RAxML (13) and annotated by using iTOL (16). B) Comparative analyses of 55 emm81 draft genome assemblies from outbreak (blue) and nonoutbreak (green) isolates mapped against a new reference GAS genome from the outbreak, DMG1800716. A large DNA sequence coinciding with a 45.4-kb ICE, ICE-SpDMG1800716, is absent in the nonoutbreak isolates compared with all outbreak isolates. The image was created by using BLAST Ring Image Generator (12). C) Schematic representation and pairwise sequence comparison (BLASTn, https://blast.ncbi.nlm.nih.gov) of ICE-SpDMG1800716 relative to the closest known homologue, ICE-Sp1108 (17). The genomic integration site of ICE-SpDMG1800716 is shown relative to a nonoutbreak emm81 isolate, DMG1800744. Red bars refer to 100% BLASTn homology as determined by Easyfig (18). The macrolide resistance gene erm(TR) is shown in dark blue and the abortive infection genes (AbiE) in green. ICE, integrative conjugative element; SNPs, single-nucleotide polymorphisms.
Although infection control measures are undoubtedly of utmost importance, the evidence as to whether targeted or mass chemoprophylaxis is preferable in eldercare settings is conflicting, because risk for secondary invasive GAS infection is higher among elderly persons than among other contacts (24, 25). Authors of a recent UK study demonstrated a considerably increased risk for invasive GAS infection among household contacts, particularly for persons >75 years of age, for whom the fatality rate for secondary cases was 19% (24). They suggested that, even in nonoutbreak settings, targeted chemoprophylaxis for elderly household contacts of invasive GAS patients should be considered (24). It is conceivable that the targeted chemoprophylaxis undertaken during the first phase of this outbreak prevented some cases, yet the occurrence of the second phase suggests that this approach alone was not sufficient. A 2007 review of 17 GAS outbreaks in long-term care facilities similarly found that in 3 facilities, targeted chemoprophylaxis was insufficient for achieving outbreak control and that control was achieved only after the facilities initiated mass chemoprophylaxis to augment existing infection control measures (5). More recently, mass chemoprophylaxis was insufficient for halting a multiphase outbreak in 2 long-term care facilities in the United States (9). In that study, mass chemoprophylaxis was initiated for all residents and consenting staff; prophylactic coverage was wider than that in our study. Nevertheless, mass chemoprophylaxis in the US outbreak was still only partially effective, and outbreak persistence was attributed mostly to continued lapses in infection control practices. During a GAS outbreak in another long-term care facility, breaches in infection control practices were also noted; prospective assessment of staff members’ wound care and hand hygiene practices found several lapses in each (26). In our study, improved infection control practices were initiated, but direct observation of staff undertaking wound care and hand hygiene might have further helped to identify exactly where lapses might have been occurring.

In summary, our data further highlight the potential for invasive GAS to cause rapid and fatal outbreaks, particularly in closed communities such as eldercare facilities. Invasive GAS disease is not notable in New Zealand, nor is there mandatory surveillance for invasive GAS infections. The incidence of invasive GAS infections in New Zealand and elsewhere is particularly high among those >75 years of age (2, 3). Our findings add to the growing body of evidence emphasizing the need for improved surveillance and response to invasive GAS infections in at-risk populations, particularly in countries such as New Zealand where active surveillance is not conducted.

Acknowledgments
We thank the laboratory staff at the Microbiological Diagnostic Unit at the Doherty Institute for Infection and Immunity, The University of Melbourne, and the core sequencing facility at the Wellcome Trust Sanger Institute, UK. We also acknowledge Seamus O’Reilly for his assistance in reviewing this manuscript.

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References


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Tick-borne relapsing fever (TBRF) is a zoonosis caused by spirochetes of the genus Borrelia. We collected data on all TBRF cases in a TBRF-endemic area in southwest Spain during 1994–2016. We analyzed data from 98 patients in whom TBRF was diagnosed by light microscopy and analyzed the relationship between climatic data and TBRF incidence. Most cases occurred in a rural environment during summer and autumn. We describe demographic, epidemiologic, clinical, and analytical characteristics, treatment, and occurrence of Jarisch-Herxheimer reaction. Most patients had fever and headache, and laboratory test results included elevated C-reactive protein, thrombocytopenia, and neutrophilia. No patients died, but 10.1% had Jarisch-Herxheimer reaction. B. hispanica was the infecting species in 12 cases with PCR results. Clinicians often do not suspect TBRF because clinical signs and symptoms vary; therefore, it is likely underdiagnosed, even in disease-endemic areas.

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SYNOPSIS

We included all patients diagnosed with ≥1 episode of TBRF during December 1994–December 2016. The area of southern Spain served by this healthcare facility (Figure 1) has a population of ≈174,000.

Diagnosis of TBRF

Diagnosis of TBRF was made by visualization of *Borrelia* spp. in thick or thin blood smears stained with 10% Giemsa and then examined by optical microscopy. We extracted blood during febrile attacks. Spirochetemia was quantified by light microscopy, at 1,000× magnification, as follows: >5 spirochetes in each field of vision was 3+; 1–5 spirochetes in each field of vision was 2+; <1 spirochete in each field of vision was 1+. During 2013–2014, we sent *Borrelia*-positive samples to the National Microbiology Center (Madrid, Spain) for species identification by multiplex PCR.

Variables and Definitions

The principal variable was confirmation of TBRF by direct diagnosis. Epidemiologic variables collected included patient age, sex, town of origin, occupation, hobbies, contact with rodents. Other variables recorded were those associated with underlying conditions, such as diabetes mellitus, arterial hypertension, chronic obstructive pulmonary disease, chronic liver disease, chronic kidney disease, cancer, neurologic disease, chronic digestive pathology, cutaneous ulcer, chronic heart failure, HIV infection, transplant recipient, and pregnancy. In addition, variables related to TBRF were recorded, including date of diagnosis, symptoms, remembered antecedent of tick bite, duration of clinical symptoms before diagnosis, main clinical signs, hospitalization, treatment, JHR, and outcome. We analyzed determinants gathered in blood samples, including hemogram and coagulation; serum, including basic biochemistry, C-reactive protein, and liver, renal, and cardiac profiles; and cerebrospinal fluid (CSF), including leukocyte count, percentage of lymphocytes, glucose, and proteins. We used the first determinant collected after symptom onset.

Meteorologic Conditions

We obtained monthly meteorologic statistics from the records of La Agencia Estatal de Meteorología (http://www.aemet.es/en/portada) of Spain during 1994–2016. We included mean temperature (°C) and percent humidity collected on day 7, 13, and 18 each month at the weather station at Morón de la Frontera (37°9′49.8767″N, 5°36′40.5219″W). The station is 87 m above sea level and 40 km from Osuna (Figure 1).

Statistical Methods

We described cases of TBRF by using epidemiologic and clinical variables and investigated the relationship...
between environmental factors and TBRF incidence by using a mixed model. We performed calculations by using R software (https://www.r-project.org). We also investigated the risk factors of JHR by performing univariate comparisons of patients who experienced JHR and those who did not by using \( \chi^2 \) or Fisher exact test, as needed, for categorical variables and Student t-test or Mann-Whitney U-test for continuous variables, as appropriate. To control for confounding factors, we performed a multivariate logistic regression analysis by using JHR as the dependent variable. We performed statistical analysis by using SPSS 21.0 (IBM, https://www.ibm.com). We express continuous variables as median and interquartile range (IQR) and categorical variables as number and percent.

Ethics Approval
The study was designed and performed according to the World Health Association’s Declaration of Helsinki (https://www.wma.net) and was approved by the ethics committee of La Merced Hospital. Because it was a retrospective study, consecutive blinded case numbers were assigned for medical histories taken from patient records to ensure a safe dissociation and prevent identification of patients included.

Results

Incidence of TBRF
We calculated the annual incidence of TBRF cases by analyzing data from 98 case-patients included in our final cohort (Figure 2). TBRF incidence increased over the study period and showed considerable variability between years. The highest incidence rates of TBRF occurred in 2015 (8.69 cases/100,000 persons), 2011 (6.31 cases/100,000 persons), and 2014 (5.77 cases/100,000 persons). Most cases of TBRF were diagnosed during June–November.

Diagnosis of TBRF
All TBRF cases in our series were diagnosed by direct visualization of \textit{Borrelia} in blood samples. Spirochete quantification was either 1+ or 2+. Blood samples were negative 1–2 days after initial Borrelia visualization. Samples collected from 12 patients during 2013–2014 were sent to the National Microbiology Center, where the bacteria species was identified as \textit{B. hispanica} by multiplex PCR targeting the 16S rRNA gene.

Clinical, Analytical, and Therapeutic Features of TBRF Cases
Among 98 patients with TBRF in our cohort, 55 (56.1%) were men and 43 (43.9%) were women (Table 1), 2 of whom were pregnant. Our data agree with the prevalence of TBRF in young persons; 19% of patients in our cohort were <14 years of age (data not shown). Of note, 80% of case-patients lived in a rural environment, and only 17 (17.3%) reported previous known contact with ticks. In most cases, TBRF diagnosis was made in the emergency department. Median time between the onset of symptoms and diagnosis was 3 days (IQR 2–5 days).

The most common clinical symptoms were fever in 99% of cases, headache in 59%, vomiting in 34.7%, and arthralgias in 30% (Table 2). A lumbar puncture was performed on 12 patients; 5 had no neck stiffness, deterioration of consciousness, or signs of Kernig and Brudzinski, but because they had fever, vomiting, and intense headaches, clinicians decided to extract CSF. Lymphocytic meningitis with moderate to high pleocytosis of mononuclear predominance, elevated proteins, and normal glucose were noted in CSF from...
Table 1. Characteristics of 98 patients with tick-borne relapsing fever, Spain*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age, y (IQR)</td>
<td>29 (17–46)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>55 (56.1)</td>
</tr>
<tr>
<td>F</td>
<td>43 (43.9)</td>
</tr>
<tr>
<td>Town of residence</td>
<td></td>
</tr>
<tr>
<td>Osuna</td>
<td>40 (40.8)</td>
</tr>
<tr>
<td>Ecija</td>
<td>20 (20.4)</td>
</tr>
<tr>
<td>Other</td>
<td>38 (38.8)</td>
</tr>
<tr>
<td>Risk activities</td>
<td></td>
</tr>
<tr>
<td>Farming</td>
<td>15 (15.3)</td>
</tr>
<tr>
<td>Hiking</td>
<td>10 (10.2)</td>
</tr>
<tr>
<td>Hunting</td>
<td>3 (3.1)</td>
</tr>
<tr>
<td>Contact with rodents</td>
<td></td>
</tr>
<tr>
<td>Tick bite</td>
<td>17 (17.3)</td>
</tr>
<tr>
<td>JHR, n = 79</td>
<td>8 (10.1)</td>
</tr>
<tr>
<td>Median time before diagnosis, d (IQR)</td>
<td>3 (2–5)</td>
</tr>
<tr>
<td>Hospitalized</td>
<td>56 (57.1)</td>
</tr>
<tr>
<td>Median length of hospital admission, d (IQR)</td>
<td>5 (4–6)</td>
</tr>
<tr>
<td>Antimicrobial drug treatment</td>
<td></td>
</tr>
<tr>
<td>Tetracycline</td>
<td>79 (80.6)</td>
</tr>
<tr>
<td>Macrolides</td>
<td>18 (18.4)</td>
</tr>
<tr>
<td>Cefalosporin</td>
<td>9 (9.2)</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Duration of antimicrobial therapy, d (IQR)</td>
<td>10 (7–14)</td>
</tr>
<tr>
<td>Death</td>
<td>0</td>
</tr>
</tbody>
</table>

*Values are no. (%) except as indicated. IQR, interquartile range; JHR, Jarisch-Herxheimer reaction. †Except where noted.

3 patients (Table 3). Meningitis was eventually ruled out in a pediatric patient (case 5), who had mild leukocytosis in the CSF without biochemical alterations. No Borrelia were observed in the CSF samples.

All 98 cases exhibited elevated C-reactive protein, and 92.9% had thrombocytopenia and neutrophilia, most without leukocytosis, likely due to compensatory reduction of lymphocytes. Anemia was detected in 32 cases, 4 of which were moderate (hemoglobin <10 g/dL). Pancytopenia was observed in 5% of cases, 23% had hypertransaminasemia, and 38% had hyperbilirubinemia.

Regarding treatment, 91 (93%) patients received 1 antimicrobial drug and 7 (7%) received >1 antimicrobial drug. Tetracycline was the first choice in adults and macrolides in persons <14 years of age. The duration of treatment was 10 days (IQR 7–14 days). We observed no notable difference between patients who received >10 days of antimicrobial therapy and those receiving <10 days of treatment (data not shown).

Eleven case-patients had >2 episodes of fever; some had as many as 4. Most recurrences of fever were caused by delay in diagnosis and treatment, except for 1 patient, 7 years of age, who received oral erythromycin for 10 days and was readmitted with the same symptoms 2 weeks later. We detected 2 reinfections: 1 in a self-described farmer and hunter who was affected in 1997 and 2005, and 1 in a hiker who was affected in 2011 and 2013.

We noted 5 clusters of TBRF; 4 occurred in family groups, and 1 involved persons with a common social activity. A 2015 cluster involved 5 family members, a 2014 cluster affected 2 family members, and 3 family members were involved in each cluster noted in 2004 and 2011. Another 2014 cluster occurred among 3 persons who all reported hunting in Osuna, Seville.

Correlation between Meteorologic Conditions and TBRF Incidence

We established a mixed model in which the temperature, humidity, and month have been established as fixed effects and the year as a random competent. When applying the different models, we found that humidity had little effect and that the risk for tick bites was dependent on the temperature and month. Bites were more frequent during summer and autumn months, when higher temperatures were recorded.

Risk Factors for JHR

Of 98 TBRF cases, we analyzed JHR risk factors for 79 patients, 8 (10.1%) of whom had JHR symptoms, including chills, worsening fever, tachycardia, hypotension, and anxiety, a few hours after starting antimicrobial drug treatment. In our analysis, male sex was a risk factor (8.9%) for JHR compared with female sex (1.3%; p = 0.13) and length of admission (median 5 days; IQR 4–7 days) was a statistically significant risk factor (8.9%) for JHR compared with female sex (1.3%; p = 0.13) and length of admission (median 5 days; IQR 4–7 days) was a statistically significant risk factor for JHR in univariate analysis (Table 4). We saw no relationship between specific antimicrobial drugs and JHR. In multivariate analysis, we found no variables independently associated with JHR (Table 4).

Discussion

We analyzed 98 cases of TBRF identified during a 23-year period from a Borrelia-endemic area of Spain. The highest incidence rates were in 2015 (8.69/100,000 persons), 2011 (6.31/100,000 persons), and 2014 (5.77/100,000 persons); no cases were reported in 1995, 2001, or 2012 (Figure 2). The highest number of cases originated in Osuna (43 cases), Ecija (21 cases), La Puebla de Cazalla (4 cases); other areas had <3 cases (Figure 1).

The TBRF incidence trended upward during the study period, probably because of increased awareness of the disease. The peaks observed in 2015, 2014, and 2011 can be justified in part by outbreaks of TBRF among persons in family groups or participating in same social activity. We do not know why years of greater incidence are interspersed among others of little (2009, 2010) or no (2012) incidence. We have not
sampled Ornithodoros ticks to evaluate densities and infection rates, nor have we collected samples from small mammals to investigate the reservoir of Borrelia spp.

We studied climatic conditions during 1994–2016 and identified a direct correlation of TBRF incidence with increased temperatures during the summer and autumn. Higher TBRF incidence rates during the warmest months can be explained by increased activity of the tick vectors and increased exposure of the general population to those vectors, both of which increase the possibility of tick bites in humans (8).

We used Giemsa staining of thin and thick blood films to identify Borrelia, but a density of $\geq 10^6$ spirochetes/1 mL of blood is needed for definitive diagnosis in thick films and $\geq 10^5$ spirochetes/1 mL of blood for thin films and microscopic visualization is not a reliable diagnostic method for TBRF (13). Larsson et al. (14) suggested the sensitivity of the method could be increased by performing a double centrifugation of the blood sample inoculated in a tube with heparin. The application of phase contrast or dark field microscopy directly on a 10× blood dilution also could be useful for cases of spirochetemia (10). Where it is technically possible, PCR is replacing microscopic visualization for diagnosis (7,15,16), but Hospital de la Merced is a small hospital in a rural area and does not have in-house PCR resources.

The most prevalent, and probably the only, Borrelia species in the environment in this area is B. hispanica, which usually causes mild infection. Patients with TBRF in our hospital had mainly nonspecific infections in pregnant women, but Rustenhoven-Sappan et al. (19) reported that pregnant women have higher spirochete loads and more severe symptoms than women who are not pregnant. Some evidence also suggests that spirochetes can cross the placenta and cause neonatal relapsing fever (20). However, we only had 2 TBRF cases in pregnant women, so the lack of clinical complications in this group likely can be attributed to the small sample size in our series.

The most common abnormality in blood cell count we noted in laboratory findings was thrombocytopenia, especially during the early stage of infection, with a median of 63,500 platelets/mm$^3$ in the hemogram. Thrombocytopenia has been reported in B. crocidurae (12), B. hermsii (21), and B. hispanica (22–24) infections, but not with B. burgdorferi (25). Thrombocytopenia could be explained by the phenomenon of bacteria binding directly to platelets during the infection, which results in increased platelet destruction, prolonged bleeding, or endothelial injury, the degree of which correlates with the degree of spirochetemia (21).

We observed anemia in 33% of patients at TBRF diagnosis with hemoglobin values $<11.8$ g/dL and hematocrits $<35\%$, likely caused by erythrocyte

### Table 2. Main symptoms, signs, and analytical findings of 98 patients with tick-borne relapsing fever, Spain

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptoms</td>
<td>Fever $&gt;38.5^\circ$C 97 (99)</td>
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<tr>
<td></td>
<td>Headache 58 (59)</td>
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<tr>
<td></td>
<td>Vomiting 34 (34.7)</td>
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<tr>
<td></td>
<td>Arthralgia 29 (30)</td>
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<tr>
<td></td>
<td>Abdominal pain 28 (28.6)</td>
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<tr>
<td></td>
<td>Myalgia 28 (28.6)</td>
</tr>
<tr>
<td></td>
<td>Chills 23 (23.6)</td>
</tr>
<tr>
<td></td>
<td>Diarrhea 6 (6.1)</td>
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<tr>
<td>Signs</td>
<td>Meningeal signs 7 (7.1)</td>
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<tr>
<td></td>
<td>Splenomegaly 7 (7.1)</td>
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<tr>
<td></td>
<td>Hepatomegaly 6 (6.1)</td>
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<tr>
<td></td>
<td>Exantheme 4 (4.1)</td>
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<tr>
<td></td>
<td>Jaundice 3 (3.1)</td>
</tr>
<tr>
<td></td>
<td>Petechiae 2 (2)</td>
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<td></td>
<td>Conjunctival injection 2 (2)</td>
</tr>
<tr>
<td>Analytical findings</td>
<td>Median platelet count, $\times 10^9$/mm$^3$ (IQR) 63.5 (45.7–87.5)</td>
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<tr>
<td></td>
<td>Thrombocytopenia, platelets &lt;13 $\times 10^9$/mm$^3$ 91 (92.9)</td>
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<tr>
<td></td>
<td>Hemoglobin, g/dL (IQR) 13.1 (11.8–14.2)</td>
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<td></td>
<td>Anemia 32 (32.6)</td>
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<td></td>
<td>Leukocytes, $\times 10^9$/mm$^3$ (IQR) 7.9 (6.6–9.8)</td>
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<td></td>
<td>Leukotysis, $&gt;1.500$/mm$^3$ 10 (10.2)</td>
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<tr>
<td></td>
<td>Neutrophils, median (IQR) 79 (74.5–86.4)</td>
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<td></td>
<td>Neutrophilia, neutrophils $&gt;65%$ 91 (92.9)</td>
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<tr>
<td></td>
<td>Prothrombin activity, median (IQR) 77 (70–87)</td>
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<td></td>
<td>Decreased prothrombin activity, $&lt;70%$ 7 (7.3)</td>
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<td></td>
<td>Median INR (IQR) 1.2 (1.1–1.3)</td>
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<td></td>
<td>INR elevation, $&gt;1.51$ 7 (13.7)</td>
</tr>
<tr>
<td></td>
<td>SGOT, U/L (IQR) 25 (20–35)</td>
</tr>
<tr>
<td></td>
<td>SGOT elevation, $&gt;37$ U/L 15 (21.7)</td>
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<td></td>
<td>SGPT, U/L (IQR) 25 (17–41)</td>
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<td></td>
<td>SGPT elevation, $&gt;40$ U/L 10 (23.3)</td>
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<tr>
<td></td>
<td>Total bilirubin, mg/dL (IQR) 1.1 (0.6–1.9)</td>
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<td></td>
<td>Elevated bilirubin, $&gt;1.2$ mg/dL 37 (57.8)</td>
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<td></td>
<td>Lactate dehydrogenase, U/L(IQR) 397.5 (320.7–481.7)</td>
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<td></td>
<td>Elevated LDH, $&gt;460$ U/L 16 (30.8)</td>
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<td>Creatinine, mg/dL (IQR) 0.94 (0.79–1.15)</td>
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<td></td>
<td>Creatinine elevation, $&gt;1.2$ mg/dL 15 (15.3)</td>
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<td></td>
<td>Creatine kinase, U/L (IQR) 41 (27–75)</td>
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<tr>
<td></td>
<td>Elevated creatine kinase, $&gt;195$ U/L 3 (14)</td>
</tr>
<tr>
<td></td>
<td>C-reactive protein, mg/L (IQR) 254.5 (218.2–335.3)</td>
</tr>
<tr>
<td></td>
<td>Elevated C-reactive protein, $&gt;5$ mg/L 98 (100)</td>
</tr>
</tbody>
</table>

Values are no. (%) except as indicated. INR, international normalized ratio; IQR, interquartile range; SGOT, serum glutamic-oxaloacetic transaminase; SGPT, serum glutamic-pyruvic transaminase.
Table 3. Findings from cerebrospinal fluid collected from 12 patients with tick-borne relapsing fever, Spain

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Patient age, y</th>
<th>Protein, mg/dL</th>
<th>Glucose, mg/dL</th>
<th>Leukocytes/μL</th>
<th>Lymphocytes, %</th>
<th>Meningitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>174</td>
<td>48</td>
<td>1,271</td>
<td>57</td>
<td>Y</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>130</td>
<td>61</td>
<td>1,760</td>
<td>90</td>
<td>Y</td>
</tr>
<tr>
<td>3</td>
<td>21</td>
<td>91</td>
<td>49</td>
<td>521</td>
<td>90</td>
<td>Y</td>
</tr>
<tr>
<td>4</td>
<td>17</td>
<td>74</td>
<td>62</td>
<td>0</td>
<td>0</td>
<td>N</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>32</td>
<td>47</td>
<td>130</td>
<td>80</td>
<td>N</td>
</tr>
<tr>
<td>6</td>
<td>42</td>
<td>23</td>
<td>74</td>
<td>1</td>
<td>100</td>
<td>N</td>
</tr>
<tr>
<td>7</td>
<td>14</td>
<td>20</td>
<td>57</td>
<td>0</td>
<td>0</td>
<td>N</td>
</tr>
<tr>
<td>8</td>
<td>19</td>
<td>20</td>
<td>62</td>
<td>4</td>
<td>75</td>
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</tr>
<tr>
<td>9</td>
<td>28</td>
<td>20</td>
<td>49</td>
<td>4</td>
<td>75</td>
<td>N</td>
</tr>
<tr>
<td>10</td>
<td>26</td>
<td>19</td>
<td>60</td>
<td>0</td>
<td>0</td>
<td>N</td>
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<tr>
<td>11</td>
<td>32</td>
<td>13</td>
<td>64</td>
<td>1</td>
<td>100</td>
<td>N</td>
</tr>
<tr>
<td>12</td>
<td>12</td>
<td>12</td>
<td>78</td>
<td>3</td>
<td>100</td>
<td>N</td>
</tr>
</tbody>
</table>

The aggregation of red blood cells around *B. hispanica* has been observed in vitro by Guo et al. (26) and has been described in infections with *B. duttonii* and *B. coricae* (26). Erythrocyte rosetting also has been reported by Shamaei-Tousi et al. (12) in an animal model infected with the *B. crocidurae*.

We also detected neutrophilia that did not produce leukocytosis because of compensatory lymphopenia. López et al. (27) studied an animal model with Rhesus macaques infected with *B. turicatae* and observed notable changes in the differential leukocyte count. They found a relative increase in the neutrophil percentage for several days after leukopenia and mild increases in monocytes several days after the initial episode of leukopenia. They noted these abnormalities to some degree in all animals studied, and the neutrophilia coincided with spirochtemia (27).

Another finding that deserves attention is pancytopenia, a potentially serious complication, which we observed in 5% of TBRF cases. Fortunately, severe pancytopenia is a rare complication described in only a few cases (13,24).

All patients in our study demonstrated known TBRF biochemical alterations in the acute phase, including high levels of C-reactive protein (22–24), mild hypertransaminasemia (13,28,29), and hyperbilirubinemia (29,30). Less frequently, elevated lactate dehydrogenase and creatinine kinase also have been reported (22); we observed elevated lactate dehydrogenase in 31% of cases in our study and elevated creatinine kinase in 15%.

*Borrelia* spirochetes are susceptible to penicillin and other β-lactam antimicrobial drugs and to tetracyclines and macrolides. Most experts recommend a dose of 100 mg doxycycline twice a day or 500 mg tetracycline four times a day for 7–14 days (1). Erythromycin or chloramphenicol are the most used alternatives (1). Parenteral therapy with ceftriaxone is recommended for patients with central nervous system involvement or severe multisystem disease (1).

Fluoroquinolones are not recommended for treating patients with TBRF (29). In our experience, we treated patients with some of the recommended regimens over a median of 10 days, and they had adequate, early responses to treatment. A shorter regimen might be equally effective for treating TBRF.

We observed JHR in 10.1% of patients. Our patients did not have severe JHR, but severe cases have been reported (31), including elevated cardiac troponin in pregnant women (23,28) and, more frequently, in children (19,23). However, neither of the 2 pregnant women in our TBRF cohort developed JHR.

Immunity to *B. hispanica* after infection is not permanent, so patients can be reinfected later. We detected 2 cases of reinfection. Reinfections with *B. crocidurae* also have been observed in patients in Senegal (32), demonstrating the importance of informing patients of this aspect of TBRF.

In conclusion, we analyzed epidemiologic data of 98 TBRF cases diagnosed in southern Spain during 1994–2016. In addition to information on the *Borrelia* life cycle, we report clinical signs, laboratory findings, prognosis, complications, and TBRF diagnosis, in patients at a rural hospital. Because clinical signs and symptoms of TBRF cover a wide range and incidence is low, clinicians have a low suspicion index for this disease. The most frequent symptoms are fever and headache and the most frequent analytical alterations are thrombocytopenia and neutrophilia without leukocytosis, in addition to the elevated C-reactive protein during the acute phase. TBRF occurs in rural environments, mainly in summer and autumn. We saw no evidence of a climate-associated increase in infection risk over the 23-year period. TBRF usually is not a severe disease in patients in this region of Spain, and they respond well to treatment. Because the spirochetemia phase is short and laboratory diagnosis is exclusively dependent on the observer, we believe TBRF is underdiagnosed, even in areas where suspicion should be relatively high. Addition of routine molecular
techniques to detect spirochetes could eliminate these diagnostic doubts in rural areas.

About the Author
Dr. Domínguez is a clinical microbiologist at La Merced Hospital, Osuna, Seville, Spain. Her primary research interests are borreliosis and epidemiology.

Table 4. Factors associated with risk for JHR among 79 cases of tick-borne relapsing fever, Spain*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
<th>p value†</th>
<th>Adjusted OR (95% CI)</th>
<th>p value‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td>0.13</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>1 (1.3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>7 (8.9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median age, y (IQR)</td>
<td></td>
<td>0.86</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Y</td>
<td>28.6 (17.3–46.3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>25.5 (18.9–48.7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rural environment</td>
<td></td>
<td>0.57</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Y</td>
<td>7 (8.9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>1 (1.3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contact with rodents</td>
<td></td>
<td>0.11</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Y</td>
<td>3 (3.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>5 (6.3)</td>
<td></td>
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<td>Hepatomegaly</td>
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<td>N</td>
<td>8 (10.1)</td>
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<td></td>
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<td>Splenomegaly</td>
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</tr>
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<td></td>
<td></td>
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</tr>
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<tr>
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<td></td>
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<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>8 (10.1)</td>
<td></td>
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<tr>
<td>Meningeal signs</td>
<td></td>
<td>0.9</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Y</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>8 (10.1)</td>
<td></td>
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<tr>
<td>Exanthema</td>
<td></td>
<td>0.28</td>
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<td></td>
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<td>1 (1.3)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>7 (8.9)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Tick bite</td>
<td></td>
<td>0.17</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Y</td>
<td>3 (3.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>5 (6.3)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Tetracycline</td>
<td></td>
<td>0.54</td>
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<tr>
<td>Y</td>
<td>7 (8.9)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>1 (1.3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cephalosporin</td>
<td></td>
<td>0.31</td>
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<td>Y</td>
<td>2 (2.5)</td>
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<tr>
<td>N</td>
<td>6 (7.6)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Macrolides</td>
<td></td>
<td>0.35</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Y</td>
<td>1 (1.3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>7 (8.9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median length of clinical signs, d (IQR)§</td>
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<td>NS</td>
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<tr>
<td>Y</td>
<td>3 (2–5.8)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>3 (1.3–4)</td>
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<tr>
<td>Median length of hospital admission, d (IQR)§</td>
<td></td>
<td>0.004</td>
<td>0.56 (0.29–1.02)</td>
<td>0.057</td>
</tr>
<tr>
<td>Y</td>
<td>5 (4–7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>3 (3–3.3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median duration of antimicrobial therapy, d (IQR)§</td>
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<td>NS</td>
<td></td>
</tr>
<tr>
<td>Y</td>
<td>10 (8–14)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>10 (9–11.8)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Values are no. (%) patients with JHR except as indicated. IQR, interquartile range; JHR, Jarisch-Herxheimer reaction; NS, not statistically significant; OR, odds ratio.
†Univariate analysis calculated by using χ² or Fisher exact test.
‡Multivariate analysis calculated by using Student t-test or Mann-Whitney U-test.
§Values represent findings from 36 patients with known length of clinical signs.

References


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Food Safety and Invasive Cronobacter Infections during Early Infancy, 1961–2018

Jonathan Strysko, Jennifer R. Cope, Haley Martin, Cheryl Tarr, Kelley Hise, Sarah Collier, Anna Bowen

Invasive Cronobacter infections among infants are associated with severe neurologic disabilities and death. Early Cronobacter reports typically featured hospitalized and preterm infants and recognized contaminated powdered infant formula (PIF) as a transmission vehicle. To clarify recent epidemiology, we reviewed all cases of bloodstream infection or meningitis among infants that were reported to the Centers for Disease Control and Prevention and in the literature (1961–2018; n = 183). Most infants were neonates (100/150 [67%]); 38% (42/112) died, and 79% (81/102) had reported recent PIF consumption. In the final quarter of the study period (2004–2018), case counts were significantly higher (global average 8.7 cases/year); among US cases, significantly higher proportions occurred among full-term (56% [27/48]) and non-hospitalized (78% [42/54]) infants. PIF contamination, most commonly from opened containers, was identified in 30% (21/71) of investigations. Our findings reaffirm the need to promote safer alternatives for infant feeding, particularly among neonates.
Cronobacter species are gram-negative bacteria known to cause severe and often life-threatening infections in infants. Invasive Cronobacter infections in infants, including bloodstream infections and meningitis (most commonly caused by *C. sakazakii*), can result in neurologic disability, as well as death; reported case-fatality rates are as high as 40% (1). Beginning in 1961, reports of invasive Cronobacter infections historically described predominantly hospitalized and preterm infants (2,3). The identification of Cronobacter in association with several cases of necrotizing enterocolitis among infants reinforced its association with hospitalized infants born prematurely (4). However, previously healthy full-term infants are also known to become infected; infants born at more advanced gestational ages might be at greater risk than early preterm infants for having Cronobacter meningitis, as opposed to isolated bloodstream infection (1).

Because reporting is not mandatory in most countries (and in most of the United States), the true incidence of invasive infant Cronobacter infections is unknown. Estimates from laboratory-based surveillance in the United States suggest that ≈18 infant cases of invasive Cronobacter infection (0.49 cases/100,000 infants) occur annually (5). In 2008, the World Health Organization (WHO) reported the yearly incidence to be at least 0.14/100,000 infants in the Philippines and 1.76/100,000 infants in England and Wales, although these are thought to be underestimates (6).

Cronobacter spp. can withstand desiccation in dried foods like powdered infant formula (PIF) and are known to thrive in reconstituted formula (7). Linked to outbreaks among hospitalized neonates in the 1980s, contaminated PIF has been identified as the transmission vehicle in nearly all Cronobacter infections for which a source was found (3,8–10). The most recently identified US outbreak of Cronobacter infections linked to intrinsic contamination of a formula product (i.e., *Cronobacter* isolated from sealed formula containers) occurred in 2001 at a Tennessee hospital (11). That outbreak helped prompt the US Food and Drug Administration (FDA) in 2002 to discourage the use of PIF in neonatal intensive care settings unless there is no alternative available (12,13). For hospitalized neonates, FDA recommended ready-to-feed (RTF) liquid formula, which is considered sterile until opened. WHO issued broader recommendations aimed at preventing Cronobacter transmission through hygienic PIF reconstitution and storage practices, emphasizing the importance of hand hygiene and advising that caregivers reconstitute PIF with water heated to ≥70°C (14). In 2014, FDA issued quality control standards aimed at safer PIF production, including requiring manufacturers to routinely test for *Salmonella* and Cronobacter before distribution (15).

In 2016, the US Centers for Disease Control and Prevention (CDC) reported a case of invasive Cronobacter infection linked to contaminated expressed human breast milk (EBM) and the associated breast pump (16). After that case, 2 additional EBM-associated cases were reported in the literature, including 1 in a full-term neonate (17,18). To characterize recent epidemiology in light of regulatory actions, enhanced surveillance and preventive efforts, and newly described modes of transmission, we analyzed all cases of invasive Cronobacter infection among infants that were reported to the CDC and documented in the literature.

### Methods

We defined an invasive case as isolated bacteremia (*Cronobacter* isolated from blood) or meningitis (*Cronobacter* isolated from cerebrospinal or brain abscess fluid, with or without bacteremia) in an infant (<12 months of age). We excluded cases of isolated *Cronobacter* urinary tract infection and *Cronobacter*-associated necrotizing enterocolitis from this analysis. We searched for invasive cases reported since the first published reports (1961–2018) (2). The *Cronobacter* genus was formerly known as the single species *Enterobacter sakazakii*. Therefore, we used the subject heading terms “Cronobacter” or “sakazakii” in combination with “newborn,” “infant,” or “neonate” to conduct a literature search of the Medline, Embase, CINAHL, Scopus, and Cochrane Libraries and review associated bibliographies. In addition, we included previously unpublished cases from CDC case consultations, cases reported in the 2008 Food and Agriculture Organization of the United Nations (FAO) and WHO meeting notes summarizing the international call for data on Cronobacter, and the PubMLST database, an internet-based repository of bacterial isolate genetic sequences (6,19). PubMLST submission profiles occasionally report clinical and demographic information, which led to the identification of additional cases. We de-duplicated redundant cases reported in multiple data sources using information provided on patient age, location, and illness onset dates.

We used “community-onset cases” and “cases among non-hospitalized patients” interchangeably to signify that symptom onset occurred outside the hospital. We defined preterm birth as <37 weeks (early preterm, <32 weeks; late preterm, ≥32 to <37 weeks) and full-term birth as ≥37 weeks.
estimated gestational age (EGA). We defined a neonate as an infant <28 days of age. For PIF, we used any commercially manufactured PIF product, including powdered human milk fortifier. (In the United States, powdered formulas, including follow-on formulas, are classified as PIF if they are intended for use among infants).

In addition to reviewing clinical information and feeding histories for each case, we reviewed results of available case investigations. Although there was no standard definition for food consumption in relation to disease onset across data sources, CDC’s reporting convention included any consumption within 7 days before symptom onset. *Cronobacter* infections are not nationally notifiable in the United States, but state and local health departments encountering new *Cronobacter* cases may contact CDC to request clinical consultation and submit clinical isolates, food, and environmental samples for laboratory testing. When possible, FDA tests PIF or other products from sealed containers of the same lots fed to the infant to assess whether contamination occurred during production. CDC used pulsed-field gel electrophoresis (PFGE) to assess similarity between clinical, food, and environmental isolates using 2 restriction enzymes, *XbaI* and *SpeI*. However, PFGE might have limited capacity to differentiate between genetically unrelated strains within the same clonal complex. In other investigations, whole-genome sequencing and multilocus sequencing typing has offered more precise determinations of genetic similarity.

We considered cases to be outbreak-associated if the clinical isolate’s PFGE pattern was indistinguishable from that of another case (invasive, noninvasive, or colonized). Alternatively, cases could be designated as outbreak-associated if they were detected in proximity with other cases both temporally (within 6 months) and spatially (in the same home or hospital). Defining the reporting period as 1961–2018, we compared reporting rates during the final quarter (2004–2018) with those during the preceding 3 quarters. We performed descriptive analysis using SAS software version 9.4 (https://www.sas.com), characterizing trends in annual reporting with negative-binomial regression, and comparing groups using χ² t-tests, and Wilcoxon signed-rank tests.

### Results

We identified 183 unique infants who met the case definition: 66 described in the literature, 61 from CDC case consultations, 53 from the WHO/FAO report, and 3 from PubMLST. Cases were reported from 24 countries across 6 continents (Table 1; Appendix (https://wwwnc.cdc.gov/EID/article/26/5/19-0858-App1.pdf)). Global annual reporting was significantly higher during the final quarter of the study period, increasing from a mean of 1.2 cases/year before 2004 to 8.7 cases/year from 2004 on (p<0.01). More than two thirds (130/183) of the cases were reported in the final quarter, when the proportion of outbreak-associated cases was significantly lower both in the United States and internationally (Table 2).

Among 79 US cases, most (61 [77%]) were reported to CDC; 15 cases were described in the literature only, most (13 [87%]) published before 2004. The proportion of cases among nonhospitalized US infants increased significantly, from 44% (8/18) before 2004 to 78% (42/54) of cases for 2004–2018 (p<0.01 by χ² test) (Figure). The proportion of cases reported among full-term US infants was also higher during this period; 22% (4/18) before 2004, compared with 56% (27/48) for 2004–2018 (p = 0.01 by χ² test) (Table 2). In contrast, outside the United States, a minority of cases occurred among nonhospitalized [15% (7/47)] or full-term [31% (11/47)] infants.

### Clinical Characteristics and Outcomes

Overall, 116 (63%) infants had meningitis and 67 (37%) had isolated bacteremia. Compared with patients with isolated *Cronobacter* bacteremia, patients with *Cronobacter* meningitis were significantly more likely to have experienced onset outside the hospital.

<table>
<thead>
<tr>
<th>Table 1. Numbers of reported invasive <em>Cronobacter</em> infections among infants, by country, 1961–2018</th>
</tr>
</thead>
<tbody>
<tr>
<td>Country</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>Argentina</td>
</tr>
<tr>
<td>Australia</td>
</tr>
<tr>
<td>Belgium</td>
</tr>
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<td>Brazil</td>
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<td>United States</td>
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</table>

feeding histories were described for 102 infants. Eighty-one (79%) reported recent consumption of PIF, with or without other supplemental feeding types, and 48 (47%) consumed PIF exclusively. Exclusive consumption of liquid infant formula was reported in 4 cases. Among 10 infants reported to consume only breastmilk in the 7 days before symptom onset, 5 received expressed breastmilk; reports did not clarify whether the breastmilk was expressed in the remaining 5 cases.

Source investigations were conducted for 71 (39%) cases. At least one contaminated environmental or food isolate was identified for 31 (44%) of these cases, and PIF contamination was identified in 30% (21/71) of investigations. Among 17 cases outside of the United States that had source investigations conducted, 14 (82%) had a transmission vehicle identified; 11 (79%) cases were linked to contaminated PIF (6 from sealed containers, 5 from open containers), 2 (14%) with blenders used to mix PIF, and 1 (7%) with contaminated EBM/breast pump (4,9,17,20–25).

Among 54 CDC-supported source investigations, ≥1 contaminated environmental or food isolate was identified for 17 (32%) investigations, each linked to a single case (Table 3). Cronobacter was isolated from 10 (22%) of the 46 open PIF containers tested; 5 (50%) of these isolates were obtained during 2004–2018. CDC identified Cronobacter from open PIF containers in 4 additional instances during 2004–2018; of these, 3 were associated with noninvasive cases and 1 was associated with a noninfant. All 4 PFGE patterns were indistinguishable from corresponding clinical isolates. FDA did not identify contaminated PIF from lot-matched sealed containers

### Table 2. Characteristics of 183 infants with invasive Cronobacter infection, overall and by clinical syndrome type, geographic location, and reporting period, 1961–2018*

<table>
<thead>
<tr>
<th>Characteristic*</th>
<th>Overall</th>
<th>Bacteremia</th>
<th>Meningitis</th>
<th>United States</th>
<th>Outside United States</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>103/130 (78)</td>
<td>27/29 (93)</td>
<td>20/26 (77)</td>
<td>27/49 (55)</td>
<td>24/41 (59)</td>
</tr>
</tbody>
</table>

**Values are no. (%); median (interquartile range). Neonatal onset defined as ≤28 d after birth; full-term birth was ≥37 weeks EGA; late preterm birth, 32–37 weeks EGA; early preterm birth, <32 weeks EGA. Outbreak-associated was defined as a clinical isolate’s pulsed-field gel electrophoresis pattern indistinguishable from that of another case (invasive, noninvasive, or colonized) or if detected in proximity with other cases both temporally (within 6 mo) and spatially (in the same home or hospital). Bold type indicates statistical significance (p<0.05) between reporting periods or syndrome types. EGA, estimated gestational age.
Food Safety and Cronobacter Infections

associated with clinical cases during 2001–2018; however, testing was not performed for all cases.

PIF preparation practices were recorded for only 4 cases in which Cronobacter was isolated from an opened PIF container. All caregivers reported proper hand hygiene; none reported reconstituting PIF using the WHO-recommended method of heating water to ≥70°C. In addition, evidence of environmental contamination was found during investigations of 6 cases: sink surfaces (n = 4), a bottle nipple (n = 1), and a pacifier (n = 1). Two cases involved contaminated EBM/breast pumps, and 2 cases involved contaminated bottled water used to reconstitute PIF. Among Cronobacter isolates identified from contaminated food or environmental samples, PFGE patterns were indistinguishable from corresponding clinical isolates 81% (13/16) of the time.

Discussion

Our findings provide continued evidence that invasive Cronobacter infections disproportionately affect infants in the neonatal period and are associated with high mortality. Early Cronobacter reports often featured hospitalized and preterm infants, but our findings suggest a rising majority of cases occurring among nonhospitalized and full-term infants in the United States. Contaminated PIF from opened containers is the most commonly identified transmission vehicle.

Reports of invasive infant Cronobacter infections appear to have increased globally, despite the lack of mandatory reporting. It is unclear whether the true incidence increased; this reporting increase might be because of more precise microbiologic identification, increased interest following publicized outbreaks and the WHO call for cases, and greater awareness about the larger public health implications (6,26). In addition, there was no name for Cronobacter spp. during the first quarter of reporting; early isolates were identified as Cronobacter spp. retrospectively. Enterobacter sakazakii was named as a species in 1980, and Cronobacter was proposed as a genus in 2007 (27). It is possible that the evolving nomenclature and identification methods may have resulted in both missed cases and inclusion of infections misclassified as Cronobacter.

Cronobacter reports outside the United States still predominantly feature hospitalized and preterm infants, whereas recent cases reported within the United States reflect higher proportions of cases among full-term, nonhospitalized infants. The reasons for these differences are likely multifactorial. Because we relied on published reports for cases among infants outside the United States, the characteristics of this group could have been influenced by a publishing bias; patients cared for in academic settings with advanced care capability might be the most likely to be detected and reported in the literature. In addition, outbreaks are more likely to be detected when cases occur in proximity, such as in neonatal intensive care units where most infants are preterm; although proportions of outbreak-associated cases declined globally in the final quarter, nearly one third of cases outside the United States were known to be associated with an outbreak (as opposed to 4% of US cases during that period). Likewise, the significant rise in cases among US full-term and nonhospitalized infants might be an

Figure. Reported invasive Cronobacter infections among infants, United States, 1979–2018, by location of patient at the time of symptom onset (n = 79). (The first case of invasive infant Cronobacter infection in the United States was reported in 1979 [13–15].)
artifact of surveillance changes during the period we examined; starting in 2001, CDC called for cases to be voluntarily reported in the United States, which likely contributed to higher proportions of sporadic cases being reported. Differential changes in risk exposure might also contribute to higher proportions of cases among full-term infants. In the United States, the 2002 FDA recommendation discouraging the use of PIF among hospitalized infants has likely helped prevent some cases among hospitalized and preterm infants; however, PIF continues to be used in neonatology units throughout much of the world (20).

High-risk groups for invasive Cronobacter infection include infants <2 months of age and infants born prematurely (even as reports of cases among full-term infants in the United States are becoming more common). Other clinical risk factors, however, are not straightforward. Immunocompromising and other concurrent conditions other than prematurity were not frequently reported and are not thought to be major drivers of disease acquisition in infants; reports of medical devices/interventions were also not common. Like previous reports, this analysis found Cronobacter meningitis was more common in full-term and late preterm infants, whereas Cronobacter bactere mia was more common in early preterm infants (1). The reason for this difference is unclear; however, timely initiation of empiric antimicrobial drug treatment among inpatients undergoing clinical monitoring could prevent invasion of bacteria beyond the blood–brain barrier (7).

PIF consumption was reported in most cases, and PIF from opened containers is the most commonly identified transmission vehicle. Even with small amounts of the remaining product available for testing, 10 (22%) PIF containers yielded Cronobacter during US investigations. More than half of these Cronobacter-contaminated containers were identified during the final quarter of the analytic period, as recently as 2017. CDC also identified Cronobacter-contaminated PIF from opened containers being used to feed 4 additional noninfant children or infants with noninvasive infections during 2004–2018, which were not included in this analysis (CDC, unpub. data). Finding Cronobacter in opened PIF containers does not prove that the PIF was intrinsically contaminated, as opposed to being contaminated after the container was opened; aside from the 2001 outbreak in a Tennessee hospital, FDA did not identify contaminated PIF from lot-matched sealed containers during this time period. Still, Cronobacter contamination during manufacturing remains a possibility; heterogeneous contamination might not be detected through even the most sensitive sampling schemes conducted by FDA and manufacturers (27).

Because PIF remains the most commonly identified transmission vehicle, prevention efforts should focus on minimizing the risk of PIF contamination through regulatory, engineering, and behavioral efforts, and promoting safer alternatives to PIF, particularly among infants 0–2 months of age. First, sustained focus is needed on identifying and maintaining effective quality control measures during PIF manufacturing. In keeping with regulatory standards, heat-labile nutrients are added to PIF after pasteurization, making it vulnerable to contamination during production, particularly with organisms like Cronobacter, which can persist for long periods in dry environments (28). The implementation of FDA’s 2014 quality control standards for safer PIF production was a crucial step toward preventing the distribution of intrinsically contaminated PIF. However, recent outbreaks of infant salmonellosis linked to contaminated PIF produced in Spain in 2010 and France in 2017 highlight the ongoing risk for PIF contamination occurring during production and the value of environmental monitoring for potential contamination (29,30).

Even if contamination does not occur during production, PIF can easily become contaminated once containers are opened and exposed to the environment. Although vigilant adherence to best hand-hygiene practices is necessary when preparing, handling, and storing PIF, engineering solutions, which remain a cornerstone of public health

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Sample type tested</th>
<th>Sample yielded</th>
<th>Sample isolate indistinguishable from clinical isolate†</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIF from opened containers</td>
<td>46/52 (87)</td>
<td>10/46 (22)‡</td>
<td>6/7 (86)</td>
</tr>
<tr>
<td>Breast pump collection kit/pump-expressed breastmilk</td>
<td>5/52 (9)</td>
<td>2/5 (40)</td>
<td>1/2(50)</td>
</tr>
<tr>
<td>Water from opened bottle</td>
<td>16/52 (31)</td>
<td>2/16 (13)</td>
<td>1/1 (100)</td>
</tr>
<tr>
<td>Environmental surfaces</td>
<td>17/52 (33)</td>
<td>6/17 (35)§</td>
<td>4/6 (67)‡</td>
</tr>
</tbody>
</table>

*Values are no. positive/no. tested (%). Results from first source investigation in the United States was reported in 1989 (9).
†Comparison made using pulsed-field gel electrophoresis.
‡Of 6 cases that yielded an isolate from an environmental surface, 4 were from kitchen sink surfaces, 1 from a pacifier, and 1 from a bottle nipple.
§Of 5 cases that yielded an isolate from an environmental surface, 4 were from kitchen sink surfaces, 1 from a pacifier, and 1 from a bottle nipple.
preventive efforts, are also needed. Redesigning PIF packaging with the aim of minimizing environmental exposure and contact with contaminated surfaces could help prevent transmission of Cronobacter and other pathogens (31). Healthcare providers, lactation consultants, and nutritionists play vital roles in supporting and educating caregivers about the risks of PIF contamination (32). They can help communicate that PIF is not a sterile product, while providing education and support for caregivers who choose to use PIF. The 2007 WHO recommendations to reconstitute PIF with water heated to $\geq$70°C should reduce pathogen load in reconstituted PIF, but these recommendations have not been universally adopted because of concerns about the potential effect on heat-sensitive nutritional components (including probiotics), impracticality, and burn risks (12). One report also suggested that the WHO-recommended method might not effectively kill all strains of Cronobacter (33). With these concerns in mind, promotion of safer alternatives to PIF, particularly for infants in the neonatal period, is needed.

Safer alternatives to PIF include direct breastfeeding, feeding with breastmilk that has been expressed safely, and feeding with liquid formula that has been safely handled and stored. Although the number of Cronobacter cases among infants who consumed PIF far outnumbered those among infants who exclusively received breastmilk, emerging reports of cases linked to contaminated EBM raise concern about hygiene practices during expression and handling of breastmilk. Comprehensive support can help caregivers to adhere to best practices. CDC offers guidance for proper breast pump hygiene, advising caregivers to wash hands thoroughly before handling pump equipment and to take apart and clean breast pump kits in the dishwasher or by hand with soap and water with a dedicated basin and brush after every use; air dry them on a clean surface in a protected area; and sanitize them at least daily by boiling, steaming, or using a dishwasher’s sanitize cycle.

The associated costs and required system supports should be taken into account when weighing the costs and benefits of PIF and PIF alternatives. RTF formula is more expensive than PIF; 2012 comparisons estimated that milk-based RTF formula cost US $0.84 (29%) more per day than milk-based PIF (12). Increased support for parental leave and onsite lactation facilities at places of employment will also make direct breastfeeding and safe breastmilk expression easier during critical exposure periods for Cronobacter and other infant illnesses.

The cases in which no source/vehicle was identified, as well as the cases occurring among infants who did not consume PIF or EBM, suggest that alternate modes of transmission are possible. Vertical transmission, although theoretically possible, is probably rare; only 4 patients experienced symptom onset in the first 48 hours after birth. C. malonaticus has been isolated from a breast abscess, but transmission during direct breastfeeding has never been reported (34). Contaminated environmental specimens suggest that fomite transmission could also occur, although the source of the contamination is unclear. Two cases involving Cronobacter-contaminated water (Table 3) highlight that even bottled and previously boiled water used to reconstitute PIF could become contaminated once opened and exposed to the environment, particularly if it does not contain residual chlorine.

This study has limitations. The changes in surveillance we describe might have affected the trend analyses, and because reporting Cronobacter cases is largely not mandatory, the analysis might have been influenced by a severity bias. Additional limitations include incomplete reporting of clinical characteristics, laboratory confirmation, feeding histories, and long-term outcomes. In some cases, clinical isolates were discarded before a public health laboratory could confirm the diagnosis. Other cases might have been excluded or the clinical syndrome misclassified if cultures were taken after initiation of antimicrobial drug therapy. Limitations of case investigations include that many food and environmental specimens were unavailable at the time of the investigation. Finally, the Cronobacter isolates included in this analysis were compared using PFGE, which might have limited capacity to differentiate between genetically unrelated strains within the same clonal complex. In other investigations, whole-genome sequencing and multilocus sequencing typing have offered more precise determinations of genetic similarity (35).

Considering the potential for severe outcomes and far-reaching policy implications, jurisdictions may consider making invasive Cronobacter infections among infants a reportable condition. Mandatory reporting with standardized reporting procedures would help better characterize incidence, elucidate risk factors, promptly detect outbreaks, and inform prevention measures. We encourage public health officials to contact CDC when investigating invasive Cronobacter cases in infants.

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References

Food Safety and Cronobacter Infections


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Candida auris is an emerging fungal pathogen that is typically resistant to fluconazole and is known to cause healthcare-associated outbreaks. We retrospectively reviewed 28 patients who had ≥1 positive culture for C. auris within a multisite health system in Illinois, USA, during May 2018–April 2019. Twelve of these patients were treated as inpatients for C. auris infections; 10 (83%) met criteria for clinical success, defined as absence of all-cause mortality, C. auris recurrence, and infection-related readmission at 30 days from the first positive culture. The other 2 patients (17%) died within 30 days. Most patients (92%) were empirically treated with micafungin. Four (14%) of 28 total isolates were resistant to fluconazole, 1 (3.6%) was resistant to amphotericin B, and 1 (3.6%) was resistant to echinocandins. Our findings describe low rates of antifungal resistance and favorable clinical outcomes for most C. auris patients.

Candida auris is an emerging, multidrug-resistant, healthcare-associated fungal pathogen that was first reported in Japan in 2009 and has now been isolated on 6 continents (1–9). C. auris has been identified as the causative pathogen in various invasive fungal infections, including bloodstream infections (2,4), and is associated with outbreaks across healthcare settings (6,10). Risk factors for C. auris infection are similar to other Candida infections including prolonged hospitalization, abdominal surgery, diabetes mellitus, intensive care unit (ICU) admission, use of central venous and urinary catheters, immunocompromising conditions, chronic kidney disease, and exposure to broad-spectrum antibiotic and antifungal agents (10–13). Investigations in the Chicago, Illinois, USA, area have found a high prevalence of C. auris colonization at ventilator-capable skilled nursing facilities (14) and have shown higher rates of C. auris colonization among patients who are mechanically ventilated, have a gastrostomy tube, or have a urinary catheter (15). Reported mortality rates attributable to invasive C. auris infection range from 30% to 59% globally (13,16) and from 22% to 57% in the United States (8,10,17).

C. auris isolates are often resistant to fluconazole and have variable susceptibility to other antifungal agents (13,16). The Centers for Disease Control and Prevention (CDC) currently recommends echinocandins as empiric therapy for suspected or confirmed C. auris colonization at ventilator-capable skilled nursing facilities (14) and have shown higher rates of C. auris colonization among patients who are mechanically ventilated, have a gastrostomy tube, or have a urinary catheter (15). Reported mortality rates attributable to invasive C. auris infection range from 30% to 59% globally (13,16) and from 22% to 57% in the United States (8,10,17).

Previous reports of C. auris infections and outbreaks have largely focused on epidemiologic information, and data on treatment strategies and clinical outcomes are limited (6,8,10,16–21). We report microbiologic data for C. auris isolates from a multisite health system in Illinois and an assessment of clinical outcomes for patients treated for C. auris infections.

Methods
This study is a retrospective cohort analysis of patients at 8 hospitals within a single health system located in the Chicago metropolitan area. We included all patients ≥18 years old who had ≥1 positive culture for C. auris from any anatomic site during January 1,
2008–April 30, 2019; we excluded pregnant patients, prisoners, and patients <18 years of age. If a patient had multiple positive cultures for C. auris, we included only the first positive culture per hospital encounter. Patients who died before culture result were not included in clinical success evaluation. The study received a non–human subjects research determination from the Advocate Aurora Health Institutional Review Board.

The microbiology laboratory for this system primarily uses matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (Vitek MS, bioMérieux, https://www.biomerieux.com) for organism identification. Because our database does not include C. auris, for isolates not identified by our MALDI-TOF mass spectrometry system, we used Vitek 2 version 8.01 (bioMérieux), which we have been using since December 2017. We sent isolates identified as C. haemulonii, C. duobushaemulonii, and Candida spp. not identified by Vitek 2 to the Illinois Department of Public Health for additional testing using MALDI-TOF mass spectrometry or genomic sequencing to rule out misidentification of C. auris. During June 2018–April 2019, we sent all isolates identified as C. auris to the Illinois Department of Public Health, which forwarded them to CDC for whole-genome sequencing. We performed antifungal susceptibility testing with colorimetric microdilution by using Sensititer YeastOne YO9 (TREK Diagnostics Systems, https://www.trekds.com). Because no C. auris susceptibility breakpoints have been established, we used tentative breakpoints published by CDC for interpretation in this study (22).

We performed manual chart review for all patients. We evaluated patient charts for demographic information, infection source, culture source and susceptibilities, empiric and definitive therapy, length of hospital and ICU stay, clinical success, and reports of adverse events associated with treatment for C. auris infection. We defined clinical success as the absence of 30-day all-cause mortality, 30-day recurrence of the same organism, and 30-day infection-related readmission. We identified adverse drug events associated with antifungal therapy by reviewing patient laboratory results and progress notes from healthcare providers.

**Results**

We evaluated records from 8 hospitals from the period of January 1, 2008, through April 30, 2019, for C. auris isolates. Cultures were obtained as part of routine clinical practice. A total of 28 patients from 5 hospitals had ≥1 positive culture for C. auris during the study period. We included 28 C. auris isolates in this study (the first isolate from our health system was collected in May 2018). Members of the cohort had a median age of 70 years (interquartile range 62–78 years), and most (20 [71%]) patients were men. Most (26 [93%]) patients were admitted from a skilled nursing facility; 1 patient was transferred from another hospital, and 1 was admitted from the community. Nine (75%) patients required chronic mechanical ventilation, and 6 (21%) were receiving hemodialysis through a central line. Most isolates were cultured from blood (12 [43%]) or urine (10 [36%]). The median time from admission to collection of the first culture positive for C. auris was 0.14 days (interquartile range 0–0.88 days). The average hospital stay for inpatients was 12 days. Thirteen patients (46%) were admitted to an ICU; the average ICU stay was 3 days.

MICs for the 28 C. auris isolates (Table 1) showed that 4 (14%) were resistant to flucytosine, 1 (3.6%) was resistant to amphotericin B, and 1 (3.6%) was resistant to echinocandins, according to tentative C. auris breakpoints published by CDC (22). One isolate was resistant to flucytosine, amphotericin B, and echinocandins. This isolate was from a patient who was considered to be colonized with C. auris in the urine and did not receive antifungal therapy.

Twelve patients (43%) were treated as inpatients for C. auris infections (Table 2). Of those patients who

<table>
<thead>
<tr>
<th>Antifungal drug</th>
<th>MIC, µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.015 0.03 0.06 0.12 0.25 0.5 1 2 4 8 16 64 128 256 &gt;256</td>
</tr>
<tr>
<td>Anidulafungin</td>
<td>3.6 64.3 28.6</td>
</tr>
<tr>
<td>Caspofungin</td>
<td>3.6 25.0 42.9 25.0</td>
</tr>
<tr>
<td>Micafungin</td>
<td>50.0 39.3 3.6 3.6</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>39.3 35.7 10.7 3.6 3.6 3.6 3.6</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>7.1 32.1 39.3 17.9 3.6</td>
</tr>
<tr>
<td>Posaconazole</td>
<td>10.7 50.0 25.0 14.3</td>
</tr>
<tr>
<td>Voriconazole</td>
<td>42.9 21.4 21.4 3.6</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>10.7 82.1 3.6</td>
</tr>
<tr>
<td>Flucytosine</td>
<td>17.9 71.4 10.7</td>
</tr>
</tbody>
</table>

*Values are percentage of isolates having the MIC shown. Shaded values are considered resistant on the basis of Centers for Disease Control and Prevention tentative C. auris breakpoints (22).
were not treated with antifungal therapy, 2 were evaluated in the emergency department and were discharged back to their skilled nursing facility in stable condition before their blood cultures results were available, 3 patients died before culture results were available, and 11 were considered to be colonized with \textit{C. auris}. Of those patients who were treated for \textit{C. auris} infections, most were found to have a central

Table 2. Demographic and clinical characteristics of patients treated for \textit{Candida auris} infections in a multisite health system, Illinois, USA*

<table>
<thead>
<tr>
<th>Patient age, y/sex</th>
<th>Culture source (infection type)</th>
<th>Empiric treatment</th>
<th>Definitive treatment</th>
<th>Treatment duration</th>
<th>Outcome</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>83/M</td>
<td>Urine (CA-UTI)</td>
<td>Micafungin 100 mg IV every 24 h</td>
<td>Micafungin 100 mg IV every 24 h</td>
<td>5 d</td>
<td>Clinical success</td>
<td>Trach to vent patient with dementia. Urine culture earlier in admission showed 10,000–50,000 CFU \textit{C. auris}, but thought to be colonization and was not treated. Repeat urine culture showed &gt;100,000 CFU \textit{C. auris}, and patient was treated.</td>
</tr>
<tr>
<td>56/M</td>
<td>Blood (CLABSI)</td>
<td>Micafungin 100 mg IV every 24 h</td>
<td>Fluconazole 200 mg per PEG every 24 h</td>
<td>15 d</td>
<td>Clinical success</td>
<td>Trach to vent patient with ESRD on HD with tunneled catheter, also had a PICC. Both lines were removed.</td>
</tr>
<tr>
<td>73/M</td>
<td>Blood (CLABSI)</td>
<td>Micafungin 100 mg IV every 24 h</td>
<td>Micafungin 100 mg IV every 24 h</td>
<td>17 d</td>
<td>Clinical success</td>
<td>Trach to vent patient with ESRD on HD with tunneled catheter, chronic osteomyelitis of the coccyx. \textit{C. auris} from culture of HD line at SNF. Tunneled catheter removed.</td>
</tr>
<tr>
<td>64/F</td>
<td>Blood (CLABSI)</td>
<td>Micafungin 100 mg IV every 24 h</td>
<td>Micafungin 100 mg IV every 24 h</td>
<td>26 d</td>
<td>Died</td>
<td>Trach to vent patient with ESRD on HD with chest port and PICC for TPN. Lines removed. 42 d of therapy planned; patient readmitted for presumed septic shock and died on day 26 after being switched to comfort care. No growth of any organisms in cultures on readmission.</td>
</tr>
<tr>
<td>61/M</td>
<td>Catheter tip</td>
<td>Micafungin 100 mg IV every 24 h</td>
<td>Micafungin 100 mg IV every 24 h</td>
<td>21 d</td>
<td>Clinical success</td>
<td>Trach patient with ESRD on HD with tunneled catheter admitted for fungemia. Started on micafungin before admission. Line removed. Azole not used because of concomitant amiodarone.</td>
</tr>
<tr>
<td>74/M</td>
<td>Urine (CA-UTI)</td>
<td>Micafungin 100 mg IV every 24 h</td>
<td>Micafungin 100 mg IV every 24 h</td>
<td>Unknown</td>
<td>Clinical success</td>
<td>Trach to vent patient. Patient transferred to SNF before culture finalized; duration of micafungin to be determined by SNF.</td>
</tr>
<tr>
<td>74/F</td>
<td>Blood (CLABSI)</td>
<td>Micafungin 100 mg IV every 24 h</td>
<td>Fluconazole 400 mg PO every 24 h</td>
<td>21 d</td>
<td>Clinical success</td>
<td>SNF patient on chronic TPN for enterocutaneous fistulas, history of line infections and infective endocarditis. Persistently fungemic for 4 d until tunneled central line was removed.</td>
</tr>
<tr>
<td>50/F</td>
<td>Abdominal wound</td>
<td>Micafungin 100 mg IV every 24 h</td>
<td>Micafungin 100 mg IV every 24 h</td>
<td>10 d</td>
<td>Clinical success</td>
<td>Patient with obesity, diabetes, and chronic abdominal/groin ulcers hospitalized for DKA; receives wound care at home. Ulcers underwent debridement; \textit{C. auris}, CoNS, and \textit{Corynebacterium} grew from operative cultures.</td>
</tr>
<tr>
<td>78/M</td>
<td>Blood</td>
<td>Fluconazole 400 mg IV every 24 h</td>
<td>Itraconazole 200 mg per PEG every 24 h</td>
<td>14 d</td>
<td>Clinical success</td>
<td>Trach to vent after cardiac arrest, midline POA for hypotension and hypoxia. Midline thought to be source. Discharged to hospice, but continued antifungal therapy. Lost to follow-up.</td>
</tr>
<tr>
<td>79/M</td>
<td>Blood (CLABSI)</td>
<td>Micafungin 100 mg IV every 24 h</td>
<td>Micafungin 100 mg IV every 24 h</td>
<td>5 d</td>
<td>Died</td>
<td>Trach, ESRD on HD with tunneled catheter. Blood culture also showed growth of \textit{Proteus mirabilis}. Died from septic shock after switching to comfort care. Repeat blood cultures showed no growth.</td>
</tr>
<tr>
<td>78/F</td>
<td>Hip synovial fluid</td>
<td>Micafungin 100 mg IV every 24 h</td>
<td>Micafungin 100 mg IV every 24 h</td>
<td>6 d</td>
<td>Clinical success</td>
<td>ESRD on HD with tunneled catheter, DM, prosthetic mitral valve, treated for drainage from hip after hip replacement 3 mo prior, had onset of septic shock after I&amp;D procedure. \textit{C auris} isolated from hip aspirate. Antifungal treatment stopped after 6 d because \textit{C. auris} was a suspected contaminant. Died in hospital &gt;30 d after \textit{C auris} isolation.</td>
</tr>
<tr>
<td>82/M</td>
<td>Blood (CLABSI)</td>
<td>Micafungin 100 mg IV every 24 h</td>
<td>Micafungin 100 mg IV every 24 h</td>
<td>14 d</td>
<td>Clinical success</td>
<td>Patient with functional quadriplegia after CVA. Trach, PEG, PICC, and chronic foley catheter POA. PICC removed.</td>
</tr>
</tbody>
</table>

*CA-UTI, catheter-associated urinary tract infection; CFU, colony forming units; CLABSI, catheter-associated urinary tract infection; CoNS, coagulase negative \textit{Staphylococcus}; CVA, cerebral vascular accident; DKA, diabetic ketoacidosis; DM, diabetes mellitus; ESRD, end-stage renal disease; HD, hemodialysis; I&D, incision and debridement; PEG, percutaneous endoscopic gastrostomy; PICC, peripherally inserted central catheter; POA, present on admission; SNF, skilled nursing facility; TPN, total parenteral nutrition; trach, tracheotomy; vent, ventilator.

868  Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 26, No. 5, May 2020
line-associated bloodstream infection (CLABSIs) (7 [59%]), whereas others were treated for catheter-associated urinary tract infection (2 [17%]), skin and skin structure infection (2 [17%]), and other bloodstream infection (BSI) (1 [8%]). All patients who were treated for C. auris infections were under the care of a physician specialized in infectious diseases.

Patients were empirically treated with micafungin (11 [92%]) or fluconazole (1 [8%]). Of those patients empirically treated with micafungin, most were being treated for CLABSIs (7 [64%]), followed by catheter-associated urinary tract infection (2 [18%]) and skin and skin structure infection (2 [18%]). For definitive treatment, patients received micafungin (9 [75%]), fluconazole (2 [17%]), or itraconazole (1 [8%]). Of those patients who received an azole as definitive treatment, all were being treated for BSIs. Treatment duration ranged from 5 to 26 days (mean 14 days). The only adverse event noted was an increase in aspartate aminotransferase from 25 to 91 U/L in 1 patient being treated with fluconazole. Fluconazole was continued, and the patient was discharged on fluconazole to complete their treatment course.

Ten (83%) patients met criteria for clinical success. No patients were found to have C. auris recurrence or infection-related readmission within 30 days of first positive culture. Two (17%) patients died within 30 days of first positive culture; both were being treated for CLABSIs.

**Discussion**

We report patient characteristics and microbiologic data for 28 patients with ≥1 positive culture for C. auris. We also describe the clinical outcomes for 12 patients treated for C. auris infections. Our observed mortality rate of 17% is lower than previously reported worldwide. A meta-analysis of 742 patients from 16 countries found an all-cause mortality rate of 30% (13). Our lower mortality rate might be a result of empirically selecting echinocandin therapy, to which >95% of our isolates were susceptible. Empiric selection of fluconazole for treatment of C. auris BSI was recently described in a case series in India (19). C. auris is often resistant to fluconazole, and lack of effective empiric therapy can result in poor outcomes. Because echinocandins are commonly empirically selected for treatment of candidemia in the United States, our lower mortality rates might be attributable to lower MICs in our geographic region. For instance, investigators of an outbreak in New York, New York, found a 45% mortality rate among 51 patients with BSIs at 90 days (10). The mortality rate in our study was 25% among patients with BSI. A separate case series from Brooklyn, New York, reported a 22% in-hospital mortality rate among a cohort of 9 patients with BSIs, which is similar to our findings (17). Small sample size and variations in underlying conditions might confound mortality rate comparisons, although another possible explanation for the lower mortality rate in our study is differences in antifungal resistance.

Previous reports of C. auris collections have noted substantially higher rates of antifungal resistance than what was observed in our cohort. In a grouping of 54 isolates from 5 countries, 93% were resistant to fluconazole, 35% to amphotericin B, and 7% to echinocandins (16). The first isolates from the United States demonstrated a similar pattern; 86% were resistant to fluconazole, 43% to amphotericin B, and 3% to echinocandins (18). An even higher rate of fluconazole resistance of 98% was noted in New York, New York (10). Drug-resistance mechanisms are genetically encoded, and some resistance mutations are linked to specific geographic clades (16). An epidemiologic investigation of 133 C. auris isolates from the United States showed that these isolates were genetically related to 1 of 4 major clades (from South America, Africa, East Asia, and South Asia) (20). Previous isolates from Illinois were found to be from the South American clade (15,20). The molecular epidemiology of the isolates we identified was beyond the scope of this investigation because the results of genomic sequencing were not shared with clinicians or laboratory personnel within our health system. Further research would be needed to determine whether these isolates are genetically distinct from clades previously noted to be present in the United States.

Most patients in this study were successfully treated with an echinocandin, which is consistent with treatment recommendations from the CDC (22). The 2 members of our cohort who died within 30 days of first positive culture attained microbiologic eradication with echinocandin therapy before they died. Three patients received azole antifungals as empiric or definitive treatment, and all 3 met our criteria for clinical success. However, treatment failures with fluconazole have been reported despite in vitro susceptibility (8,21). Echinocandin-resistant C. auris, possibly induced by antifungal pressure, has also been reported in the United States and in other countries (16,21). Patients with persistent or recurrent C. auris infections might require repeat susceptibility testing.

Several antifungal agents currently in development have activity against C. auris, including SCY-078, the first orally bioavailable 1,3-β-D-glucan synthesis inhibitor; VT-1598, a tetrazole-based lanosterol 14α-demethylase inhibitor; APX001, which interrupts
glycosylphosphatidylinositol biosynthesis by inhibiting the fungal enzyme Gwt1; and CD101, an echinocandin that can be administered once weekly (12). In the future, these agents might become treatment options for C. auris infections, including those caused by isolates resistant to conventional therapies.

Most patients in our study who required treatment for C. auris were previously exposed to skilled nursing facilities and had multiple risk factors for invasive Candida infections, including central venous and urinary catheters (12). No patients were thought to have acquired C. auris infection during their hospital admission. The possibility of transmission within hospitals is a concern, given that C. auris has been shown to colonize the skin, persist on surfaces in the healthcare environment, and cause healthcare-associated outbreaks (10,21). Contact precautions, hand hygiene, and environmental cleaning and disinfection are essential to preventing the spread of C. auris, and these infection control practices are used within our health system (22).

Our study is limited by a small sample size and the inherent limitations of a retrospective, observational case series. In addition, evaluation of clinical outcomes at 30 days from the first positive culture prevented us from capturing any adverse outcomes that might have occurred after 30 days. However, our data provides insight on patient exposures. Most of these patients came from skilled nursing facilities. Targeted infection prevention and antimicrobial stewardship measures within these facilities might help to reduce the emergence and progression of resistance of this organism. Our experiences with C. auris are notable for favorable clinical outcomes and low rates of antifungal resistance. However, the development of resistance remains a concern, and patient response to treatment should be monitored closely.

**Acknowledgments**

We thank Eric Beck for his assistance with describing our laboratory’s identification and susceptibility testing protocols.

**About the Author**

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**References**


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Mosquito Control Activities during Local Transmission of Zika Virus, Miami-Dade County, Florida, USA, 2016

Janet C. McAllister, Mario Porcelli, Johana M. Medina, Mark J. Delorey, C. Roxanne Connelly,† Marvin S. Godsey, Nicholas A. Panella, Nicole Dzuris, Karen A. Boegler, Joan L. Kenney, Linda Kothera, Lucrecia Vizcaino, Audrey E. Lenhart, John-Paul Mutebi, Chalmers Vasquez

Zika virus (ZIKV), a flavivirus that can cause birth defects and is associated with Guillain-Barré syndrome, has rapidly spread throughout the Western Hemisphere (1–3). The virus is spread primarily by the bite of infected *Aedes aegypti* mosquitoes; sexual transmission and bloodborne transmission also have been documented (4,5). The southern United States is habitable for *Ae. aegypti* mosquitoes, which are predominantly an urban species. Miami-Dade County, Florida, has well-established *Ae. aegypti* mosquito populations and is a major travel destination (15.8 million visitors reported in 2016 [http://partners.miamiandbeaches.com/tools-and-resources/research-and-statistics]). In addition, the county has a large population of residents who routinely visit countries that had Zika outbreaks in 2016.

In January 2016, the county documented its first travel-associated case of ZIKV infection (6). The first cluster of local vector-transmitted cases was identified through epidemiologic investigation in the Wynwood neighborhood of Miami on July 21, 2016, and was confirmed and announced on July 29 (7). Following Centers for Disease Control and Prevention (CDC) guidelines (https://www.cdc.gov/zika/public-health-partners/cdc-zika-interim-response-plan.html) a “red zone” or travel warning was declared for pregnant women to avoid unnecessary travel to areas within ≈1 square mile around the cluster of cases (Figure 1). Subsequent clusters were identified south of 28th Street in Miami Beach on August 19, initiating a second red zone of 1.5 square miles. On September 16, a cluster was identified north of 28th Street in Miami Beach, expanding the second red zone by another 1.5 square miles to the north. On October 13, a fourth cluster was identified, and a red zone was declared in the Little River area of Miami, although all but 1 case occurred before October 13.

As each cluster was identified, Miami-Dade County Health Department began intensified epidemiologic surveillance to detect additional cases. Concurrently, the Miami-Dade County Mosquito Control Division (MCD) initiated intensive mosquito control activities within each new cluster of local transmission. We describe the mosquito control activities used by MCD during the local transmission of ZIKV in Miami-Dade County, 2016.

In 2016, four clusters of local mosquito-borne Zika virus transmission were identified in Miami-Dade County, Florida, USA, generating “red zones” (areas into which pregnant women were advised against traveling). The Miami-Dade County Mosquito Control Division initiated intensive control activities, including property inspections, community education, and handheld sprayer applications of larvicides and adulticides. For the first time, the Mosquito Control Division used a combination of areawide ultralow-volume adulticide and low-volume larvicide spraying to effectively control *Aedes aegypti* mosquitoes, the primary Zika virus vector within the county. The number of mosquitoes rapidly decreased, and Zika virus transmission was interrupted within the red zones immediately after the combination of adulticide and larvicide spraying.

SYNOPSIS

In 2016, four clusters of local mosquito-borne Zika virus transmission were identified in Miami-Dade County, Florida, USA, generating “red zones” (areas into which pregnant women were advised against traveling). The Miami-Dade County Mosquito Control Division initiated intensive control activities, including property inspections, community education, and handheld sprayer applications of larvicides and adulticides. For the first time, the Mosquito Control Division used a combination of areawide ultralow-volume adulticide and low-volume larvicide spraying to effectively control *Aedes aegypti* mosquitoes, the primary Zika virus vector within the county. The number of mosquitoes rapidly decreased, and Zika virus transmission was interrupted within the red zones immediately after the combination of adulticide and larvicide spraying.
Figure 1. Locations of declared zones where clusters of locally acquired vectorborne Zika virus transmission were identified and aerial mosquito control activities conducted, Miami-Dade County, Florida, USA, 2016.
to address the clusters of locally acquired ZIKV and their effect on subsequent mosquito numbers and Zika transmission.

Mosquito Control Methods
The Miami-Dade County Health Department notified the county MCD of all suspected or confirmed ZIKV infections. Relevant addresses (i.e., home, work) associated with each notification were inspected for the presence of Ae. aegypti mosquitoes. On the basis of the inspection, source reduction and application of larvicide, adulticide, or both were performed as needed. In addition, MCD attempted to inspect all properties in a 150-meter radius of the case-patient’s house. MCD made multiple visits to reach all homes. At a minimum, front yards of all properties were evaluated, and when house occupants granted permission, backyards as well. MCD left educational materials at all properties.

In the red zones, control activities expanded to include all properties within the zone. Inspection of individual properties helped the MCD define the most common containers that served as larval habitats. The MCD recorded only presence or absence of larvae and did not attempt to quantify or identify the species; thus, mosquito species other than Ae. aegypti might have been present. Regardless of the mosquito species present, the MCD treated them either by removing the water (dumping) or applying a larvicide. During July 23–December 29, MCD conducted 352,209 property inspections countywide. The Wynwood red zone had 1,721 parcels on which 5,974 inspections occurred. During August 19–December 29, MCD conducted 8,755 inspections in the southern Miami Beach and 6,872 inspections in the northern Miami Beach (2,783 parcels) red zones. In Little River, MCD conducted 3,239 inspections on the 2,075 parcels within the red zone during October 14–December 29.

The 24,795 inspections in the 4 red zones identified a total of 2,720 containers with larval mosquitoes. Most (92%) containers with larval mosquitoes were of 3 types: drains, predominately storm drains (33%); plants, predominately bromeliads (35%); and small containers that were easily dumped (25%). Saucers beneath potted plants were included in the small containers–dumpable category. The next most common larval mosquito habitat was tires, constituting 4% of larvae-positive containers. The remaining container types represented <1% of the total: small containers–permanent, plastic construction barriers, fountains, pools, boats, ponds, ditches, and hot tubs.

The distribution of the most common container types was not uniform across the county. In Wynwood, plants were the most abundant container with larvae (26%) (Figure 2, panel A). In northern Miami Beach, plants accounted for 61% of the containers with larvae (Figure 2, panel B). In southern Miami Beach, drains contributed almost half (47%) of the larval sites (Figure 2, panel C). In Little River, small containers–dumpable accounted for 39% of containers with mosquito larvae (Figure 2, panel D). In addition, red zones received ultraslow-volume (ULV) spraying of adulticide and low-volume spraying of larvicide delivered by airplane or truck-mounted equipment (Table 1).

Mosquito Surveillance
A routine surveillance system for Ae. aegypti mosquitoes was not in place before August 2016. In each red zone, surveillance for adult Ae. aegypti mosquitoes was initiated as soon as a new zone was identified. BG Sentinel traps enhanced with BG-lures (Biogents, https://eu.biogents.com) and dry ice were deployed. Trap density was 17–19 traps/zone/night. Adult mosquitoes from each trap were counted and identified daily until the red zone designation was removed. The predominant species collected in the BG Sentinel traps was Ae. aegypti (86%), followed by Culex quinquefasciatus (L.) (14%). All other mosquito species comprised <1%. To compare different treatment strategies in Wynwood, we set additional traps in an area around the red zone that received aerial adulticide applications only and inside the red zone where both aerial adulticide and larvicide were applied. Because traps were not readily available in August and early September, traps were moved after 2 weeks from the Wynwood adulticide only area for use in subsequent red zones. As a result, continued surveillance in the area that received aerial adulticide only was not available for longer-term (6 weeks) comparison to aerial adulticide plus larvicide treatments.

Insecticide Resistance
Laboratory Assays
The insecticide resistance status of Ae. aegypti mosquitoes in Miami-Dade County was not known at the beginning of the outbreak. At the same time that the intensified mosquito control activities began in Wynwood and Miami Beach, Ae. aegypti eggs and adults were collected to evaluate their susceptibility to the active ingredients found in various commercial adulticide products, including those
routinely used by the MCD. Eggs were reared in an insectary at 27°C and 80%-90% humidity, with 14 h daylight, and the resulting adults were used in the laboratory bioassays. CDC bottle bioassay (8) was performed using technical-grade perme-thrin, 43 µg/bottle; deltamethrin, 0.75 µg/bottle; etofenprox, 12.5 µg/bottle; sumithrin, 20 µg/bottle; naled, 2.25 µg/bottle; and malathion, 400 µg/bottle. Bottle concentrations and threshold times were based on prior calibration of the assay as described previously (8). All technical-grade insecticides came from ChemService Inc. (https://www.chemservice.com). Ae. aegypti Orlando strain mosquitoes were used as a susceptible comparison colony. This colony was started in 1952 at what is now the US Department of Agriculture’s Agricultural Research Service, Center for Medical, Agricultural and Veterinary Entomology (Gainesville, FL, USA). The CDC bottle bioassay revealed high levels of resistance to all synthetic pyrethroids at the diagnostic time; sumithrin (3%-14% death), etofenprox (1%-7% death), permethrin (2%-12% death), and deltamethrin (5%-65% death). We found no resistance to malathion or naled (Table 2).

Field Assays
Because resistance in laboratory assays does not directly translate to product failure in the field, we field-tested commercial products to find the most efficacious pyrethroid product for use in truck-mounted ULV spraying. The MCD used the midlabel rate (Table 1) for product application before and early in the outbreak. Mosquitoes collected in BG sentinel traps were held in a BugDorm2 Insect Tent (BioQuip, https://www.bioquip.com) and supplied with 10% sucrose until use in field testing. Field testing consisted of placing adult mosquitoes in cages, then exposing them to the commercial product applied at the mid-label rate with a truck-mounted Grizzly ULV Sprayer (Clarke, https://www.clarke.com). Cages were 7.6 m and 15.2 m from the road. Fifteen minutes after insecticide exposure, mosquitoes were transferred to clean holding containers (236.5-mL cardboard ice cream cups covered with netting) and given
access to a 10% sucrose solution. Deaths were recorded 24 h after treatment (Table 2). Additional field testing was conducted using the highest label rate (Table 1) of DeltaGard (deltamethrin; Bayer CropScience LP, https://www.environmentscience.bayer.com) and Zenivex (etofenprox; Central Life Sciences, https://www.centrallifesciences.com). We chose Zenivex for additional testing because the wind appeared to shift direction during the initial application, causing the treatment to not fully reach all cages. We chose DeltaGard because it performed the best in both bottle bioassay and the previous field testing using the midlabel rate. DeltaGard was selected as the best performing product when applied at the highest label rate (0.00134 lb/acre) and was used for truck-mounted spraying in the northern Miami Beach and Little River red zones where aerial spraying did not occur.

**Insecticide Treatments**

In addition to treating vegetation with a synthetic pyrethroid during property inspections, MCD applied adulticides using trucks throughout the county during property inspections, MCD applied adulticides using trucks throughout the county. Insecticide field testing showed that the highest label rate of DeltaGard could be effective (93% death). Thus, in the red zones in northern Miami Beach and Little River, a 1.5-square mile each were treated with DeltaGard using a truck-mounted Grizzly ULV Sprayer. Treatments occurred on a similar schedule as was used for aerial spraying, with the initial 2 treatments within 4 days of each other followed by 2 more treatments at weekly intervals.

**Table 1. Mosquito control products used by the county Mosquito Control program, Miami-Dade County, Florida, USA, 2016**

<table>
<thead>
<tr>
<th>Product name</th>
<th>Active Ingredient</th>
<th>Life stage targeted</th>
<th>Method of application</th>
<th>Application rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomist*</td>
<td>Permethrin/piperonyl butoxide</td>
<td>Adult</td>
<td>Backpack/truck</td>
<td>0.0035 lb/acre</td>
</tr>
<tr>
<td>Duet*</td>
<td>Sumithrin and pallethrin</td>
<td>Adult</td>
<td>Backpack/truck</td>
<td>0.0035 lb/acre</td>
</tr>
<tr>
<td>Zenivex†</td>
<td>Etofenprox</td>
<td>Adult</td>
<td>Truck</td>
<td>0.0035 lb/acre</td>
</tr>
<tr>
<td>DeltaGard‡</td>
<td>Deltamethrin</td>
<td>Adult</td>
<td>Truck</td>
<td>0.0035 lb/acre</td>
</tr>
<tr>
<td>Dibrom§</td>
<td>Naled</td>
<td>Adult</td>
<td>Airplane</td>
<td>0.1 lb/acre</td>
</tr>
<tr>
<td>Vectobac WGD¶</td>
<td>Bacillus thuringiensis israelensis</td>
<td>Larva</td>
<td>Truck/airplane/hand</td>
<td>0.5 lb/acre</td>
</tr>
<tr>
<td>Abate#</td>
<td>Temephos</td>
<td>Larva</td>
<td>Backpack/hand</td>
<td>**</td>
</tr>
<tr>
<td>Allosid†</td>
<td>Methoprene</td>
<td>Larva</td>
<td>Hand</td>
<td>**</td>
</tr>
</tbody>
</table>

‡Bayer CropScience LP, https://www.bayer.com. Rate of application was increased to the maximum amount allowable by the label after field trials to determine effective rate were concluded October 10, 2016.
**Application rate depended on the container.

**Table 2. Percentages of mosquito populations susceptible to active ingredients or products used for adult mosquito control in laboratory bioassays and field tests of Aedes aegypti mosquitoes, Miami-Dade County, Florida, USA, 2016**

<table>
<thead>
<tr>
<th>Chemical/product</th>
<th>Bottle dosage, µg/bottle</th>
<th>CDC bottle bioassay</th>
<th>At 1/2 label rate in field assay</th>
<th>At full label rate in field assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naled†</td>
<td>2.25</td>
<td>100</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Malathion†</td>
<td>400</td>
<td>100</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Deltamethrin/DeltaGard‡</td>
<td>0.75</td>
<td>5–65</td>
<td>80</td>
<td>93</td>
</tr>
<tr>
<td>Etofenprox/Zenivex§</td>
<td>12.5</td>
<td>1–7</td>
<td>19</td>
<td>57</td>
</tr>
<tr>
<td>Permethrin/Biomist‖</td>
<td>43</td>
<td>2–12</td>
<td>33</td>
<td>NA</td>
</tr>
<tr>
<td>Sumithrin/Duet‖</td>
<td>20</td>
<td>3–14</td>
<td>44</td>
<td>NA</td>
</tr>
</tbody>
</table>

*NA, test not conducted because mosquitoes were susceptible to active ingredient or field test results excluded it from further testing.
†Used in bottle bioassays only. No field tests were conducted because mosquitoes were susceptible to this chemical.
Bacillus thuringiensis israelensis, was applied every 7 days for 4 weeks in all 4 red zones. This larvicide was applied by aircraft in Wynwood and by a CSM3 Turbine Vector Sprayer/Duster (Buffalo Turbine, https://buffaloturbine.com) mounted on a truck in southern Miami Beach, northern Miami Beach, and Little River. In Wynwood, a 2 m² area was treated, but in the other red zones, larvicide treatments covered the same areas as the adulticide treatments.

Effect on Mosquito Abundance and Zika Infections
Because the response to the Zika outbreak in southern Florida was an emergency public health intervention, there was no time to set up proper controls. Therefore, we cannot evaluate properly using common comparison techniques the effect of the interventions. Instead, we used a changepoint analysis. A changepoint occurs if a time at which the statistical properties of the ordered sequence of observed case counts change. Case counts evaluated here are adult Ae. aegypti counts from BG Sentinel traps. A sequence can have >1 changepoint. In this analysis, the characteristic we assessed is the mean Ae. aegypti count change during the time observed. We consider 2 hypotheses: 1) Ae. aegypti counts during the entire period derive from a Poisson distribution with a constant mean, and 2) ≥2 time intervals exist, in each of which the Ae. aegypti counts derive from Poisson distributions with different means. We used a likelihood approach using binary segmentation, as described previously (9), and implemented in the R package changepoint (10) to identify whether the data were consistent with hypothesis 1 or hypothesis 2. With each binary segmentation of the sequence, the Akaike information criterion with a correction for small sample size (AICcs) of the models with and without a changepoint, were computed. A model with the changepoint was considered a better fit if its AICc was smaller by ≥6 than the AICc of the model without the changepoint, which corresponds roughly with a type I error of 0.05.

We obtained dates of human cases from the Florida Department of Health website (http://www.floridahealth.gov/newsroom/2016/11/113016-zika-update.html). Because the Little River red zone was declared when mosquito numbers were naturally dropping due to seasonality and mosquito-borne transmission had ceased by the time that red zone was identified, we did not evaluate changepoint analysis and transmission after spraying.

Although we cannot make a statistical association with the location of the changepoints in the other red zones, it is interesting that the first changepoints occurred after adulticide treatments began. In the Wynwood red zone that received both aerial adulticide and larvicide (Figure 3, panel A), this changepoint represents a large drop in mean Ae. aegypti counts. With further adulticiding and larviciding, the counts remained low and, in fact, dropped further on
August 24. On approximately this date, insecticide applications stopped, and this date is followed by a third changepoint at which mean *Ae. aegypti* counts increased again.

The only changepoint in the 10-mile area around Wynwood that received adulticide only occurred after the adulticiding began. We do not know what the mean *Ae. aegypti* counts were before August 9 (Figure 3, panel B). However, superimposing the counts for 10-mile region around the Wynwood neighborhood over those for the Wynwood neighborhood (as shown in Figure 3, panel C) showed that the mean *Ae. aegypti* counts for August 9 were comparable. Counts before August 9 might also have been comparable, but we have no way to verify that possibility. *Ae. aegypti* counts then increased in the region around Wynwood, whereas mean counts within Wynwood remained low. One possible explanation for this increase is that larviciding was not done in the 10-mile region around Wynwood. This observation reinforces the concept that both adulticiding and larviciding are needed to quickly reduce mosquito populations and maintain suppression. As reported previously (7), detection of new Zika virus infections in Wynwood stopped after adulticide treatments began (Figure 4).

In the southern Miami Beach red zone, we again saw that the first changepoint occurred after the first adulticide treatments (Figure 3, panel D). There was a slight increase in mosquito count after the initial decrease (although this is not statistically a changepoint). Once the larvicide treatments began on September 6, mean *Ae. aegypti* counts decreased again, and 2 changepoints in mean counts followed the start of the larviciding. New cases of Zika virus ceased immediately after the first aerial adulticide treatments. However, for 4 weeks, single cases occurred roughly weekly after the last aerial adulticide treatment.

In the northern Miami Beach red zone, the first changepoint again occurred after the first adulticide treatments (Figure 3, panel E). After larviciding, the
mean counts remained low and were followed by 2 more changepoints in mean *Ae. aegypti* counts. Again, for a third time no new Zika virus infections occurred after the first adulticide treatments.

We do not know what the *Ae. aegypti* counts were ahead of treatments or what would have occurred if treatments had not been initiated. However, graphing the *Ae. aegypti* counts from Wynwood and Miami Beach together (Figure 3, panel F) suggests that, before treatments began, the mosquito counts remained consistently high throughout the season (=30–50 mosquitoes per trap). Only after the first adulticiding in each area did the mean mosquito counts drop statistically and vector-transmitted Zika virus infections cease.

**Lessons Learned**

The purpose of using adulticides in an outbreak is to immediately reduce the number of adult mosquitoes (particularly older ones) that might be capable of transmitting disease. We observed interruption of vectorborne Zika virus transmission in Wynwood and both red zones in Miami Beach after beginning intensive adulticiding. In the United States, adulticide treatments using space-spraying techniques against *Ae. aegypti* mosquitoes have been shown to quickly knock down adult populations (11). However, these adult mosquito reductions are transient because not all mosquitoes will be active (and thus exposed) during application; in addition, adulticides do not control larvae and pupae, new adult mosquitoes will quickly repopulate an area. Therefore, repeated adulticide treatments are needed to eliminate newly emerging mosquitoes.

The use of larvicides alone does not immediately control adult mosquito populations, and it is not unusual to see the effect of larvicides until several weeks after their application (12). Our observation in Wynwood, where mosquito numbers remained suppressed when both adulticide and larvicide applications occurred, compared with the area that received only adulticide treatments, reinforces the necessity of a combination approach to achieve and sustain impact. Observations that aerial adulticiding and combinations of adult and larval mosquito control can successfully interrupt vectorborne disease transmission have been previously reported. Aerial adulticiding in California stopped West Nile virus transmission in an area that received the treatment, whereas cases continued to occur in untreated surrounding areas (13). Although larvicides are typically not recommended as part of a malaria control program, an example of the effect of both adulticide and larvicide contributing to reduction of disease was documented in Kenya, where transmission of malaria decreased substantially after a combination of larvicide and insecticide-treated nets were used (14). The combination approach can prolong the recovery of a treated mosquito population because adult mosquitoes are killed, thereby immediately interrupting virus transmission and deposition of new eggs, and emergence of new adults is interrupted by the larvicide, keeping the population from quickly rebounding and thus preventing ongoing virus transmission.

**Acknowledgments**

We recognize all the employees from the cities of Miami and Miami Beach and Miami-Dade County, Florida. Many individuals from Miami-Dade County departments played a role in containing the outbreak: Lilian Rivera, Reynald Jean, and staff of the Department of Health; Samir Elmir and staff of the Department of Health’s Environmental Health and Engineering Division; and Alina Hudak, Paul Mauriello, Aimee Cabrera, Lee Casey, and staff of the Department of Solid Waste Management. We extend special thanks to the leaders of Miami Beach, the city of Miami, and Miami-Dade County, whose leadership and cooperation were crucial in successfully stopping the spread of Zika virus. Alden Estep graciously supplied the *Ae. aegypti*-susceptible strain. Finally, we thank the Florida Department of Health and Florida Department of Agriculture and Consumer Services for their support of the vector control activities.

**About the Author**

Dr. McAllister is a research entomologist with the Arboviral Diseases Branch, Division of Vector-Borne Diseases, National Center for Emerging and Zoonotic Infectious Diseases, CDC. Her primary research interests include insecticide resistance and mosquito control research.

**References**

SYNOPSIS


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EID Podcast: WWI and the 1918 Flu Pandemic
CDC’s Dr. Terence Chorba discusses his EID cover art essay about the 1918 flu pandemic and the WWI painting by John Singer Sargent

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Blastomycosis is a systemic disease caused by *Blastomyces* spp. fungi. To determine its epidemiology in blastomycosis-endemic Minnesota, USA, we evaluated all cases reported to public health officials during 1999–2018. We focused on time to diagnosis, exposure activities, and exposure location. A total of 671 cases and a median of 34 cases/year were reported. Median time to diagnosis was 31 days; 61% of patients were not tested for blastomycosis until they were hospitalized. The case-fatality rate was 10%, and patients who died were 5.3 times more likely to have a concurrent medical condition. Outdoor activities and soil exposure were reported by many patients, but no specific activity or exposure was common to most. Almost one third of patients were probably exposed in geographic areas other than their home county. Providers should consider alternative etiologies for patients with pneumonia not responding to antibacterial treatment, and public health officials should increase awareness in blastomycosis-endemic areas.
Blastomycosis is a systemic disease caused by thermally dimorphic *Blastomyces* spp. fungi found in soil. Infection with *B. dermatitidis* or *B. gilchristii* occurs primarily by inhalation of conidia and most often causes pneumonia, although direct inoculation of soft tissue can occur (1). Infections can disseminate hematogenously, most commonly to skin, bone, and the central nervous system (2). Case-fatality rates range from 6% to 22% (3–5). Most patients are male (60%–75%) (3–8) and middle-aged (median age 41–44 years) (3,5,7,8). Diagnosis is often delayed because community-acquired bacterial pneumonia has a similar presentation (9,10) and index of suspicion for blastomycosis is low among healthcare providers (1,11). The standard diagnostic method is isolation and identification of *Blastomyces* spp. in culture from clinical specimens, but also used are histopathology, cytopathology, antigen testing, and antibody testing (11).

In North America, blastomycosis occurs primarily in areas surrounding the Great Lakes, the Mississippi and Ohio River valleys, and the St. Lawrence River, which include many US states and Canada provinces (1,2). Recent phylogenetic studies and ecologic niche modeling reports have increased our knowledge of the distribution and ecology of *Blastomyces* spp. (12–15). However, the difficulty of isolating the organism directly from environmental samples limits our ability to determine its true endemic ranges (12). Case series and outbreak reports have provided insight into the ecology of *Blastomyces* spp. and possible risk factors for human infection (16–19). Outbreaks have been associated with outdoor recreation (17,20–22) and with construction, excavation, or local environmental sources such as yard waste compost (18). Incidence or mortality rates are increased among black (3,4,23), Asian (24), American Indian/Alaska Native (23), and Aboriginal Canadian persons (5). Risk factors for sporadic cases are less well documented; a retrospective case-control study did not find associations with classic outbreak exposures (4).

To better describe the epidemiology of blastomycosis in Minnesota, an endemic area, we evaluated all cases reported to public health officials during 1999–2018. We also examined delayed recognition and diagnosis of the disease.

**Methods**

Blastomycosis has been reportable to the Minnesota Department of Health (MDH) since 1985. Beginning in 1999, MDH routinely collected information on demographics, illness history, diagnostic test results, treatment, outcomes, and any exposures by using a standardized case report form. Data collection evolved over time; during 1999–2015, case report forms were completed by providers or their staff, and during 2016–2018, MDH staff abstracted medical records and completed case report forms. During the entire study period, a confirmed case of blastomycosis was defined as illness in a Minnesota resident with any of the following: a positive *Blastomyces* culture, *Blastomyces* organisms visualized in tissue or body fluid, or a positive *Blastomyces* antigen test result and compatible clinical illness (e.g., cough, fever, abnormal pulmonary imaging, or skin lesions). Cases were classified as pulmonary only, nonpulmonary (localized disease outside the pulmonary system with no clinical pulmonary illness), or disseminated (disease in both the pulmonary system and at least 1 other system/site). We collected illness onset date, date of first visit to a healthcare provider, and date of the first test for blastomycosis regardless of test result. To assess diagnostic delays, we defined the patient interval as the time between illness onset and first visit to healthcare and the provider interval as the time between first healthcare visit and sample collection date for the first blastomycosis test (which indicates that a blastomycosis diagnosis was under consideration). Total time to diagnosis was defined as the time from illness onset to the first test for blastomycosis. We used the date of first test regardless of result to evaluate the time until healthcare providers considered a systemic mycotic infection.

We attempted to interview all patients or next of kin regarding patients’ illness and exposure history during the 3 months before illness onset, including home and neighborhood environment, occupation, outdoor activities and travel, concurrent medical conditions, immunosuppressive medications, smoking history, and family members or pets with a blastomycosis diagnosis. Underlying conditions included diabetes mellitus, chronic lung disease (e.g., chronic obstructive pulmonary disease, asthma), chronic liver disease (e.g., cirrhosis, hepatitis), and other chronic illnesses (e.g., HIV infection/AIDS, sarcoidosis, heart disease, kidney disease). Immunosuppressive medications included corticosteroids, tumor necrosis factor-α blockers, chemotherapy, or posttransplant medications. Patients were also asked about any information missing from case report forms regarding demographics, symptoms, and prescribed antibacterial and antifungal drugs. On the basis of exposure information obtained during
Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 26, No. 5, May 2020

interviews, we assigned the most likely location of Blastomyces exposure for each patient, either a specific Minnesota county or an out-of-state location. This subjective assessment considered incubation period, travel, and activities.

We included in our analysis confirmed cases with a positive specimen collection date of 1999 through 2018. We did not include patients with positive antigen test results but no compatible illness, positive serologic antibody tests only, or other fungal infections.

We calculated incidence by race by using the number of cases and race population in Minnesota for each year (25) and then averaged the yearly incidence rates. We calculated incidence by county by using the average population of each county for the entire period and the average number of cases in each county. We classified counties with an incidence rate of >3 cases/100,000 population as highly blastomycosis-endemic counties, based on a natural break in the distribution of incidence by county.

We analyzed data by using SAS 9.2 statistical software (https://www.sas.com) and conducted univariate analysis by using $\chi^2$, Fisher exact, Student $t$, Wilcoxon rank-sum, and Kruskal-Wallis tests. To control for race and sex in analyses of outcome and concurrent conditions, we used multivariate logistic regression. We considered 2-sided p-values of <0.05 to be significant.

Results

Demographics

During the 20-year study period, 671 confirmed cases of blastomycosis were reported in Minnesota; the median number of cases per year was 34 (range 22–58) (Figure 1). A total of 32 (5%) cases were part of outbreaks with patient exposure in Minnesota or Wisconsin, including a large 1999 outbreak in St. Louis County, Minnesota, which involved humans and dogs and was associated with wet weather and an excavation site for a new neighborhood. Except for 1999, more cases were reported during 2016–2018 than during previous years.

The statewide average annual incidence was 0.64 cases/100,000 population. Average annual incidence ranged from 0 to 7.6 cases/100,000 for individual counties (Figure 2). The median patient age was 44 years (range 3–93 years), and 474 (71%) patients were male (Table 1; Figure 3). The average annual incidence was highest for American Indian/Alaska Natives (2.7/100,000 population), followed by white (0.53/100,000), Asian/Pacific Islander (0.51/100,000), and black (0.48/100,000) persons.

Clinical Characteristics

Reported symptoms included cough (83%), fatigue (79%), fever (69%), weight loss (62%), night sweats (61%), poor appetite (57%), chills (57%), joint pain (30%), back pain (28%), and skin lesions (25%). A total of 456/663 (69%) patients were hospitalized for a median of 8 (range 1–197) days (hospitalization data were not available for 8 patients). Most (72%) infections involved only the pulmonary system, 21% of infections were disseminated, and 7% were nonpulmonary localized infections (Table 1). The most common site was skin or soft tissue for disseminated infections (108 cases, 79%) and nonpulmonary infections (38 cases, 83%), followed by bones or joints (22 [16%] disseminated cases, 6
[13%] nonpulmonary cases) and the central nervous system (13 [9%] disseminated cases, 2 [4%] nonpulmonary cases). For 47% of patients, ≥3 courses of antibacterial drugs were prescribed before blastomycosis was diagnosed.

Diagnostic Methods
The most commonly reported diagnostic test used was culture; 557/617 (90%) positive results were reported. Positive cytopathology results were reported for 250/539 (46%) patients and positive histopathology results for 83/467 (18%). Samples obtained by bronchoalveolar lavage or tracheal swab were the most common sources for culture (269/567 [47%] patients) and cytopathology (123/273 [45%] patients). Antigen testing became available in 2003 but was not widely used until 2008. Of the 401 patients from 2008–2018, a positive urine or serum antigen test was included in the diagnostic testing for 167 (42%). Use of antigen tests to evaluate treatment efficacy was not tracked.

Time to Diagnosis
Among all patients, the median total time to diagnosis was 31 (range 0–1,130) days, interquartile range [IQR] 16–64 days) (Figure 4). The median patient interval (time from illness onset to first visit) was 5 (range 0–409, IQR 0–15) days. The median provider interval (time from first visit to first blastomycosis test) was 14 (range 0–517, IQR, 6–32) days (Figure 4). Provider interval was >30 days for 27% of patients. The median total time to diagnosis for patients with nonpulmonary disease was 67 (IQR 39–150) days. For 61% of patients, blastomycosis testing was not performed until they were admitted to the hospital. The median time to diagnosis was 40.5 (IQR 22–58) days for Asian/Pacific Islander patients and 34 (IQR 12–60) days for American Indian/Alaska Native patients compared with 31 (IQR 16–66) days for white patients. However, these differences were not statistically significant.

The overall time to diagnosis was the same in both highly blastomycosis-endemic and less blastomycosis-endemic counties. Hospitalization was more likely for patients living in less blastomycosis-endemic counties (72%) than for patients living in highly blastomycosis-endemic counties (64%) (odds ratio [OR] 1.4, 95% CI 1.02–1.99; p = 0.036).

Treatment
Medications used for blastomycosis treatment were not reported for all case-patients. Among those for whom they were reported, 462 (84%) case-patients received itraconazole, 145 (26%) amphotericin B, 37 (7%) voriconazole, and 28 (5%) other or unknown medications.

Outcomes
The overall case-fatality rate was 10% (yearly range 3%–16%). Although fatality rates were higher among persons in some racial groups (18% for American Indian/Alaska Natives, 17% for Asian/Pacific Islanders), no statistically significant differences were observed. Patients who died were significantly older than patients who survived; mean difference was 15 years (Table 1). No patients with nonpulmonary infections died. Among patients who did die, the median time to diagnosis was significantly shorter than for those who survived (Figure 4).

Concurrent Conditions
Concurrent medical conditions or an immunocompromised status resulting from illness or medication were reported by 195 (35%) patients (Table 1). Patients who were hospitalized were twice as likely as those not hospitalized to have a concurrent condition (OR 2.12, 95% CI 1.4–3.2; p<0.001). Patients who died were 5.3 times more likely to have a concurrent condition than were patients who survived (Table 1). The most common concurrent condition was diabetes. Current smoking was reported by 109 (20%) patients, and any history of smoking was reported by 194 (39%).
Exposures
Interviews were conducted with 541 (81%) of 671 patients or their next of kin (Table 2). In the 3 months before illness onset, 317 (59%) of 539 patients participated in ≥1 outdoor activity including hunting, fishing, swimming, boating, camping, hiking, or bike riding. Of these, the most commonly reported were boating (40%), fishing (30%), and hiking (28%). At least 1 specific soil exposure was reported by 375 (78%) of 480 patients; soil exposures included gardening, clearing or cutting wood, gathering wild plants, or using an all-terrain vehicle (ATV), or being near excavation. Occupational exposure to soil, wooded, or boggy areas was reported by 97 (21%) of 468 patients. None of these activities or other exposures were reported by 31 (6%) patients. Owning a dog was reported by 283 (53%) of 539 patients; of those, 29 (10%) reported owning a dog that had ever had blastomycosis. Having a family member who had ever had blastomycosis was reported by 22 (4%) of the 541 patients. A significantly higher proportion of male than female patients participated in outdoor activities (OR 0.49, 95% CI 0.33–0.71; p<0.001). The individual activities of fishing, camping, hiking, and swimming were reported significantly less often by patients with concurrent conditions (data not shown).

A total of 340 (51%) of 671 patients were most likely exposed to Blastomyces in their county of residence; 195 (29%) were exposed outside their county of residence, either in other Minnesota counties or other states (Figure 5). These locations included the highly blastomycosis-endemic northern Minnesota counties of St. Louis (27 patients), Cass (24), and Itasca (10); Wisconsin (52); and Canada (10). The most probable location of exposure was unknown for 136 patients (20%) because of multiple possible locations (21 patients [3%]) or because no interview could be conducted (115 patients [17%]).

Discussion
The epidemiology and clinical courses of blastomycosis cases in Minnesota are similar to those in other disease-endemic regions. The case-fatality rate, sex ratio, age distribution, and reported symptoms are consistent with those reported from other disease-
endemic areas \((3,5,7,8)\). Although case counts and fatality rates were higher in the most recent 2 years, we found no overall trends by year, race, or sex. The typical blastomycosis patient in Minnesota is a 45-year-old white man who spends time outdoors. However, this report highlights underrecognized features of blastomycosis epidemiology, particularly patients who do not fit the typical presentation or demographic.

Although the blastomycosis incidence rate was highest among American Indian/Alaska Natives, the incidence difference between that group and white, Asian, and black persons was not statistically significant. However, in Minnesota, the population of American Indian/Alaska Natives is much smaller than that of other races, which, combined with other factors such as prevalence rates for concurrent conditions or geographic location of residence, may influence incidence rates in the American Indian/Alaska Native population. For example, a larger proportion of American Indian/Alaska Native blastomycosis patients (70%) than patients of other races (38%) live in highly blastomycosis-endemic counties (OR 3.9, 95% CI 1.9–7.8; \(p<0.001\)). Genetics may also play a role. Others have discussed increased susceptibility to disease or severe disease after *Blastomyces* infection for Asian, black, and American Indian/Alaska Native persons \((3,4,19,23,24,26)\). Our data show higher case-fatality rates for Asians and American Indians/Alaska Natives; however, the difference was not significant, even after controlling for sex and underlying conditions. Another study evaluating mortality rates found that the likelihood of dying from blastomycosis-related complications was higher for American Indian/Alaska Native and black patients than for white patients \((23)\). Variation in blastomycosis incidence and outcomes by race warrants further exploration \((26)\).

Hospitalization and mortality rates were higher among patients with underlying conditions or immunocompromised status, as previously reported \((5,7,27)\). Those patients reported outdoor and soil exposures less frequently than did previously healthy patients, which could lead a clinician to discount blastomycosis as a diagnostic possibility. Because the odds of death are dramatically higher for patients with an underlying condition than for previously healthy patients, earlier consideration of alternative pneumonia etiologies (beyond antimicrobial drug–resistant bacterial infection) for those patients is warranted.

Diagnosis of blastomycosis is often delayed. For half of the patients in this study, \(\geq1\) full month elapsed between illness onset and the patient’s first test that could diagnose blastomycosis. Diagnosis took even longer for those with nonpulmonary infections. Provider interval accounted for a larger proportion of this time than did patient interval. Medical record abstraction provided ample anecdotal evidence that patients visited healthcare providers numerous times before their first blastomycosis test. These delays have many possible consequences, including unnecessary antibacterial drug use and higher hospitalization rates. Being hospitalized seemed to be a key factor in being tested for blastomycosis; 60% of patients were not tested until hospital admission. Earlier testing may have prevented some of these admissions. Relatively few patients underwent urine or serum antigen testing, which, despite cross-reactivity with
other fungal pathogens, may have provided guidance toward a general diagnosis of fungal disease and appropriate treatment.

Time to diagnosis should logically be shorter for patients with skin lesions because visible lesions might trigger sampling or consideration of blastomycosis in a patient with concurrent pneumonia. This result was found for 123 Mississippi patients, for whom clinicians correctly diagnosed 64% of blastomycosis cases for patients with skin lesions on their initial visit but only 18% of blastomycosis cases for all patients (28). However, in our study, we compared patients with skin lesions with patients without skin lesions and found that provider delay and total time to diagnosis were significantly longer for those with skin lesions than without skin lesions (data not shown).

Time to diagnosis was also significantly shorter for patients who died than for those who survived. Both patient interval and provider interval were shorter for patients who died, probably because their blastomycosis was more severe from the onset, which may have resulted in earlier presentation to healthcare and more aggressive initial diagnostics. However, patients who died were not tested for a median of 11 days after their first healthcare visit, and perhaps some might not have died had a diagnosis been reached sooner.

We anticipated that time to diagnosis would be shorter for patients residing in highly blastomycosis-endemic counties because local providers would be more familiar with the disease and would order testing earlier. Although the median time to diagnosis was the same in highly and less disease-endemic counties, patients living in highly disease-endemic counties were 50% less likely to be hospitalized. This finding may indicate that where providers were more familiar with blastomycosis, they more frequently ordered testing before hospitalization was required.

Although incidence by county of residence provides a measure of disease frequency, 29% of patients were probably exposed outside their home county. Mapping of case totals for county of exposure compared with county of residence illustrates that many patients live in more populated, less blastomycosis-endemic counties, such as Hennepin and Ramsey (i.e., the Minneapolis–St. Paul metropolitan area) but are exposed in highly blastomycosis-endemic northern counties (e.g., Cass, Itasca). By tracking suspected exposure locations, we can more accurately distinguish highly
Further refinement of such areas and future exposure location data will provide more information about the ecologic niche of blastomycosis and help focus awareness campaigns among healthcare providers, residents, and visitors. Many of the highly blastomycosis-endemic counties attract seasonal residents and tourists in summer, underscoring the value of travel histories for patients with infectious disease and provider familiarity with geographic areas where risk for Blastomyces exposure is greater.

As previously reported, blastomycosis cases were skewed heavily by patient sex. This finding is often attributed to a perceived higher likelihood of

![Figure 5. Blastomycosis cases, by county of residence (A; n = 670) and probable county of exposure (B; n = 463), Minnesota, USA, 1999–2018.](image-url)
male patients having engaged in recreational and occupational outdoor activities that increase risk for exposure to Blastomyces (1,5). In Minnesota, 2 other outdoor-associated diseases that occur in similar locations, Lyme disease and anaplasmosis, also occur more often in men, but the male:female ratio (60:40) is less dramatic (29). Male blastomycosis patients reported participation in some outdoor activities at significantly higher proportions than did female patients. However, even when evaluating an exposure that should not intuitively differ by sex, such as nearby excavation, 72% of patients were male. Other factors may explain differences by sex, such as hormonal effects, as have been proposed for other diseases (30–32).

A previous retrospective case–control study did not find any association between infection and factors typically associated with blastomycosis in outbreaks, such as proximity to water, recreational activities, or soil-related activities (4). A prospective case–control study would help determine whether those who acquire blastomycosis participate in these exposure activities at higher rates. Most patients in this study reported ≥1 activity typically considered a risk factor for blastomycosis, but no activities were common to all or most patients. In addition, this study does not enable us to determine which exposures present the highest risk because background exposure rates for Minnesota residents are not readily available.

Study limitations include those characteristic of surveillance data. Because the surveillance system is passive, undercounting is possible if cases were not reported. Race and ethnicity were not always documented in medical records. Data collection methods changed in 2016 when medical record abstraction was added. Back and bone pain were added to case report forms in 2001; joint pain, boating, and ATV use were added in 2010. Data regarding the number of healthcare visits were not consistently available. We used the date of first test for blastomycosis as the endpoint for determining time to diagnosis. However, negative test results are not routinely reported. Although we made every effort to collect this information from medical record abstraction or providers, some could have been missed.

In conclusion, to reduce the time to diagnosis and case-fatality rates for patients with blastomycosis, providers should consider alternative etiologies for patients with pneumonia that is unresponsive to antibacterial drugs. Complete travel and exposure histories should be obtained, and antigen testing should be considered as a screening test. Blastomycosis should be considered an emerging risk for immunocompromised or chronically ill patients in disease-endemic regions, even for those who do not report classic exposures. Public health officials should work to increase awareness among persons who live and visit blastomycosis-endemic areas so they can advocate for themselves.

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890

Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 26, No. 5, May 2020
Human infections with avian influenza A(H7N9) virus were laboratory confirmed in China in the spring of 2013 (1). Since then, 1,567 human cases and 615 fatal cases have been officially reported in 5 epidemic waves (February–September 2013, October 2013–September 2014, October 2014–September 2015, October 2015–September 2016, and October 2016–September 2017) as of March 2, 2018 (2). Compared with the previous 4 epidemic waves, the 2016–17 fifth wave raised global concerns because of several characteristics. First, a surge in laboratory-confirmed cases of H7N9 virus infection was observed in wave 5, along with some clusters of limited human-to-human transmission (3,4). Second, a highly pathogenic avian influenza H7N9 virus infection was confirmed in Guangdong Province and has caused further human infections in 3 provinces (5,6). The genetic divergence of H7N9 virus, its geographic spread (7), and a much longer epidemic duration raised concerns about an enhanced potential pandemic threat in 2016–17.

Live poultry markets (LPMs) are a major source of human infections with H7N9 virus; the maintenance, amplification, and dissemination of H7N9 viruses have occurred in LPMs (8,9). Most human patients were exposed to H7N9 viruses through direct exposure to infected poultry or indirect exposure in contaminated environments, which increased the risk of H7N9 infections (9). Closure of LPMs is thus considered to play a key role in reducing the risk of animal-to-human transmission of H7N9. Different levels of LPM interventions were implemented in different geographic areas during 2013–2018. Permanent and temporary LPM closures were the main measures used to reduce the exposures of human population to H7N9 virus and reduce transmission (10,11). In some counties, alternative practices to complete bans of LPMs have also been put in place, such as bans on overnight poultry storage combined with regular cleaning and disinfection or market rest days (12).

So far, the effectiveness of LPM interventions in controlling H7N9 epidemics has been discussed in several studies. In comparison with the previous 4
epidemic waves, a quantitative effectiveness assessment of LPM closure on the fifth H7N9 epidemic wave has not yet been conducted. Moreover, previous studies investigated the effect of the occurrence of LPM closure on controlling the H7N9 epidemic only by directly comparing the detection and isolation rates of H7N9 virus in the environment (13,14), investigating the number of H7N9 cases (10,15), or evaluating the posterior estimates of H7N9 incidence using transmission models before and after LPM closure (16–18). Although such modeling studies have quantified the effectiveness of LPM closure, inaccurate estimates of the effectiveness may have arisen because they did not account for the full characteristics of the LPM interventions (e.g., the type, start date, and duration of the interventions) and the underlying natural transmission dynamics of H7N9. In particular, neglecting the natural transmission dynamics of H7N9 may have led to underestimates or overestimates of the effectiveness of LPM closure if the interventions were implemented before or after the epidemic peak. Given the limitations of previous studies and variations in the implementation of LPM interventions in different geographic areas, there is a need to consider the potential effects of the characteristics of various interventions on the control of H7N9 epidemics.

Our study aimed to assess the differences in the effectiveness of various LPM interventions across 5 epidemic waves, especially during the 2016–17 epidemic wave. Specifically, we compared 4 LPM interventions: permanent, long-period, short-period, and recursive closures. We compared the daily incidence rates of H7N9 for different types and closing levels of LPM closure across 5 epidemic waves and quantified the effect of 4 LPM interventions on H7N9 transmission in the 2016–17 epidemic wave.

Materials and Methods

Data Sources
We compiled a database recording the characteristics (e.g., the type, start date, and end date) of LPM closure (Appendix, https://wwwnc.cdc.gov/EID/article/26/5/19-0390-App1.pdf). We initially identified 32 types of LPM closure in cities with >1 H7N9 case (Appendix Table 1, Figure 1) and classified them based on the duration of LPM closure and the proportion of closing days. The duration of LPM closure refers to the total number of closing days; the proportion of closing days is equal to the duration of LPM closure divided by the duration of each epidemic wave. Given variations in duration, start dates, and end dates of the 5 H7N9 epidemic waves, it was not reasonable to use similar start and end dates for all epidemic waves to estimate daily incidence rates (DIRs). To give more comparable estimates of DIRs, we set the duration of each epidemic as the period separating the 5th from the 95th percentiles of the days of onset of illness in each wave. First, taking the duration of closure into consideration, we classified LPM closure measures into 4 categories: permanent closure, whereby LPMs were permanently closed within the epidemic wave or for the entire epidemic wave duration; long-period closure (≥14 days within the epidemic wave [10,17]); short-period closure (<14 days within the epidemic wave); and recursive closure, whereby LPMs were closed for 1 or 2 days with a repetition of the closing over time (the closing might be implemented weekly, biweekly, or monthly). Second, we classified LPM closures according to the proportion of days of closing out of the total epidemic wave duration, using a quantile classification method (i.e., <25%, 25%–75%, and >75% of epidemic wave duration) because of abnormal distributions of the proportions of closing days in waves 1–5 (Appendix Figure 2). We collected the onset date and information on residence for all laboratory-confirmed H7N9 human cases during March 2013–September 2017 from the World Health Organization (https://www.who.int/csr/don/17-january-2017-ah7n9-china), Monthly Risk Assessment Summary reports (https://www.who.int/influenza/human_animal_interface/avian_influenza/archive), websites of the national and provincial Health and Family Planning Commission of China (http://www.nhc.gov.cn), FluTrackers (http://www.flutracker.com), HealthMap (https://healthmap.com.au), and avian influenza reports from the Centre of Health Protection of Hong Kong (https://www.chp.gov.hk/tc/index.html).

Statistical Analyses

Assessment of Type of LPM Closure on H7N9 DIR
We first assessed the effect of 4 types of LPM closures (recursive, short-period, long-period, and permanent closures) on H7N9 DIRs. We calculated DIR estimates only for counties where >1 H7N9 case was reported in 2013–2017 (Appendix). In addition to looking at the type of the intervention, we also explored the influence of the closing levels of LPM closure (<25%, 25%–75%, and >75% of epidemic wave duration) on DIRs. We used a generalized linear mixed effect model (GLMM) followed by a multiple comparison procedure (Tukey test) to compare DIRs by contrasting counties with no measures to counties with different
Assessment of LPM Interventions on Risk for Animal-to-Human and Human-to-Human Transmission in the 2016–17 Epidemic Wave

To further assess the effect of the type of LPM closure on reduction in H7N9 transmission risk in each site, we constructed an H7N9 transmission model similar to that developed by Yu et al. (16) and Virlogeux et al. (18) using data from the 2016–17 epidemic wave (Appendix). We included 17 sites (60 districts/counties) with ≥5 urban and semiurban cases in wave 5 (Appendix Figure 3). We compared the reduction in the number of animal-to-human infections before and after closure among 4 LPM interventions using Welch’s analysis of variance and multiple comparison (Tamhane’s T2 test).

The H7N9 epidemics in 2013–2017 followed a seasonal pattern, with peaks in the winter months and sporadic cases in the summer months. Thus, we considered the reductions in number of infections, together with LPM interventions, to be correlated with the seasonal pattern of the H7N9 epidemics. We incorporated absolute humidity, the most dominant contributor to the H7N9 epidemic, into transmission models to modulate the seasonal pattern of H7N9 epidemic in a sensitivity analysis (Appendix) (19,20).

We assumed the transmissibility of H7N9 virus to be higher at lower absolute humidity in accordance with previous studies (19,20) and an observed pattern of H7N9 epidemic in the 17 study sites (Appendix Figure 4). In addition, we separated the effect of LPM closure from the natural transmission dynamics of H7N9 viruses by comparing the differences in
Figure 2. Estimated daily incidence rates in counties with various levels of live poultry market closures across waves of influenza A(H7N9) infections, by duration of closure, China, 2013–2017. A) Wave 1; B) wave 2; C) wave 3; D) wave 4; E) wave 5. Error bars indicate 95% CIs. Asterisks (*) above bars indicate statistically significant (p<0.05) differences between daily incidence rates and reference category (Ref.) rates. Duration categories: no closure during epidemic wave; permanent closure, permanently closed within the epidemic wave or for the entire epidemic wave duration; long-period closure (>14 days within the epidemic wave [10,17]); short-period closure (<14 days within the epidemic wave); and recursive closure, whereby LPMs were closed for 1 or 2 day with a repetition of the closing over time (the closing might be implemented weekly, biweekly, or monthly).
the reductions in the number of infections between 2 sites (1 with and 1 without LPM closure) where a similar season pattern of human H7N9 infections had been observed. We created hypothetical start and end dates of LPM closures in sites without such closures and assumed them to be consistent with those in sites with closures. We used the Mann-Whitney U test to compare the differences of the reductions in the number of infections between the 2 sites.

Results
The comparison over time of DIRs between counties with and without LPM closures (Figure 1) showed that counties with measures had higher DIRs than counties free of closures during 2013–2017. In wave 5, DIRs decreased over time in counties with closures, whereas DIRs for counties without measures remained fairly high. Comparisons of DIRs for counties with different types (Figure 2) and levels (Figure 3) of LPM closure showed that, with the exception of wave 1 and wave 4, showed that DIRs were significantly lower in counties after closure than before (p<0.001) (Appendix Table 2). The DIRs in counties after LPM closure were also significantly lower than those estimated for counties without closures (p<0.001) (Appendix Table 2). We observed no statistically significant difference between counties with recursive, short-period, long-period, or permanent closures except for counties with recursive, long-period, and permanent closures in wave 2; for counties with short-period and long-period closures in wave 3; and for counties with recursive and long-period closures in wave 5. No DIRs were significantly different among counties with different levels of closing days, but the difference was significant in 25%-75% versus >75% of epidemic wave duration in wave 2.

To further quantify the effectiveness of LPM intervention in each site in wave 5, we compared the reduction in number of daily infections before and after closure among counties with 4 LPM interventions. A total of 142 laboratory-confirmed cases were located in 17 sites in wave 5 (Table 1), which is much higher than the total number of H7N9 cases in these sites in waves 1–4 (n = 116). A compilation of the onset dates of illness for these cases (Appendix Figure 5) shows that, with the exception of 4 study sites where human H7N9 epidemics ended before closing LPMs (study sites 8, 10–11, and 14), there was an observable drop in the number of H7N9 cases after LPM intervention in each site. After LPM closure, Gusu District in Suzhou, with permanent closure, had a higher reduction (97.0%, 95% CI 94.0%-100.0%) than other sites. The mean posterior estimates of the reductions ranged from 48% to 98% in sites with long-period closure. Guangzhou implemented recursive measures at the beginning of the epidemic but had a much lower reduction (34.0%, 95% CI 15.0%–70.0%). Compared with Guangzhou, which had short-period closures in the second intervention (73.0%, 95% CI 53.0%–77.0%), Foshan (96%) and Fuzhou (95%) showed larger relative reductions in the daily number of infections. Overall, the mean reduction in daily number of infections increased successively among sites with recursive, short-period, long-period, and permanent closures (p<0.001) (Appendix Table 3).

When we examined potential for human-to-human transmission, we found that the estimated effective reproduction number was 0.147 (95% CI 0.034–0.285) (Table 2; Appendix Figure 6). The slightly higher daily number of infections estimated by the model incorporating animal-to-human and human-to-human transmission (Appendix Figure 5) also suggests the potential for human-to-human transmission when compared with those estimates in an animal-to-human transmission model (Appendix Figure 7). Sensitivity analyses examined the influence of mean serial interval and of the proportion of unreported cases on the effective reproduction number. A decrease in the effective reproduction number was observed when the mean serial interval increased (Appendix Table 4). After accounting for the seasonality of H7N9 affected by absolute humidity, estimates of the reduction in number of daily infections changed slightly in some sites (Appendix Table 5), which should not be surprising, because the season pattern of H7N9 epidemics may well vary from one site to another (Appendix Figure 4). After we adjusted for the potential effect of the natural transmission dynamics of H7N9 virus, the net effect of LPM closure varied in study sites with long-period (range 0.5%-52.0%) and permanent (45.0%, 95% CI 32.0%-88.0%) closures in wave 5 (Table 3). In all study sites except 1, the differences in reductions in number of infections among sites with and without closures were statistically significant (p<0.001).

Discussion
LPM closing measures have often been implemented reactively, after the occurrence of human H7N9 cases in a given county (Appendix Figure 8); it is thus not surprising to find generally high DIRs in counties that undertook such measures (Figure 1). However, what matters most is what happened to the DIR and mean daily number of illnesses after these closing measures were taken. Both DIRs and mean daily number of illness onsets decreased in counties or sites following
**Figure 3.** Estimated daily incidence rates in counties with various levels of live poultry market (LPM) closures across waves of influenza A(H7N9) infections, by proportion of closure days during epidemic wave, China, 2013–2017. A) Wave 1; B) wave 2; C) wave 3; D) wave 4; E) wave 5. Error bars indicate 95% CIs. Asterisks (*) above bars indicate statistically significant (p<0.05) differences between daily incidence rates and reference category (Ref.) rates. Proportion categories: no closure; before closure, incidence rate before market was closed; <25%, closed <25% of the days of the wave duration; 25%–75%, closed 25%–75% of the days of the wave duration; >75%, closed >75% of the days of the wave duration.
LPM interventions, but the effect varied depending on the type of intervention and epidemic wave.

In general, permanent, long-period, and short-period closures provided comparable estimates in terms of DIR reduction. However, the association between the type and closing levels of LPM measures and DIRs showed different results across waves. For example, the difference in DIRs in counties with dif-

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**Table 1. Characteristics of study sites in the 2016–17 epidemic wave of influenza A(H7N9), China.**

<table>
<thead>
<tr>
<th>Province</th>
<th>City</th>
<th>District/county, n = 60</th>
<th>Site no., n = 17</th>
<th>No. cases</th>
<th>Type of LPM closure</th>
<th>Start date</th>
<th>End date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jiangsu</td>
<td>Suzhou</td>
<td>Gusu District</td>
<td>1</td>
<td>19</td>
<td>Permanent</td>
<td>2016 Dec 31</td>
<td>Unreported</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Huqiu District, Wuzhong District, Xiangcheng District</td>
<td>2</td>
<td>12</td>
<td>Long-period</td>
<td>2016 Dec 27</td>
<td>Unreported</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kunshan City</td>
<td>3</td>
<td>5</td>
<td>Long-period</td>
<td>2016 Dec 19</td>
<td>Unreported</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Xishan District, Binhu District, Liangxi District (Chongan District, Nanchang District, Beilang District), Xinwu District, Jiangyin City</td>
<td>4</td>
<td>9</td>
<td>Long-period</td>
<td>2016 Dec 29</td>
<td>2017 Apr 27</td>
</tr>
<tr>
<td>Wuxi</td>
<td></td>
<td>Zhonglou District, Tianming District, Wujin District, Xinbei District, Jintan City, Liyang City, Chongchuan District</td>
<td>5</td>
<td>8</td>
<td>Long-period</td>
<td>2016 Dec 30</td>
<td>2017 Apr 30</td>
</tr>
<tr>
<td>Changzhou</td>
<td></td>
<td>Haizhu District, Panyu District, Baiyun District</td>
<td>6</td>
<td>5</td>
<td>Long-period</td>
<td>2017 Feb 25</td>
<td>Unreported</td>
</tr>
<tr>
<td>Nantong</td>
<td></td>
<td>Chongchuan District</td>
<td>7</td>
<td>5</td>
<td>Recursive</td>
<td>2017 Jan 1</td>
<td>2017 Feb 15</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>Short-period</td>
<td>2017 Feb 16</td>
<td>2017 Feb 28</td>
</tr>
<tr>
<td>Guangdong</td>
<td>Guangzhou</td>
<td>Haizhu District, Panyu District, Huangpu District</td>
<td>7</td>
<td>5</td>
<td>Recursive</td>
<td>2017 Jan 1</td>
<td>2017 Feb 15</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>Short-period</td>
<td>2017 Feb 16</td>
<td>2017 Feb 28</td>
</tr>
<tr>
<td>Foshan</td>
<td></td>
<td>Nanhai District, Shunde District</td>
<td>8</td>
<td>1</td>
<td>Short-period</td>
<td>2017 Jan 16</td>
<td>2017 Jan 25</td>
</tr>
<tr>
<td>Zhejiang</td>
<td>Ningbo</td>
<td>Yuyao City, Cixi City, Fenghua City, Ninghai District</td>
<td>9</td>
<td>1</td>
<td>Long-period</td>
<td>2017 Feb 11</td>
<td>Unreported</td>
</tr>
<tr>
<td></td>
<td>Hangzhou</td>
<td>Yuhang District, Xiaoshan District, Linan City, Fuyang City, Chunan County, Dongtou District, Yueqing City, Ruiian County, Cangnan County</td>
<td>10</td>
<td>2</td>
<td>Long-period</td>
<td>2017 Feb 11</td>
<td>Unreported</td>
</tr>
<tr>
<td></td>
<td>Wenzhou</td>
<td>Yueqing City, Ruiian County, Cangnan County, Chongding District</td>
<td>11</td>
<td>1</td>
<td>Long-period</td>
<td>2017 Feb 11</td>
<td>Unreported</td>
</tr>
<tr>
<td></td>
<td>Lishui</td>
<td>Suichang County, Jingning County, Jinyun County, Tongyang County</td>
<td>12</td>
<td>0</td>
<td>Long-period</td>
<td>2017 Feb 11</td>
<td>Unreported</td>
</tr>
<tr>
<td>Hunan</td>
<td>Xiangtan</td>
<td>Yuetang District, Yuhu District, Xiangtan County</td>
<td>13</td>
<td>3</td>
<td>Long-period</td>
<td>2017 Jan 24</td>
<td>Unreported</td>
</tr>
<tr>
<td>Anhui</td>
<td>Suzhou City</td>
<td>Yongqiao District</td>
<td>14</td>
<td>5</td>
<td>Long-period</td>
<td>2017 Feb 15</td>
<td>2017 Apr 30</td>
</tr>
<tr>
<td>Fujian</td>
<td>Fuzhou</td>
<td>Jinan District, Gulou District, Taijiang District</td>
<td>15</td>
<td>5</td>
<td>Short-period</td>
<td>2017 Feb 7</td>
<td>2017 Feb 17</td>
</tr>
<tr>
<td>Sichuan</td>
<td>Aba</td>
<td>Jinchuan County, Ruoergai County, Xiaojin County</td>
<td>16</td>
<td>0</td>
<td>Recursive</td>
<td>2017 May 10</td>
<td>Unreported</td>
</tr>
<tr>
<td>Shanghai</td>
<td>Shanghai</td>
<td>Chongqing District, Fengxian District, Jiading District, Jingan District, Jinhua District</td>
<td>17</td>
<td>2</td>
<td>Long-period</td>
<td>2017 Jan 28</td>
<td>2017 Apr 30</td>
</tr>
</tbody>
</table>

*Unreported indicates that the end of the LPM closure was not observed before May 31, 2017. LPM, live poultry market.
different levels of closing days was observed only in wave 2. During this wave, long-period and permanent closures represented the large majority of the measures (82.4% of the closing measures). During wave 5, short-period and recursive closures became available to authorities as potential measures and were implemented more abundantly, especially in cities with few H7N9 cases; thus, long-period and permanent closures represented only 55% of the total closing measures.

For wave 5, we also evaluated the effectiveness of different types of LPM interventions in controlling H7N9 epidemics in several key sites. Overall, the effectiveness of LPM closure varied with the type of the interventions in these sites during 2016–17. Permanent closure was more effective than long-period closure, short-period closure, and recursive closure. The relatively lower effectiveness of short-period closure was observed in wave 5, but the point estimates of the reduction in daily number of infections inferred from the transmission model were consistent with the effectiveness assessment of a 14-day LPM closure (range 53.0%–89.0%) (17). Accompanying the effectiveness assessment of consecutive LPM closure, Yuan et al. (21) quantified the effectiveness of periodic LPM closure together with daily cleaning and disinfection (range –47.0% to 34.0%), which was consistent with our minimum point estimates of the effectiveness of recursive closure.

The decline in the number of human infections with H7N9 virus varied among study sites. In addition to being a factor of the type of the intervention, the variations in these declines may have been influenced by the underlying natural transmission dynamics of H7N9. After adjustment for absolute humidity, the most dominant environmental driver for influenza seasonality, the reduction in number of infections did not change significantly in any one of the study sites. Therefore, overall estimates of the effect of LPM closure is unlikely to be confounded by those climatic factors. However, we cannot exclude the possibility that the effectiveness of LPM closure may be delayed because of climatic factors at a specific time, as low temperature and higher humidity always drive the spread of H7N9 virus. In addition, we cannot definitively exclude other unknown seasonal confounders, such as the seasonality of poultry movement. Available evidence supports the seasonal effects of poultry movement on human infection with H5N1 virus around Chinese New Year (22). Although we found no quantitative evidence that seasonal variation in poultry trade played a role in human infection with H7N9 virus, the fact that the high-risk season of the H7N9 epidemic was consistent with the peak time of poultry trade around Chinese New Year is notable.

Limited human-to-human transmissibility of H7N9 virus was previously observed during waves 1–4 (3). Our low estimates of reproduction number in wave 5 were consistent with previous descriptive analysis of possible clusters of human infection with H7N9 virus (3,23), confirming that human-to-human transmissibility of H7N9 virus remained unsustainable.

Other factors, such as societal economic costs and residents’ behavior toward banning live poultry trade, may affect the effectiveness of LPM closure (24) and lead to a displacement effect. LPM closure has threatened the wholesale and retail market chain (25); local

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**Table 2. Parameter estimation of infection rates before and after live poultry market closures in the 2016–17 influenza A(H7N9) epidemic wave, China.**

<table>
<thead>
<tr>
<th>Site no.</th>
<th>Type of closure</th>
<th>Expected daily no. infections (95% CI)</th>
<th>Reduction in no. infections after closure, % (95% CI)</th>
<th>Reproduction number (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Permanent</td>
<td>0.230 (0.121–0.372)</td>
<td>97.0 (94.0–100.0)</td>
<td>0.147 (0.034–0.285)</td>
</tr>
<tr>
<td>2</td>
<td>Long</td>
<td>0.340 (0.171–0.540)</td>
<td>90.0 (87.0–95.0)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Long</td>
<td>0.120 (0.037–0.248)</td>
<td>92.0 (87.0–100.0)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Long</td>
<td>0.390 (0.183–0.648)</td>
<td>95.0 (90.0–100.0)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Long</td>
<td>0.460 (0.231–0.763)</td>
<td>98.0 (96.0–100.0)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Long</td>
<td>0.040 (0.014–0.089)</td>
<td>48.0 (35.0–81.0)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Recursive</td>
<td>0.162 (0.037–0.229)</td>
<td>34.0 (15.0–70.0)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Short</td>
<td>0.107 (0.012–0.301)</td>
<td>73.0 (53.0–77.0)</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Short</td>
<td>0.190 (0.062–0.379)</td>
<td>96.0 (93.0–99.0)</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Long</td>
<td>0.120 (0.050–0.220)</td>
<td>84.0 (75.0–96.0)</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Long</td>
<td>0.090 (0.032–0.176)</td>
<td>89.0 (80.0–99.0)</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Long</td>
<td>0.110 (0.037–0.229)</td>
<td>92.0 (84.0–99.0)</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Long</td>
<td>0.090 (0.029–0.193)</td>
<td>78.0 (72.0–92.0)</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Long</td>
<td>0.220 (0.048–0.518)</td>
<td>86.0 (83.0–87.0)</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Short</td>
<td>0.190 (0.066–0.374)</td>
<td>92.0 (86.0–100.0)</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Recursive</td>
<td>0.350 (0.117–0.728)</td>
<td>95.0 (92.0–98.0)</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Long</td>
<td>0.210 (0.068–0.400)</td>
<td>71.0 (47.0–97.0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Long</td>
<td>0.140 (0.044–0.295)</td>
<td>84.0 (79.0–94.0)</td>
<td></td>
</tr>
</tbody>
</table>
This study has several limitations. First, the timing of the implementation of LPM closures in relation to the progress of the H7N9 epidemic was not considered in the effectiveness assessment of LPM closures, which would lead to an overestimation of the effects of LPM closure if LPM interventions were implemented after the epidemic peak. The incidence reduction might also not be comparable in cities with LPM interventions implemented before reaching the epidemic peak with those implemented near the end of the epidemic. Second, our findings focus only on human cases occurring in urban and semiurban areas in China in wave 5, ignoring H7N9 cases in rural areas, where LPMs are rarely located. More rural cases were reported in wave 5 than in previous epidemic waves, and exposure to poultry in farms and backyards were the main sources of these rural human cases (9,31). Therefore, LPM closure might be less effective in controlling H7N9 epidemics in these rural areas, and other effective interventions (e.g., vaccination of poultry) need to be further explored. Third, because of the ecologic nature of our study, some anthropogenic factors may have acted as potential confounders that can bias our findings, such as the number of LPM visitors, frequency of LPM visits, improvements in biosecurity for poultry-handling practices, or which live bird species were found in LPMs. These factors and LPM interventions have always existed in parallel, so we cannot rule out the possibility that differences in the reduction in the daily number of infections among different sites may be partially explained by these anthropogenic factors, especially in sites with

Table 3. Estimates of the net effect of LPM closures by comparing the reductions in the number of influenza A(H7N9) infections between study sites with and without closures, adjusting for similar season pattern of absolute humidity, China

<table>
<thead>
<tr>
<th>Study sites with LPM interventions</th>
<th>Reference sites without LPM interventions</th>
<th>Study sites with LPM interventions, Reduction in no. infections, % (95% CI)</th>
<th>Reference sites without LPM interventions, Difference in reduction, p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site 1 1 Jiangsu Huaiian (Huaiian District, Qingpu District, Nantong)</td>
<td>97.0 (93.0–100.0)</td>
<td>52.0 (12.0–81.0)</td>
<td>45.0 (32.0–88.0)</td>
</tr>
<tr>
<td>Site 2 1 Qingpu District, Nantong</td>
<td>90.0 (88.0–94.0)</td>
<td>57.0 (16.0–87.0)</td>
<td>33.0 (21.0–78.0)</td>
</tr>
<tr>
<td>Site 3 1 Haimen City, Rugao City, Xuzhou</td>
<td>90.0 (84.0–99.0)</td>
<td>70.0 (42.0–76.0)</td>
<td>20.0 (8.0–57.0)</td>
</tr>
<tr>
<td>Site 4 1 (Shuangfeng District), (Shuangfeng District)</td>
<td>94.0 (89.0–99.0)</td>
<td>49.0 (10.0–48.0)</td>
<td>44.0 (30.0–89.0)</td>
</tr>
<tr>
<td>Site 5 1 (Suining County)</td>
<td>98.0 (96.0–100.0)</td>
<td>48.0 (20.0–58.0)</td>
<td>51.0 (38.0–80.0)</td>
</tr>
<tr>
<td>Site 6 1 (Dongtai City), Yancheng</td>
<td>50.0 (32.0–74.0)</td>
<td>32.0 (21.0–50.0)</td>
<td>18.0 (10.0–23.0)</td>
</tr>
<tr>
<td>Site 14 1 (Huangshan District)</td>
<td>93.0 (86.0–100.0)</td>
<td>40.0 (27.0–63.0)</td>
<td>52.0 (36.0–59.0)</td>
</tr>
<tr>
<td>Site 17 1 (Huangzhou)</td>
<td>84.0 (78.0–94.0)</td>
<td>46.0 (39.0–61.0)</td>
<td>38.0 (33.0–39.0)</td>
</tr>
<tr>
<td>Site 13 2 Hunan Chenzhou (Beihu District, Yongxing County), Hengyang (Hengdong County, Shigu District, Zhuhui District), Loudi (Shuangfeng District), Shaoyang (Shaodong County, Xinning County, Xinshao County), Zhongxiang (Yongding District)</td>
<td>86.0 (81.0–87.0)</td>
<td>85.0 (84.0–86.0)</td>
<td>0.5 (–3.0 to 1.0)</td>
</tr>
</tbody>
</table>

*Similar seasonal patterns of absolute humidity had been observed among study sites with and without closures (e.g., study site 1–6, site 14, site 17, and reference site 1). LPM, live poultry market.

...authorities in epidemic areas even tried to control the spread of H7N9 virus by banning live poultry trade. Consequences of such interventions included loss of consumer confidence, decreases in prices of poultry products, and loss of market shares. In an attempt to reduce adverse effects in economic, less disruptive interventions were introduced, such as rest days, banning live poultry overnight, or periodic cleaning and disinfection (26,27). These LPM interventions proved to be less effective (28).

Besides LPM interventions, several key measures (e.g., culling known infected poultry and direct contacts, vaccinating poultry, or improving biosecurity for poultry-handling practices) have been taken to control zoonotic infection with H7N9 viruses (29). These measures are always applied in parallel and have gradually changed human behaviors related to the management, transportation, and trade of poultry. Specifically, traditional poultry handling and trade practices have been replaced by central slaughtering and frozen poultry products in major cities in China, which may have substantially reduced the risk of human exposure to infected poultry. The government of China implanted vaccination of poultry against H7N9 virus to control the 2017–18 epidemic wave after the surge in the reported number of cases in wave 5. The introduction of this H5/H7 bivalent inactivated vaccine substantially reduced the number of cases in wave 5. The introduction of this H5/H7 bivalent inactivated vaccine substantially reduced the number of cases in wave 5. The introduction of this H5/H7 bivalent inactivated vaccine substantially reduced the number of cases in wave 5. The introduction of this H5/H7 bivalent inactivated vaccine substantially reduced the number of cases in wave 5.

...biosecurity for poultry-handling practices, or which live bird species were found in LPMs. These factors and LPM interventions have always existed in parallel, so we cannot rule out the possibility that differences in the reduction in the daily number of infections among different sites may be partially explained by these anthropogenic factors, especially in sites with...
recursive or short-period closures. To more precisely differentiate the effectiveness of each type of LPM interventions, future studies could incorporate additional datasets to try to separate the effects of LPM closure from the natural transmission dynamics of H7N9 virus and other anthropogenic factors. Furthermore, the estimate of the reproduction number in this study relies on the assumption that this parameter is constant among locations. Although the estimate of this parameter did not involve the geographic locations of these cases and the likelihood that these human cases might have been in contact, the estimate was consistent with previous epidemiologic studies (3,23).

A number of research questions need to be further clarified in future studies. The optimal time and duration to implement LPM closure to balance the economic loss and transmission risk reduction needs further investigation, combined with a time-varying force of infection. Moreover, it could be possible to estimate key epidemiologic parameters (e.g., animal-to-human transmissibility and reproduction number) by considering the spatial–temporal dynamics of H7N9 epidemics in poultry and related environments, potential market functioning effects, and the frequency of human exposure to H7N9 virus to explain the differences in effectiveness.

In conclusion, the characteristics of LPM interventions can potentially affect their effectiveness. Although possibly more challenging from an operational point of view, permanent and long-period closures were found to be more effective in reducing human H7N9 cases during waves 1–5. In the long term, structural changes in the poultry value chain linked to permanent LPM closure may be required to maintain sufficient effectiveness of interventions and prevent the occurrence of H7N9 epidemics.

Acknowledgments
We thank Joseph T. Wu for technical support.

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References

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The island of Hispaniola remains the last location with endemic malaria in the Caribbean region, and ongoing elimination efforts aim to achieve zero cases from local transmission by the year 2025 (http://www.malariazeroalliance.org) (1). The western nation within Hispaniola, Haiti, has renewed interest in malaria elimination after the devastating 2010 earthquake in the southern part of the country, and local and international partners are collaborating to achieve this goal. In Haiti, chloroquine with a single dose of primaquine remains the first-line treatment for uncomplicated Plasmodium falciparum malaria, and strong evidence indicates that parasites in the country remain largely sensitive to chloroquine (2–4), although some researchers recommend monitoring patients after chloroquine treatment to ensure parasite clearance (5,6). Concerns about the potential importation of chloroquine-resistant P. falciparum haplotypes into Haiti persist because of the proximity to South America and because travelers from Africa commonly visit Haiti (1,7,8).

Until recently, sulfadoxine/pyrimethamine (SP) was the second-line treatment for malaria in Haiti. SP works to inhibit the protozoal folate pathway, can be administered inexpensively in a single dose, is generally well-tolerated by the recipient population (9,10), and has a long half-life in humans (both drugs remain in plasma well beyond 1 month) (11). These attributes have made SP an attractive option for malaria chemoprophylaxis (9,12) and other types of population-based mass drug administration (MDA) campaigns (13). Other antimalarial medications such as dihydroartemisinin/piperaquine are used in other settings for MDA but are hampered by the need for multiday dosing. As a nation moves toward malaria elimination and very low P. falciparum transmission rates, the residual infections in the human population are predominantly asymptomatic and even below the limit of detection for diagnostic tests (14,15). Having an effective


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Haiti is striving for zero local malaria transmission by the year 2025. Chloroquine remains the first-line treatment, and sulfadoxine/pyrimethamine (SP) has been used for mass drug-administration pilot programs. In March 2016, nationwide molecular surveillance was initiated to assess molecular resistance signatures for chloroquine and SP. For 778 samples collected through December 2017, we used Sanger sequencing to investigate putative resistance markers to chloroquine (P. falciparum Pfcrt codons 72, 74, 75, and 76), sulfadoxine (P. falciparum Ptdhps codons 436, 437, 540, 581, 613), and pyrimethamine (P. falciparum Pfdhfr codons 50, 51, 59, 108, 164). No parasites harbored P. falciparum point mutations. Prevalence of the P. falciparum Pfdhfr S108N single mutation was 47%, and we found the triple mutant P. falciparum Pfdhfr haplotype (108N, 51I, and 59R) in a single isolate. We observed no P. falciparum variants except in 1 isolate (A437G mutation). These data confirm the lack of highly resistant chloroquine and SP alleles in Haiti and support the continued use of chloroquine and SP.

The island of Hispaniola remains the last location with endemic malaria in the Caribbean region, and ongoing elimination efforts aim to achieve zero cases from local transmission by the year 2025 (http://www.malariazeroalliance.org) (1). The

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antiparasitic drug therapy capable of clearing this malaria reservoir through MDA could be a tool to interrupt *P. falciparum* transmission in a population (16).

In areas of the world with low levels of *P. falciparum* transmission, in vivo drug efficacy studies with sufficient statistical power are difficult to perform; thus, screening parasite populations for well-characterized genetic drug-resistance markers can provide a viable alternative to monitor the emergence of drug-resistant haplotypes (17,18). In particular, the *Pfcrt* K76T polymorphism is known to be the strongest single molecular predictor of chloroquine resistance; numerous global studies have linked this mutation with clinical failure (18–20). For SP treatment, each antifolate compound has multiple putative molecular markers that confer resistant phenotypes; mutations in the *Pfdhps* gene show the highest risk for resistance to sulfadoxine, and *Pfdhfr* mutations show the highest risk for resistance to pyrimethamine (21). More important, beyond a single missense mutation, multiple concurrent mutations (≥3) in a *P. falciparum* isolate’s *Pfdhps* and *Pfdhfr* genes have been shown to be a robust predictor of parasite resistance to SP (18).

As Haiti moves toward malaria elimination, verification of drug efficacy will be crucial for ensuring *Plasmodia* parasites can be successfully cleared from infected persons in the population. However, because malaria prevalence has declined to low levels in Haiti, conducting in vivo therapeutic efficacy studies becomes increasingly difficult. Therefore, routine molecular surveillance for markers of antimalarial drug resistance was established at the 11 sentinel sites in Haiti in 2016. In this article, we outline the molecular surveillance data related to chloroquine- and SP-resistant alleles from samples collected during the first 2 years of surveillance (2016–2017).

Materials and Methods

Surveillance Network and Population

We selected a health facility in each of Haiti’s 10 administrative departments, plus an 11th site in Grand Anse (the department that consistently has the highest number of reported malaria cases [1]), as sentinel sites to participate in antimalarial molecular resistance marker surveillance. Treatment-seeking persons of all ages who sought care at any of the 11 sentinel sites for symptoms of malaria and who also tested positive for malaria by either rapid diagnostic test (RDT) or microscopy were eligible to participate and provide a blood sample for malaria testing. Experienced microscopists performed thick-smear microscopy on Giemsa-stained slides. RDT testing was performed with RDTs available at each health facility and would have been by 1 of the 3 best-in-class HRP2-based tests: First Response Malaria Ag HRP2 (Premier Medical Corporation, http://premiemed-corp.com), CareStart Malaria HRP2 (Pf) (Access Bio, http://www.accessbio.net), and SD Bioline Malaria Ag Pf (Standard Diagnostics, https://sdinc.en). Patients who were clinically unstable and requiring urgent medical care were ineligible for participation.

We collected 2,016 samples from consenting patients from the 11 sentinel sites during March 2016–December 2017. The protocol for molecular surveillance was approved by the Haitian Ministry of Public Health and Population Bioethics Committee as a nonresearch programmatic activity. This protocol was also reviewed by the US Centers for Disease Control and Prevention (CDC) Center for Global Health and approved as a nonresearch surveillance activity. Blood specimens were collected only when participants (parents or guardians for children) consented to participate.

Because the sentinel health facility in Artibonite (Centre de Santé Clinique Jolivert) had not provided any malaria-positive specimens during 2016–2017, we chose samples from a separate malaria prevalence survey conducted in southern Artibonite in April 2017 from 2 health facilities ≈1 km apart to represent genotypes from this important malaria transmission area. This survey in Artibonite was approved by Haiti’s Ministry of Public Health and Population Bioethics Committee and the institutional review boards of Tulane University and the London School of Hygiene and Tropical Medicine. Adult participants provided written consent, consent for children (<18 years) was provided by a parent or guardian, and children >6 years of age gave written assent to participate. Persons 16 or 17 years of age who were married, a head of household, or a parent were considered mature minors and consented directly. Thumbprint consent or assent (countersigned by a witness) was used for illiterate participants. Persons <6 months of age or who required immediate medical attention were excluded.

Sample Collection and DNA Extraction in Laboratory

For consenting participants in sentinel surveillance and cross-sectional survey study sites, health facility workers drew a sample of ≥200 µL by fingerprick onto Whatman 903 Protein Saver cards (GE Healthcare Life Sciences, https://www.gelifesciences.com). To ensure consistency in blood sample collection, all health facility workers had previously been trained on appropriate dried blood spot sample preparation. Each filter paper was air-dried overnight and
individually stored in a sealed plastic bag containing a desiccant packet. Filter papers were stored at health facilities at room temperature away from sunlight until transfer. Three times per year, sentinel site samples were transferred to Haiti’s National Public Health Laboratory (Laboratoire National de Santé Publique [LNSP]). For the separate Artibonite survey, samples were transported to LNSP on a weekly basis.

At LNSP, filter paper cards were cut in half, with 1 section remaining at LNSP and 1 shipped to CDC (Atlanta, Georgia, USA) for molecular analysis and sequencing. On the Whatman 903 card, the center circle was not filled with blood to allow cutting of the card down the middle without introducing DNA contamination risk. Scissors were wiped with 70% ethanol between card cuttings. DNA extraction was performed by using the DNA Mini Kit (QIAGEN, https://www.qiagen.com) according to the manufacturer’s protocol, with the exception that double the amount of filter paper blood and proteinase K were used. After elution, DNA samples were kept at 4°C for short-term storage and −40°C for long-term storage.

*P. falciparum* Photo-Induced Electron Transfer–PCR and Gene Sequencing

We confirmed the presence and quantity of *P. falciparum* DNA by using the *P. falciparum* photo-induced electron transfer PCR (PET-PCR) real-time assay as described previously (22). We multiplexed primer targets for *Plasmodium* genus and *P. falciparum* 18S rDNA in a single reaction, which we then amplified for 45 cycles. We determined cycle threshold (Ct) on the basis of the beginning of the log phase increase in fluorescence intensity, and we considered a Ct value <40.0 to be DNA-positive signal. We converted Ct values to estimated parasite densities on the basis of a separate DNA dilution series of 3D7 and Dd2 *P. falciparum* blood cultures that had been dried on Whatman 903 filter paper and on the basis of DNA extracted according to the same protocol we have described. We estimated parasite density by extrapolating from Ct values on the basis of the average regression curve of the 2 *P. falciparum* culture strains (22).

We performed Sanger sequencing for *Pfcrt*, *Pfdhfr*, and *Pfdhps* as described previously (2) by using primer sets and reaction conditions for nested PCR and sequencing PCR (Appendix Table 1, https://wwwnc.cdc.gov/EID/article/26/5/19-0556-App1.pdf). We amplified the *Pfcrt* and *Pfdhfr* genes by using primary and secondary nested PCR reactions and amplified *Pfdhps* genes by using a single PCR reaction. We conducted all amplification reactions by using the Expand High Fidelity PCR buffer (Roche, https://www.roche.com) and the Sigma dNTP kit (Sigma Aldrich, https://www.sigma-aldrich.com), with addition of 1 µL of each primer (at 15 mmol/L) and 2 µL DNA per reaction, yielding a final reaction volume of 20 µL. We subjected amplified products to a sequencing PCR with the Applied Biosystems Big Dye kit (ThermoFisher, https://www.thermofisher.com) with 1 µL of 15 mmol/L primer (Appendix Table 1). We cleaned sequencing PCR products by ethanol precipitation and resuspended them in Applied Biosystems Hi-Di formamide (2). We sequenced samples on an Applied Biosystems 3130 XL Genetic Analyzer and read gene sequences on MacVector 7.2 software (MacVector, https://macvector.com).

**Results**

For samples collected during March 2016–December 2017, 757 were analyzed for molecular drug resistance markers associated with chloroquine and SP resistance. Of 11 sentinel sites participating in the program, 9 provided samples (Figure 1). A total of 21 samples for Artibonite were gathered through the separate survey in April 2017. Most samples analyzed (689, 88.6%) came from four health facilities in the western half of the southern peninsula (departments: Sud, Grand Anse, and Nippes).

As most persons were enrolled through positivity to an antigen-based RDT, presence of malaria DNA with a person’s blood sample was confirmed through PCR reactions at both the LNSP and the CDC malaria laboratory. The 2 laboratories showed good concordance in detection of parasite DNA through quantitative PET-PCR assays (Appendix Figure 1). Quantitative estimates for parasite densities in the 778 samples analyzed found the majority of *P. falciparum* infections to be at low estimated parasite densities; 299 (38.4%) were estimated at a parasite density of <1,000 p/µL blood and 544 (69.9%) <5,000 p/µL blood (Figure 2). Only 8 (1.0%) of the samples analyzed were estimated to contain a parasite density of ≥50,000 p/µL blood.

Of 778 samples analyzed, we successfully sequenced 741 (95.2%) for reporting of *Pfcrt* polymorphisms at codons 72, 74, 75, and 76 (Table; Appendix Table 2). All samples successfully sequenced showed the CVMNK genotype (codons 72–76), indicating an absence of the molecular markers for chloroquine resistance in the *Pfcrt* gene. Sequencing for *Pfdhfr* was less successful; 548 (70.4%) of all samples analyzed provided results for codons 50, 51, 59, 108, and 164 (Appendix Table 2). We found No polymorphisms at the C50R and I164L codons. A single isolate from the Cazale facility in the Ouest department showed a triple mutation in the N51I, C59R, and S108N codons,
but we observed no other N51I or C59R mutants in other isolates. Of interest, 46.9% (257/548) of Pfdhfr sequenced isolates carried the S108N mutation, associated with low level pyrimethamine resistance. The S108N mutation was found in all sites except the Pignon facility in the Nord department (although this facility only provided 2 samples) and ranged from 6% to 67% of isolates sequenced from each health facility (Table). Sequencing of Pfdhps was least successful, with 406 (52.2%) samples providing interpretable results. Not considering the 540 codon, we successfully 465 (59.8%) samples analyzed sequenced for Pfdhps.

The only mutations in Pfdhps were for the five considered codons, except for a single polymorphism of A437G found in 1 sample from the Les Anglais facility in Sud department.

The rate of success for sequencing the Pfcr, Pfdhfr, and Pfdhps genes was largely dependent upon the estimated parasite density via P. falciparum DNA content in the sample. At parasite densities >1,000 p/µL blood, all codons were reported for Pfcr in >98% of samples (Figure 3). Even at estimated densities <1,000 p/µL blood, reportable results were possible for the Pfcr codons in 91% of the isolates. More striking differences in success of gene sequencing were observed for Pfdhfr and Pfdhps genes. Patient samples with estimated densities >1,000 p/µL blood generated reportable results for the 10 codons (5 for Pfdhfr and 5 for Pfdhps) >75% of the time, and in samples >25,000 p/µL blood sequencing was successful 89% of the time (if not including Pfdhps K540E at 79%). However, at densities <1,000 p/µL blood, the 5 Pfdhfr codons were only sequenced in 50% of samples and the 5 Pfdhps codons in 17% of samples.

Discussion

Our study aimed to describe Haiti’s nationwide sentinel site surveillance program for monitoring antimalarial drug resistance for samples collected during 2016–2017 (1). Because patient samples will continue to be collected from these participating health facilities during the country’s progress toward malaria elimination, changes in the parasite population (e.g., changes in molecular signatures related to drug resistance) should be quickly detected through this surveillance system. Epidemiologic findings from the
first 2 years of the surveillance program will be reported elsewhere.

We used a total of 778 samples, representing 9 of the 10 departments in Haiti (no samples were taken in the Nord-Ouest department), for this initial report to establish a molecular baseline for polymorphisms in the \emph{P. falciparum} genes \emph{Pfcrt}, \emph{Pfdhfr}, and \emph{Pfdhps}. Being able to draw from samples in many different areas of the country enables a higher confidence of genetic representation in identifying any drug-resistant populations; however, isolated foci of drug-resistant haplotypes might exist in the country. Because the RDT used in this survey relies on detection of histidine-rich protein (HRP) 2 for primary diagnosis, any \emph{P. falciparum} isolates lacking functional HRP2 and HRP3 production might have been overlooked through this surveillance strategy (23). However, this possibility is remote, given that no evidence to date suggests any deletion of the HRP2 gene in this population.

All persons in this study were enrolled at health facilities, so the data do not represent the asymptomatic or non–treatment seeking reservoir in Haiti, and our enrollment procedures might also have missed smaller foci of inbred or clonal \emph{P. falciparum} drug-resistant haplotypes in the country outside of the catchment areas of these health facilities (24, 25).

Most (689 [88.6%]) of the samples tested were from patients enrolled in 1 of 4 health facilities in the southwest corner of Haiti. This proportion is indicative of current \emph{P. falciparum} transmission in Haiti, where higher case counts have been observed in the departments of Grand Anse, Sud, and Nippes compared with the rest of the country (1, 5, 26).

<table>
<thead>
<tr>
<th>Site (no. samples)</th>
<th>\textit{Pfcrt}†</th>
<th>\textit{Pfdhfr}</th>
<th>\textit{Pfdhps}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arnaud (211)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Capotille (13)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Cazale (23)</td>
<td>ND</td>
<td>1 (4)</td>
<td>1 (4)</td>
</tr>
<tr>
<td>Thienne/Dauphine (21)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Lavanneau (12)</td>
<td>ND</td>
<td>1 (6)</td>
<td>ND</td>
</tr>
<tr>
<td>Les Anglais (211)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Pignon (2)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Roseaux (68)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Sainte-Hélène (199)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Thononde (18)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Total (778)</td>
<td>ND</td>
<td>1 (0.2)</td>
<td>1 (0.2)</td>
</tr>
</tbody>
</table>

*Values are no. (%) unless indicated. ND, not detected.
†Mutations in any of the \emph{Pfcrt} C72S, M74I, N75E, or K76T codons.
‡S436A, S436Y, or S436F mutations.
§A613S or A613T mutations.
to future drug pressure will be of particular focus for timely acquisition of molecular drug-resistance data.

Investigations of *P. falciparum* resistance to chloroquine in Haiti have been numerous and ongoing since 1981. Even the first reports in the early 1980s found in vivo and in vitro evidence for possible resistance to chloroquine (27,28), which had been in use in Haiti at least since the 1950s (29). With the advent of mutations in the *Pfcrtr* gene serving as molecular markers for chloroquine resistance (18,19), the *Pfcrtr* K76T haplotype has been reported sporadically in Haiti since 2009, but always at very low rates (2,30). Our study found that all 741 isolates collected from sites around Haiti that were successfully sequenced for *Pfcrtr* have codons 72–76 of the CVMNK genotype, which indicate chloroquine susceptibility (31,32). Our data and previous reports indicate that chloroquine-resistant *Pfcrtr* alleles evidently are rarely found in Haiti, a finding which supports the continued use of this drug for the primary treatment of malaria in Haiti. As antimalarial treatment practices are improved nationwide during elimination efforts, ongoing monitoring will be necessary to ensure the appropriate use of this important drug in the country.

During malaria elimination efforts in Haiti in the 1960s, MDA campaigns distributed chloroquine/pyrimethamine tablets (at an adult dose of 600 mg chloroquine and 50 mg pyrimethamine) to the population in areas with a high level of transmission, with particular focus on the southern peninsula (33). Drug uptake in the population was high (>90%), and in total, >2 million persons in Haiti were reached by these campaigns, some receiving up to 15 rounds of MDA. The MDA campaigns were begun in 1964, and by 1966, widespread success in reducing parasite rates led to focal drug distribution in outbreak areas and eventual cessation in the late 1960s (34). With termination of malaria elimination efforts in the 1970s, *P. falciparum* cases rapidly increased throughout the nation to the point where most of the population was again at risk for exposure (34).

One of the most consistent findings in this 2016–2017 study was the pervasive prevalence of the *Pfdhfr* S108N mutation in Haiti; 47% of all successfully sequenced isolates showed this polymorphism. This point mutation is well known to develop under pyrimethamine drug pressure (18), and previous studies in Haiti have found evidence to both in vivo and in vitro pyrimethamine resistance (35,36), and, more recently, the specific S108N mutation at frequencies of 36% (2) and 33% (37). Because the *P. falciparum* parasite population in Haiti has been shown to be of low genetic diversity and distinct from that of South America (2,3,38), presence of this genotype in Haiti probably could be traced back to MDA campaigns in the 1960s. It is remarkable that *P. falciparum* parasites in Haiti would continue to carry this polymorphism after 50 years, although the S108N mutation has been shown to be the first to arise during pyrimethamine drug pressure (39,40). This observation is consistent with the hypothesis that the S108N mutation does not affect fitness of the parasites carrying this allele (41). S108N mutation confers only low-level resistance to pyrimethamine, and the presence of ≥3 mutations (leading to the N51I/C59R/S108N triple mutant) is required for high-level resistance (42–45). Outside of pyrimethamine, the atovaquone/proguanil chemoprophylactic regimen often used by travelers to Haiti might also have the potential to induce drug pressure on the *Pfdhfr* gene and might lose efficacy if multiple codon mutations would arise in the gene (46). Because the *P. falciparum* parasite population in Haiti is currently only showing incomplete penetration of the S108N mutation, if SP were used for MDA in Haiti, then this ongoing drug-resistance surveillance program will be crucial to detect any emergence of *Pfdhfr* double or triple mutants, especially in conjunction with the A437G and K540E mutations in the *Pfdhps* gene (47). The presence of 1 *Pfdhtr* triple mutant in Cazale and 1 *Pfdhps* single mutant in Les Anglais is notable, but both represent well under 1% of the parasite isolates in the our study, which is consistent with previous reports (2,37).

Categorizing the samples by quantified parasite DNA levels was very predictive of success in Sanger sequencing for the *Pfdhfr* and *Pfdhps* genes, although *Pfcrtr* Sanger sequencing performed well at any level of DNA content. Using this strategy of DNA quantification will assist in excluding samples for *Pfdhfr* and *Pfdhps* Sanger sequencing that would have a low likelihood of interpretable results. Although the success rate for Sanger sequencing of *Pfdhfr* and *Pfdhps* was low compared with the success rate for *Pfcrtr*, especially in low-density parasitemia samples, resistant chloroquine or highly resistant SP resistant genotypes clearly are very rare across Haiti. Furthermore, our results support the rationale for using SP for MDA in certain areas of Haiti with the aim of interrupting *P. falciparum* transmission as part of malaria elimination efforts. If different antimalarial drug strategies are proposed for Haiti in the future, this nationwide sentinel surveillance system could also be used for monitoring of other putative genetic markers of resistance. In addition, employing next-generation sequencing methods will also enable the comprehensive characterization of this parasite population for
well-established resistance markers in other target genes besides only Pfert, Pfdhfr, and Pfdhps. Continued monitoring for drug-resistance markers, especially during MDA, will be critical for detecting any potential emergence of highly resistant genotypes associated with resistance to SP and chloroquine.

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Systematic Review and Meta-Analysis of Sex Differences in Social Contact Patterns and Implications for Tuberculosis Transmission and Control

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Tuberculosis (TB) is the leading infectious cause of death worldwide; there were an estimated 1.3 million deaths during 2017 (1). Approximately 25% of the world’s population is infected with Mycobacterium tuberculosis (2), the bacterium that causes TB (3). Of 1.7 billion persons infected with M. tuberculosis, TB developed in 10 million persons during 2017 (1,4). Despite major investment in disease control efforts since the 1990s, progress has been slow; incidence is currently decreasing by only 1.5%/year (3).

TB predominantly affects men, who have 60% of reported cases and 65% of reported deaths globally (1). Men are less likely than women to access timely TB diagnosis and treatment (5,6) and remain infectious in the community for a much longer period (5,7). The impact is apparent from recent prevalence surveys of undiagnosed TB, which offer the most accurate measure of disease burden (1) and confirm pronounced sex disparity; men account for 70% of infectious cases in the community (5).

Critically, M. tuberculosis is spread person-to-person by airborne transmission. Undiagnosed infectious TB is the key driver of ongoing transmission, and most TB episodes reflect recent transmission from adult contacts (3). The excess burden of TB in men might be a result of broader socialization patterns that emerge during adolescence (8,9). The risk for TB in men might be amplified if sex-assortative (like-with-like by sex, male or female) mixing is prevalent, such that men have greater contact with other men than with women (5). Sex-specific social contact patterns might also be useful in understanding TB in women and children, as shown by analytical results suggesting most new M. tuberculosis infections among men, women, and children in South Africa and Zambia can be attributed to contact with men (10).

Data from social contact surveys provide insight into how individual behaviors drive disease dynamics at the population level (11), providing better predictions of patterns of infection for respiratory pathogens (12,13) than can be made from assumptions of homogenous or proportionate mixing (14). Several analyses have examined sex differences in social contact patterns, although most
analyses report sex differences in the number of reported contacts. Only a few analyses have assessed the sex assortativity of contacts in sufficient detail to provide major insights into the transmission potential for diseases with major sex disparities, such as TB (10,15,16).

We conducted a systematic review and meta-analysis to examine sex differences in the number, sex assortativity, and location of social contacts reported by children and adults. Our main aims were to evaluate sex-based social contact patterns in children and adults, sex-assortative mixing among adults, and the frequency of contact between men and boys, men and girls, and men and women.

Methods

Search Strategy

We conducted this systematic review according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Appendix 1 Checklist 1, https://wwwnc.cdc.gov/EID/article/26/5/19-0574-App1.pdf) and Meta-Analyses of Observational Studies in Epidemiology (MOOSE) guidelines (Appendix 1 Checklist 2) in accordance with a published protocol (17). We identified publications describing social contact surveys conducted during January 1, 1997–August 5, 2018, through searches of PubMed, Embase, Global Health, and the Cochrane Database of Systematic Reviews (Appendix 1 Table 1). We searched reference lists from included publications by hand and contacted researchers with expertise in these surveys, particularly authors of a recent systematic review (18), to assist with identification of relevant publications.

Two authors (K.C.H. and A.L.H.) independently reviewed titles and then abstracts, in parallel, for relevance and included publications identified by either author for full-text review. These authors also reviewed full texts to determine which publications met inclusion criteria and then reviewed texts and supplemental materials to determine whether data on sex were recorded for participants and contacts. These authors contacted publication authors if it was unclear whether these data had been collected.

K.C.H. extracted data on methods from included surveys by using a piloted electronic form and gathered datasets from supplemental materials or a social contact data repository (https://www.social-contactdata.org) if results were not reported in a format necessary for meta-analyses. When datasets were not publicly available, K.C.H contacted authors and asked them to share relevant results or data.

Inclusion and Exclusion Criteria

The review included cross-sectional surveys conducted to assess social contact patterns relevant to airborne disease transmission that recorded participant sex and contact sex. We included only surveys that recorded all contacts over the survey period; we excluded surveys that examined only a subset of participants’ contacts (e.g., only those within a workplace or with other participants). We also excluded surveys that included only participants or contacts of a single sex and, because of limited sources for translation, publications in languages other than English. When we identified >1 report for a single survey, we included the earliest source or most complete dataset and excluded other records.

Survey Quality

We assessed each survey by using the Appraisal Tool for Cross-sectional Studies (AXIS tool). This tool evaluates survey design, reporting quality, and risk for bias (19).

Definitions

We considered participation equitable by sex if each sex made up 45%–55% of the survey population. We adjusted numbers of participants for analyses of physical and location-based contacts to exclude participants who did not report this information.

We stratified participants and contacts by age as children (boys and girls) and adults (men and women). For most surveys, adults were defined as persons ≥15 years of age (1); in instances where aggregate age categories did not enable disaggregation at this cutoff point, we used the nearest possible value. We defined close contacts, including physical and nonphysical contacts, according to survey-specific definitions, typically by a conversation longer than a greeting or ≥3 words.

We defined sex-assortative mixing as like-with-like contacts according to sex (male or female), either within age groups (e.g., men-with-men) or between age groups (e.g., men-with-boys). We defined preferential mixing as more mixing with 1 sex/age group than another.

Data Analysis

For each survey, we calculated the average number of contacts over a 24-hour period for each sex/age category of participants with each sex/age category of contacts. For surveys in which data were collected over a 48-hour period, we divided the number of contacts by 2. For surveys in which data were collected over a 72-hour period, we divided the number of
contacts by 3. We compared the average number of contacts across sex and age groups by using the Mann-Whitney-Wilcoxon test.

We calculated the percentage of sex-assortative mixing with 95% Clopper-Pearson CIs as contacts with the same sex divided by total contacts. We assessed sex-assortative mixing in children’s contacts with children and adults and in adults’ contacts with children and adults. We also compared the proportion of sex-assortative mixing by contact location: contacts within the home and contacts outside the home and, among contacts outside the home, contacts at work (for adults), school (for children), and elsewhere. We assessed heterogeneity by using the I² statistic (20) and summarized findings across surveys by using the median and interquartile range (IQR).

We estimated the percentage of boys’, girls’, men’s and women’s adult contacts with men for subgroups based on survey setting characteristics (region, setting, and TB burden) and survey methods (sampling methods, reporting duration, age cutoff values for adults, and participation by sex). We excluded contact events for which the participant’s sex or age or the contact’s sex or age was missing. We made no adjustments for nonparticipation or nonsampling and used no weighting. We performed all analyses by using R version 3.2.2 (21).

Results
Of 124 full-text publications reviewed for eligibility, we excluded 76 (Appendix 1 Table 2), and identified 48 that had eligible methods (Figure 1). Twenty-three publications described surveys that did not, to our knowledge, record sex and age for participants and contacts (Appendix 1 Table 3); 25 publications described surveys that were known to have recorded

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**Figure 1.** Preferred reporting items for systematic reviews and meta-analyses flowchart used for analysis of sex differences in social contact patterns and tuberculosis transmission and control.
sex and age for participants and contacts (Appendix 1 Table 4). Data were available for meta-analysis from 14 publications describing 21 surveys (10,13–16,22–30) (Table, https://wwwnc.cdc.gov/EID/article/26/5/19-0574-T1.htm; Appendix 2, https://wwwnc.cdc.gov/EID/article/26/5/19-0574-App2.xlsx).

Included surveys had >22,146 participants and 270,308 sex-specific/age-specific contacts. Surveys were conducted in 17 countries: 4 surveys with 5,085 participants in Africa, 1 survey with 558 participants in the Americas, 11 surveys with 11,260 participants in Europe, and 5 surveys with 5,243 participants in the Western Pacific region. Thirteen surveys were conducted in high-income countries, 5 in upper-middle-income countries, 2 in lower-middle-income countries, and 1 in a low-income country. Ten surveys were conducted at a national scale; 11 were subnational. All surveys were during 2005–2016. Seventeen surveys included child participants; 20 adult participants, and 16 both children and adults.

**Participation by Sex**

Participation by children was considered equitable by sex in 15 (88%) of 17 surveys. In 2 (12%) surveys, participation by boys substantially exceeded that by girls: boys made up 56% and 57% of the population of each survey. Participation by adults was considered equitable by sex in 11 (55%) of 20 surveys. In 8 (40%) of 20 surveys, participation by women substantially exceeded that by men; women made up 56%–83% of the population of each survey. In 1 (5%) survey, participation by men substantially exceeded that by women; men made up 60% of the survey population.

**Social Contacts by Boys and Girls**

The median number of contacts reported over a 24-hour period was 12.9 (IQR 9.3–15.9) for boys and 13.5 (IQR 9.5–15.9) for girls (Appendix 1 Table 5); the difference in numbers of contacts was not significant (p = 0.92). Approximately half of contacts reported by boys (median 53%, IQR 43%–55%) and girls (median 51%, IQR 45%–56%) were with other children.

Among contacts of children with other children, we found strong evidence of sex-assortative mixing reported by boys in 15 (88%) of 17 surveys and by girls in 15 (88%) of 17 surveys (Figure 2, panels A, C; Appendix 1 Table 6). The median percentage of sex-assortative mixing in contacts with children was 62% (IQR 59%–63%) for boys and 59% (IQR 59%–65%) for girls. Summary measures are not reported because of substantial heterogeneity between surveys ($I^2 = 96.3\%$ for boys, $I^2 = 95.6\%$ for girls).

Among contacts of children with adults, there was no evidence of sex-assortative mixing reported by boys and strong evidence reported by girls in 17 (100%) of 17 surveys (Figure 2, panel B, D, Appendix 1 Table 6). The median percentage of sex-assortative mixing was 42% (IQR 41%–43%) for boys and 61% (IQR 60%–63%) for girls. Boys reported preferential mixing with women in 15 (88%) of 17 surveys. Summary measures are not reported because of substantial heterogeneity between surveys ($I^2 = 73.8\%$ for boys, $I^2 = 44.3\%$ for girls).

Most contacts reported by children took place outside the home (median 65% [IQR 62%–72%] for boys, median 67% [IQR 56%–73%] for girls) (Appendix 1 Table 7). The sex assortativity of children’s contacts outside the home was similar to that at home. Among contacts with children, boys and girls reported more sex-assortative mixing in contacts outside the home than at home in 6 (43%) of 14 surveys for boys and 5 (36%) of 14 surveys for girls (Figure 3, panels A, C; Appendix 1 Table 8). Among contacts with adults, boys reported no more sex-assortative mixing in adult contacts outside the home than at home in 14 (100%) of 14 (100%) surveys, and girls reported more sex-assortative mixing outside the home than at home in 6 (42%) of 14 surveys (Figure 3, panels B, D; Appendix 1 Table 8). Summary measures are not reported because of substantial heterogeneity between surveys ($I^2 = 88.4\%$ for boys, $I^2 = 83.0\%$ for girls).

Among contacts of children outside the home, ≈50% of contacts of boys and girls contacts (median 56% [IQR 39%–62%] for boys, median 55% [IQR 38%–63%] for girls) occurred at school (Appendix Table 9). We found few differences in the sex assortativity of contacts at school compared with those at other locations outside the home (Appendix 1 Table 10, Figure 1). Summary measures are not reported because of substantial heterogeneity between surveys ($I^2 = 84.7\%$ for boys, $I^2 = 74.1\%$ for girls).

**Social Contacts by Men and Women**

The median number of contacts reported over a 24-hour period was 11.1 (IQR 8.1–15.3) for men and 11.6 (IQR 7.8–14.3) for women (Appendix 1 Table 11); the differences were not significant (p = 0.88), and the total number of contacts reported by adults did not differ from the total number of contacts reported by children (p = 0.26). Most contacts reported by men (median 91% [IQR 88%–93%] and women (median 87% [IQR 83%–90%]) were with other adults, which was significantly more than the number of adult contacts reported by children (p = 0.01).
Among contacts of adults with children, there was strong evidence of sex-assortative mixing reported by men in 4 (20%) of 20 surveys and by women in 4 (20%) of 20 surveys (Figure 4, panels A, C; Appendix 1 Table 12). In 15 (75%) of 20 surveys, there was no major evidence of preferential mixing by sex reported by men or women in contacts with children. The median percentage of sex-assortative mixing was 53% (IQR 50%–57%) for men and 52% (IQR 50%–54%) for women. Summary measures are not reported because of substantial heterogeneity between surveys ($I^2 = 76.3\%$ for boys, $I^2 = 81.6\%$ for girls).

Among adult contacts with other adults, there was strong evidence of sex-assortative mixing reported by men in 16 (80%) of 20 surveys and by women in 19 (95%) of 20 surveys (Figure 4, panels B, D; Appendix 1 Table 12). The median percentage of sex-assortative mixing was 56% (IQR 54%–58%) for men and 59 (IQR 57%–63%) for women. Summary measures are not reported because of substantial heterogeneity between surveys ($I^2 = 98.1\%$ for men, $I^2 = 97.0\%$ for women).

Most contacts reported by adults took place outside the home (median 74%, IQR 62%–77% for men; median 70%, IQR 54%–76% for women) (Appendix 1 Table 13). Contacts of adults with children showed similar sex assortativity at home and outside the home (Figure 5, panels A, C; Appendix 1 Table 14). Among contacts of adults with adults, there was more sex-assortative mixing by men and women in contacts outside the home than in contacts within the home in 14 (93%) of 15 surveys (Figure 5, panel B, D; Appendix 1 Table 14). Summary measures are not reported because of substantial heterogeneity between surveys ($I^2 = 63.1\%$ for men, $I^2 = 28.6\%$ for women).

Among adult contacts outside the home, ≈33% of contacts of men and women (median 35% [IQR 28%–39%] for men, median 29% [IQR 26%–34%] for women) occurred at work (Appendix 1 Table 15). Because adults reported few contacts with children at work,
**Sex Differences in Social Contact Patterns and TB**

CIs are wide for sex-assortative mixing estimates for men and women in most surveys (Appendix 1 Table 16, Figure 2, panels A, C). Men reported more sex-assortative mixing in contacts with other adults at work compared with contacts elsewhere outside the home in 12 (80%) of 15 surveys and elsewhere in 1 (7%) of 15 surveys (Appendix 1 Table 16, Figure 2, panels B, D). Women reported more sex-assortative mixing at work compared with contacts elsewhere outside the home in only 2 (13%) of 15 surveys and elsewhere in 1 (7%) of 15 surveys. Summary measures are not reported because of substantial heterogeneity between surveys ($I^2 = 32.3\%$ for men, $I^2 = 87.0\%$ for women).

**Subgroup Analyses**

Subgroup analyses did not show clear differences in the frequency of contact with men by survey setting or method. There was little variation in survey characteristics measured by the AXIS tool (Appendix 1 Table 17). Substantial heterogeneity remained in summary measures for subgroups examined (Appendix 1 Table 18).

**Discussion**

The main finding of this systematic review and meta-analysis of 21 social contact surveys in 17 countries is that sex differences in social contact patterns are profound, to an extent likely to be amplifying sex disparities in the adult burden of TB in many settings. Differences in sex-specific and age-specific social contact patterns between children and adults suggest a behavioral shift during adolescence, potentially driving the emergence of sex difference in TB epidemiology in adults. Sex-assortative mixing in adult contacts was reported by men in 80% of surveys and women in 95% of surveys. These findings have critical implications for men’s health and for broader TB prevention efforts because half of men’s contacts, one third
of women’s contacts, and one fifth of children’s contacts were with adult men.

Social contact patterns clearly differ for children and adults. There was no major difference in the total number of contacts reported by children and adults. However, half of children’s contacts were with other children, who are less likely than adults to have TB or to transmit *M. tuberculosis* (31), and most adult contacts were with other adults. Children of both sexes frequently reported preferential mixing with women in adult contacts, and men and women both reported sex assortivity in contacts with other adults.

Among children, sex-specific patterns of contact with adults were similar at home and outside the home, and preferential mixing with women was reported across locations. Although many contacts were reported at school and substantial child contact time occurs at school (25), those contacts include few adult contacts and therefore limited opportunity for exposure to *M. tuberculosis*. These differences in contact patterns among children and adults support recent genetic epidemiology studies suggesting that only a small proportion of adult infections occur within the household (32,33) but that the odds of household transmission of *M. tuberculosis* are much higher among children (34). The higher number of adult contacts outside the home and greater sex assortativity of those contacts compared with children might partially explain the emergence of sex differences in TB epidemiology in adults.

In nearly all of the surveys examined, strong sex-assortative mixing in adult contacts was reported by men and women, as noted in previous studies that have examined sex assortativity (10,15,16). Results from our study indicate that in many settings, sex-assortative mixing might exacerbate the disproportionate burden of disease for men by amplifying risk for infection in a population already at greater risk for disease because of a nexus of biological, sociobehavioral, and health systems factors (5). Further research

![Figure 4](image-url)
is needed to determine the relative contribution of
sex-assortative mixing among these factors.

Among adults, reports of sex-assortative mixing
were not symmetric; men reported less sex-assor-
tative mixing than women in nearly half of surveys
conducted among adults. In 3 surveys in which men
did not report strong sex-assortative mixing, women
did (13,29,30), raising questions of reporting bias. Pre-
vious studies that used wireless sensor devices have
shown greater concordance between sensor and self-
report methods for women than men (35), suggesting
that inconsistencies might, in part, reflect less accu-
rate reporting by men.

Only 1 survey, from rural and periurban Zim-
babwe, reported no assortative mixing by adult
respondents (26). This survey provided strong evi-
dence of true negative sex assortativity among boys,
girls, men, and women, suggesting underlying
differences in social behavior that affect social
interactions might pertain in some settings. This sur-
vey was similar in design to other surveys, but also
reported a young age structure and substantial inter-
gerational mixing with extremes of age (26). Sex
differences were less pronounced in the 2014 nation-
al TB survey in Zimbabwe than in other countries
in Africa (I).

Our analysis of social contact patterns across sex
and age groups has implications for M. tuberculosis
transmission beyond understanding the excess bur-
den of TB in men. Although sex-assortative mixing
among adults to some extent protects women from
exposure to M. tuberculosis transmission, one third of
women’s contacts and one fifth of children’s contacts
were with men. Therefore, the excess burden of TB
among men has implications for M. tuberculosis trans-
mision across the population, making strategies to
provide early diagnosis of TB for men of potentially
high public health value.
Our study had several limitations. Less than half of eligible publications had data on sex and age for participants and contacts, limiting the number of surveys included in our analyses. We recommend that future social contact surveys collect and report these data, ideally by using standardized tools to try to reduce high intersurvey heterogeneity that prevented us from reporting summary measures. In addition, our focus on close contacts will have excluded some contacts relevant to the spread of *M. tuberculosis* (36) but was dictated by data availability because no surveys reported casual contacts by sex. We also did not assess the intimacy or duration of contacts by sex.

Our analysis in only 2 age categories (children and adults) also reflects the nature of available data but might have led us to overlook more nuanced age differences in sex-based social contact patterns. Some surveys deliberately oversampled certain age groups, and we made no adjustments in our analyses for sampling bias and used no weighting, because of a lack of data on which to weight. Response bias might also have affected results, but few surveys reported the response rate, and none distinguished the response rate by sex.

Men are often overlooked in discussions of sex and TB, and strategies to assess and address men’s excess burden of disease and barriers to TB care are notably absent from the global research agenda. However, because men have most TB cases and remain untreated, and therefore infectious, longer than women, a better understanding of the factors that drive their disproportionate burden of disease is essential to appropriately direct resources to address these disparities. Our results show that social contact patterns likely contribute to the emergence of sex disparities in the adult burden of TB by amplifying men’s burden of disease. Contacts of men with women, boys, and girls show that the excess burden of TB among men also has serious implications for *M. tuberculosis* transmission across sex and age groups. Addressing the excess burden of TB in men is essential to improve men’s health and to meet the ambitious targets for reducing TB incidence and deaths (37,38).

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About the Author

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Sex Differences in Social Contact Patterns and TB
Effects of Air Pollution and Other Environmental Exposures on Estimates of Severe Influenza Illness, Washington, USA

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Seasonal influenza is associated with an estimated 3,300–48,000 annual deaths in the United States (1) and has a major global impact on economies and health (2–4). Prospective surveillance with specific laboratory testing for influenza is expensive and may underestimate the true burden of influenza if such tests are underused or insensitive or if influenza results in complications or hospitalizations beyond the period in which virus may be detected in patient samples (5). Therefore, the Centers for Disease Control and Prevention (CDC) and other public health organizations use modeling studies to estimate the incidence of severe influenza illness to inform public health actions (1,3,6–10). Typically, modeling of the influenza disease burden links aggregate data for outcomes identified in vital statistics or hospitalization administrative databases to influenza virologic surveillance data over time. The difference between estimates with and without influenza covariates is attributed to influenza activity. Such models have been used extensively in the United States (10–14), in other countries (15–17), and to produce global estimates of influenza disease burden (2,3,18–20). The resulting estimates of excess influenza-associated events inform public health actions, such as vaccine or treatment recommendations, as well as patient and healthcare provider communications.

In the United States, influenza and most other respiratory infections are seasonal and follow an approximately sinusoidal curve with winter peaks. Climatic and air pollutant parameters, such as temperature, humidity, and ambient fine particulate matter, vary during the putative influenza season and are associated with acute respiratory infections (21). Because these other factors share a seasonality similar to influenza, neglecting them may overestimate the effects of influenza on health outcomes. Influenza models that include meteorological data have improved predictive accuracy for viral circulation and peak seasonality (21–23). National and global models of influenza disease burden do not account for environmental and meteorological
parameters, which may be important confounding variables (1–3,6–8).

Given the importance of influenza disease burden estimates on public health decision making and the reliance on ecologic models that exclude environmental exposure covariates, we undertook this study to evaluate the effect of including environmental exposures in traditional models on estimates of influenza disease. We hypothesized that environmental exposures would be associated with severe respiratory and circulatory (RC) hospitalizations and that adjustment for these covariates would have a clinically meaningful effect on estimates of severe influenza disease incidence.

Materials and Methods

Design Overview
We conducted a study using aggregated datasets from 3 counties (King, Pierce, and Snohomish) in western Washington state during 2001–2012. We used administrative hospitalization data, respiratory virus surveillance data, and environmental exposure data collected prospectively from the study area. The primary analysis was to estimate the incidence of influenza-associated RC hospitalizations using standard CDC ecologic models and to assess the effect that inclusion of environmental exposure variables in these models had on the incidence estimates. In a secondary analysis, we added respiratory syncytial virus (RSV) as an additional exposure covariate into the model. This study received exempt review status from the Human Subjects Division at the University of Washington and the Washington State Department of Health Institutional Review Board.

Hospitalization Database
We obtained the Washington State Department of Health Comprehensive Hospital Abstract Reporting System (CHARS) dataset for the study area and time periods. The CHARS database contains publicly available deidentified discharge information derived from hospital billing systems for patients in all of the public and private hospitals in Washington (24). The CHARS data contain information on age, home ZIP code, and other demographics, as well as patient diagnoses, procedures, and billed charges. We defined RC hospitalizations as any listed hospitalizations with codes 390–519 from the International Classification of Diseases, 9th Edition (5,6,8). We categorized age as 0–6 months, 7–23 months, 2–4 years, 5–14 years, 15–49 years, 50–64 years, and ≥65 years. We calculated aggregate RC counts per day, age category, and county and merged them with the other datasets for statistical analysis. The unit of observation was RC hospitalization, which we also call “event” in this report.

Respiratory Virus Surveillance Data
We accessed influenza virus surveillance data from 3 sources in the study area: University of Washington Clinical Virology Laboratory, Public Health–Seattle & King County, and Seattle Children’s Hospital. Each laboratory was in King County and participated in the United States Influenza Virologic Surveillance System during the study period. Influenza testing data were available for September 30, 2001, through December 29, 2012, except for the third quarter of 2002 (25). Clinical specimens collected as part of routine care were tested in laboratories for evidence of influenza virus, and results were reported to local and the state health departments and CDC. The 3 sites used viral culture or reverse transcription PCR (RT-PCR), with an increasing use of RT-PCR over the study period. We did not include influenza data from Tacoma and Snohomish counties. Public health respiratory virus surveillance was not conducted in the counties during the study period. We reviewed limited influenza testing data from the largest hospital systems in each county. Total influenza tests from Tacoma (23,741) and Snohomish counties (<3,000) were very low compared with those from King County (372,022) and were available for only part of our study period (2007–2008 and 2008–2012 for Tacoma and 2010–2013 for Snohomish). Influenza seasonality and peak seasons were similar in all 3 sites. Laboratory reports did not consistently distinguish between influenza A subtypes or influenza B lineages; therefore, we included only influenza A and B as exposure variables. The seasonality and temporal peaks of the proportion positive of influenza A and influenza B data among these sites were similar, so we aggregated each across all 3 counties for analyses.

RSV laboratory data were collected as part of routine care by the University of Washington Clinical Virology Laboratory and reported to the National Respiratory and Enteric Virus Surveillance System; these data were available for the period September 30, 2007–December 29, 2012 (26). RSV tests used antigen detection, viral culture, and RT-PCR testing, with RT-PCR use increasing over the period. RSV subtypes were not available.

We used weekly surveillance data for our model. We divided the weekly number of influenza A and influenza B detections by the weekly number of
influenza tests performed and multiplied the result by 100 to calculate a weekly percentage of positive tests. We calculated the weekly percentage of positive RSV tests similarly.

Environmental and Meteorology Exposure Time Series
We accessed daily meteorology data including temperature, relative humidity, and dew point for 6 meteorological stations from each of the 3 counties studied (27,28) (Appendix Figures 1–5, https://wwwnc.cdc.gov/EID/article/26/5/19-0599-App1.pdf). The values for the 6 stations were highly correlated (Pearson correlation coefficient range 0.93–0.99). Because the data from Boeing Field station in King County were the most complete and the station was the closest to the urban centers, we used data from this station to represent the meteorological exposures for the entire study area. For the meteorological data, including temperature, relative humidity, and dew point, 680 weeks (4,736 days) of data were available, leaving <0.005% of days with missing data during the study period.

We also used daily ambient outdoor air pollution data in the form of concentration of particulate matter with a diameter <2.5 μm (PM$_{2.5}$) (29,30) (Appendix Figures 6, 7). The PM$_{2.5}$ concentration data were available for 21 stations from each of the 3 counties, giving a total of 4,581 days of data. Some of the stations were distant from urban centers (e.g., the Mount Rainier National Park station in Pierce County); others had substantial periods with missing data during the study period. Three stations, Seattle–Beacon Hill (King County), Tacoma (Pierce County), and Marysville (Snohomish County), were close to urban centers and had less missing data; we used these sites to define the daily PM$_{2.5}$ exposures. Because the daily PM$_{2.5}$ exposures for the 3 stations were highly correlated with only small systematic differences (Pearson correlation coefficients among pairs of stations 0.74–0.91), we averaged daily PM$_{2.5}$ exposures across the available values for the stations. The resulting daily average was available in 96% of the study period.

Population Estimates
We obtained annual age-specific population estimates for each of the 3 counties for 2001–2012 from Washington State Office of Financial Management (OFM) (30). OFM population estimates for 2000–2010 are based on the 2000 and 2010 US Census and an interpolation in the intermediate years (31). OFM population estimates for 2011 and 2012 were developed using the component method, which derives the estimated population by adding natural population change (births minus deaths) and net migration to the base-year population (32). Population estimates for the 0–6 month, 7–23 month, and 2–4 year age groups were not available from OFM data and were estimated from the annual birth data from the Washington State Department of Health (32). We carried the annual birth numbers for each county forward in time to estimate the population sizes for the 0–6 month, 7–23 month, and 2–4 year categories at a specific time point. Because births were reported annually and our age categories included half-year fractions (0–6 month and 7–23 month), we used halves of the appropriate annual birth numbers to estimate population sizes in these age categories.

Statistical Analysis
To describe all-cause RC hospitalizations, we calculated rates of any RC hospitalization divided by person-time under observation for each age category. To estimate influenza-associated events, we adapted negative binomial regression models used previously by CDC to estimate the incidence of influenza-associated hospitalizations from surveillance data and administrative hospitalization datasets (5,6,8,33,34). We fitted age-specific negative binomial regression models to daily events in the 3 counties of interest (Appendix). Covariates were time (day expressed as a fraction of the year), daily RC hospitalizations in a particular county on a particular day, the county’s population size in that calendar year, the percentage of specimens testing positive in the corresponding week for influenza A and influenza B, daily environmental effects, and terms accounting for secular and seasonal trends. The offsets for county and population in the model account for different population sizes across counties and years. The environmental effects include the effect of the temperature, humidity, dew point, and PM$_{2.5}$ concentration. We modeled the effect of each of these 4 variables by exposure on the same day and by exposure on the previous day (one-day lag term). We used a cubic B-spline with 3 degrees of freedom for both the same day and 1-day lag terms for a total of 24 adjustment coefficients for the 4 environmental variables.

For each age category, we fit a base model, which was similar to CDC ecologic models and excludes environmental exposures, and an expanded model, which included the environmental exposures. Using each fitted model, we calculated the number of age-specific influenza-associated RC hospitalizations as the difference between model-predicted RC hospital-
izations estimated from the original data and model-predicted RC hospitalizations with all influenza terms set to 0. We calculated the number of type-specific influenza-associated RC hospitalizations (influenza A or B) in a similar fashion but by setting only one of the influenza terms to 0. To express the influenza-associated RC hospitalizations as rates, we divided them by the age-specific population estimates (presented as the number of events per 10,000 person-months or 100,000 person-years). We calculated population-attributable risks (PARs) for influenza-associated RC hospitalizations for each age category as the number of influenza-associated RC hospitalizations divided by the number of all-cause RC hospitalizations. We calculated 95% CIs for the number of influenza-associated RC hospitalizations, rates, and PARs using the nonparametric bootstrap (35).

To assess the effect of inclusion of RSV in our models, our secondary analysis expanded the model by adding an additional term, $\beta_{l>5}\text{RSV}$, for the effect of the percentage of specimens testing positive in the corresponding week for RSV. We calculated the numbers of virus-specific (disaggregated) and the total (influenza + RSV) attributable RC hospitalizations as the corresponding rates and PARs. We calculated incidence rates for RSV-associated outcomes similarly to the influenza outcomes. We limited the fit of the RSV model to the period of RSV data availability.

We conducted 3 sensitivity analyses based on the primary analysis model to assess the effect of alternative modeling choices: 1) analysis with the environmental exposure modeled as linear instead of as the cubic B-spline; 2) analysis without the 1-day-lag environmental exposure variables; and 3) analysis with weekly events instead of daily events. We compared the results of the primary analysis to these alternative modeling choices and found no major differences. Statistical diagnostics of the models included added variable plots and likelihood ratio tests for distributed lags (day 2 through day 6 lags) and illustrated adequate model fit. We performed analyses with R version 3.1.0 statistical software (https://r-project.org).

**Results**

The study populations ranged from 1,758,779 (King), 708,230 (Pierce), and 615,435 (Snohomish) in 2001 to 1,960,782 (King), 808,316 (Pierce), and 723,301 (Snohomish) in 2012. A total of 1,503,081 all-cause RC hospitalizations occurred in these 3 counties during September 30, 2001–December 29, 2012, for an overall incidence rate of 4,600/100,000 person-years. Incidence rates were highest at the extremes of age (0–6 months, 5,949/100,000 person-years; 50–64 years, 6,503/100,000 person-years; and ≥65 years, 23,077/100,000 person-years).

Using the base model, incorporating time and seasonality, and excluding environmental exposures, the overall incidence rate of influenza-associated RC hospitalizations was 31/100,000 person-years with 0.7% PAR. Event rates varied across age groups and had a marked winter seasonality over the study period (Figure 1). In the base model, influenza-attributable event rates were highest in the 0–6 months age group (118.7/100,000 person-years) and the ≥65 years age group (157.0/100,000 person-years). Of these, the influenza A attributable event rate was highest in the same 2 age groups (0–6 months, 159.9/100,000 person-years; ≥65 years, 81.3/100,000 person-years), and the influenza B rate was highest in the ≥65 years age group (76.2/100,000 person-years) (Table 1). Overall, influenza A was associated with higher hospitalization rates than influenza B (21.3 vs. 10.3/100,000 person-years).

In the expanded model incorporating environmental covariates, all the environmental and air pollution covariates were significantly associated with RC hospitalizations (p < 0.01) for each of the 7 age groups (Appendix Table 2); however, the influenza-associated event rates did not change appreciably in any age group (Table 1; Figure 2). The overall influenza-attributable rate was similar at 31.4/100,000 person-years (influenza A, 21.3/100,000 person-years; influenza B, 10.3/100,000 person-years). The influenza-associated event rates were highest in the 0–6 month (111.9/100,000 person-years) and the ≥65 years (147.3/100,000 person-years) age groups. PAR was highest in the 5–14 years age group (4.8%; 95% CI 3.7%–6.0%) (Table 2). When assessed by influenza type, influenza A had a greater number of attributable events in all age groups with the exception of the ≥65 years age group (influenza A, 69.3/100,000 person-years; influenza B, 78.4/100,000 person-years). Similarly, PAR was greater for influenza A across age groups with the exception of the ≥65 years group (Table 2). PAR for influenza was similar in both the base (without environmental covariates) and expanded (with environmental covariates) models.

**Secondary Analysis—Influenza and RSV Models**

Among data with other covariates available, RSV data were available for 1,811 days and were analyzed with influenza in models with and without environmental covariates (Appendix Table 1). In the base model incorporating time and seasonality, similar to influenza, RSV-attributable event rates were highest in the youngest and oldest age groups. In the expanded model...
incorporating environmental covariates, the attributable event rates for influenza or RSV did not appreciably change. PAR for influenza- or RSV-associated RC hospitalizations in the expanded model was 1.0%–12.9% and did not differ from the base model results (Table 3).

**Sensitivity Analysis—Examining Alternative Modeling Choices**

We performed sensitivity analysis evaluating alternative modeling choices: environmental covariates modeled as linear; environmental covariates modeled without lag terms; and models run on weekly aggregates. We ran these alternative models for influenza alone (primary analysis) and for influenza with RSV (secondary analysis). We found, as in our primary analysis, that age-specific models with all assessed environmental and air pollution parameters were significantly associated with RC hospitalizations (p<0.05 for each age group). However, we saw no meaningful changes in the attributable event rates (Appendix Figures 8, 9).

**Discussion**

We conducted a population-based study incorporating hospitalization, laboratory, and meteorological data from 3 Washington counties over 12 years to estimate the burden of influenza- and RSV-associated RC hospitalizations. Hospitalization rates peaked during the winter, corresponding to periods of influenza circulation, and the highest rates were at the extremes of age for both influenza and RSV events. Our overall influenza-associated hospitalization rate estimate was 31/100,000 years. This is similar to CDC estimates of 55.0/100,000 person-years (95% CI 22.5–125.4) over a period including the 1990s, which was notable for high rates of severe influenza (6,7). Our age-specific models with all assessed environmental and air pollution parameters demonstrated that factors of temperature, relative humidity, dew point, and particulate matter were significantly associated with RC hospitalizations (p<0.01 for each age group). However, the inclusion of environmental covariates did not result in clinically meaningful changes in respiratory virus-associated event estimates. In addition, we conducted several alternative models, including linear adjustments for environmental parameters (instead of cubic-splines), models without lags for environmental parameters (versus models with lags), and models on weekly-aggregated data (versus

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**Figure 1.** Influenza detections and respiratory and circulatory hospitalizations in western Washington, USA, 2001–2012. A) Total detections of influenza by clinical laboratories and public health surveillance. B) Incidence of all-cause respiratory and circulatory hospitalizations by age group.
Several environmental parameters have been found to improve forecasts of influenza activity, and they may contribute to influenza illness in several ways. Climatic variables, such as temperature and humidity, increase the survival and spread of influenza in the environment (23,36). These same factors change human behaviors and enhance virus transmission by driving people indoors and increasing crowding. Air pollution increases every winter and is significantly associated with respiratory infections (37). Certain climate conditions, including temperature, humidity, and particulate

### Table 1. Influenza-associated respiratory and circulatory hospitalizations by age group modeled with and without environmental covariates, October 2001–December 2012*

<table>
<thead>
<tr>
<th>Model type and age group</th>
<th>All influenza-attributable events</th>
<th>All influenza-attributable events/100,000 person-years</th>
<th>Influenza A-attributable events</th>
<th>Influenza A-attributable events/100,000 person-years</th>
<th>Influenza B-attributable events</th>
<th>Influenza B-attributable events/100,000 person-years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without environmental covariates‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–6 mo</td>
<td>254</td>
<td>118.7</td>
<td>342</td>
<td>159.9</td>
<td>−92</td>
<td>−42.9</td>
</tr>
<tr>
<td>7–23 mo</td>
<td>88</td>
<td>13.8</td>
<td>176</td>
<td>27.6</td>
<td>−90</td>
<td>−14.1</td>
</tr>
<tr>
<td>2–4 y</td>
<td>218</td>
<td>17.5</td>
<td>242</td>
<td>19.4</td>
<td>−25</td>
<td>−2.0</td>
</tr>
<tr>
<td>5–14 y</td>
<td>735</td>
<td>17.5</td>
<td>547</td>
<td>13.0</td>
<td>199</td>
<td>4.7</td>
</tr>
<tr>
<td>15–49 y</td>
<td>2,108</td>
<td>12.4</td>
<td>1,835</td>
<td>10.8</td>
<td>276</td>
<td>1.6</td>
</tr>
<tr>
<td>50–64 y</td>
<td>2,204</td>
<td>37.3</td>
<td>1,849</td>
<td>31.3</td>
<td>358</td>
<td>6.1</td>
</tr>
<tr>
<td>&gt;65 y</td>
<td>5,376</td>
<td>157.0</td>
<td>2,782</td>
<td>81.3</td>
<td>2,609</td>
<td>76.2</td>
</tr>
<tr>
<td>With environmental covariates‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–6 mo</td>
<td>239</td>
<td>111.9</td>
<td>308</td>
<td>143.9</td>
<td>−71</td>
<td>−33.2</td>
</tr>
<tr>
<td>7–23 mo</td>
<td>87</td>
<td>13.6</td>
<td>174</td>
<td>27.2</td>
<td>−89</td>
<td>−13.9</td>
</tr>
<tr>
<td>2–4 y</td>
<td>215</td>
<td>17.2</td>
<td>240</td>
<td>19.3</td>
<td>−26</td>
<td>−2.1</td>
</tr>
<tr>
<td>5–14 y</td>
<td>741</td>
<td>17.6</td>
<td>571</td>
<td>13.6</td>
<td>181</td>
<td>4.3</td>
</tr>
<tr>
<td>15–49 y</td>
<td>1,885</td>
<td>11.1</td>
<td>1,618</td>
<td>9.5</td>
<td>270</td>
<td>1.6</td>
</tr>
<tr>
<td>50–64 y</td>
<td>2,064</td>
<td>34.9</td>
<td>1,667</td>
<td>28.2</td>
<td>401</td>
<td>6.8</td>
</tr>
<tr>
<td>&gt;65 y</td>
<td>5,043</td>
<td>147.3</td>
<td>2,371</td>
<td>69.3</td>
<td>2,685</td>
<td>78.4</td>
</tr>
</tbody>
</table>

*The number of all influenza-attributable events is not equal to the sum of influenza A and influenza B events because the 2 types of influenza exposure are not independent and their attribution can overlap.
†We could not discern between influenza A(H3N2) and influenza A(H1N1) because of testing limitations over the study period.
‡Environmental covariates included daily averages of temperature, relative humidity, dew point, and particulate matter with a diameter <2.5 μm.
matter can affect susceptibility to upper respiratory infections (38–41). Despite these well-known associations between environmental exposures and respiratory events, our study found that their inclusion in a model designed to estimate influenza illness in western Washington had a negligible effect. Whether their influence on disease burden estimates remains small in regions with more extreme weather or air pollution is unclear.

Our study should be interpreted in light of its strengths and limitations. Because our data were from western Washington state, the study may not be generalizable to other regions in which environmental factors may differ. Our focus on a limited number of covariates means that our results may not be applicable to other regions or settings. Additionally, the study was conducted during a period of high influenza activity, which may have influenced our findings. However, this period also highlights the importance of understanding the role of environmental factors in influenza transmission.

### Table 2. Population attributable risk of influenza-associated respiratory and circulatory hospitalizations by age group modeled with and without environmental covariates, October 2001–December 2012

<table>
<thead>
<tr>
<th>Model type</th>
<th>Age group</th>
<th>All influenza, %</th>
<th>Influenza A, †%</th>
<th>Influenza B, %</th>
<th>Difference†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without environmental covariates*</td>
<td>0–6 mo</td>
<td>2.0 (0.6–3.4)</td>
<td>0.8 (0.8–0.8)</td>
<td>2.0 (0.6–3.4)</td>
<td>–0.7</td>
</tr>
<tr>
<td></td>
<td>7–23 mo</td>
<td>4.8 (3.7–6.0)</td>
<td>0.8 (0.3–1.1)</td>
<td>0.6 (0.2–1.0)</td>
<td>–1.6</td>
</tr>
<tr>
<td></td>
<td>2–4 y</td>
<td>1.5 (0.2–2.2)</td>
<td>0.7 (0.3–1.0)</td>
<td>0.5 (0.1–0.8)</td>
<td>–0.2</td>
</tr>
<tr>
<td></td>
<td>5–14 y</td>
<td>0.6 (0.1–0.6)</td>
<td>0.1 (0.1–0.6)</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15–49 y</td>
<td>0.3 (0.4–1.0)</td>
<td>–0.1 to 0.3</td>
<td>(0.1–0.5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50–64 y</td>
<td>0.7 (0.4–1.0)</td>
<td>–0.1 to 0.3</td>
<td>(0.1–0.5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>≥65 y</td>
<td>0.0</td>
<td>–0.1 to 0.3</td>
<td>(0.1–0.5)</td>
<td></td>
</tr>
</tbody>
</table>

*Environmental covariates included daily averages of temperature, relative humidity, dew point, and particulate matter with a diameter <2.5 μm.
†We could not discern between influenza A(H3N2) and influenza A(H1N1) because of testing limitations over the study period.
‡With covariates minus without covariates.

### Table 3. Secondary analysis of population attributable risk (PAR) of influenza or respiratory syncytial virus (RSV)-associated respiratory and circulatory hospitalizations by age group modeled with and without environmental covariates, September 2007–December 2012

<table>
<thead>
<tr>
<th>Model type</th>
<th>Age group</th>
<th>All influenza and RSV, %</th>
<th>Influenza A, †%</th>
<th>Influenza B, %</th>
<th>RSV, %</th>
<th>Difference†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without environmental covariates*</td>
<td>0–6 mo</td>
<td>14.0 (10.0–17.6)</td>
<td>2.7 (0.4–1.4)</td>
<td>–0.8 to 0.8</td>
<td>0.8</td>
<td>–0.1</td>
</tr>
<tr>
<td></td>
<td>7–23 mo</td>
<td>6.9 (2.6–10.9)</td>
<td>2.7 (0.5–4.6)</td>
<td>–1.1 to 1.4</td>
<td>2.7</td>
<td>–0.7</td>
</tr>
<tr>
<td></td>
<td>2–4 y</td>
<td>8.3 (5.1–11.3)</td>
<td>5.8 (4.0–7.5)</td>
<td>–0.1 to 1.7</td>
<td>5.8</td>
<td>–0.6</td>
</tr>
<tr>
<td></td>
<td>5–14 y</td>
<td>1.2 (0.2–2.3)</td>
<td>0.7 (0.2–1.2)</td>
<td>–1.7 to 3.0</td>
<td>0.7</td>
<td>–0.7</td>
</tr>
<tr>
<td></td>
<td>15–49 y</td>
<td>1.4 (0.1–2.6)</td>
<td>0.7 (0.0 to 1.1)</td>
<td>–0.0 to 0.2</td>
<td>0.7</td>
<td>–0.6</td>
</tr>
<tr>
<td></td>
<td>50–64 y</td>
<td>0.6 (0.2–1.5)</td>
<td>0.6 (0.0 to 0.6)</td>
<td>–0.1 to 0.6</td>
<td>0.6</td>
<td>–0.5</td>
</tr>
<tr>
<td></td>
<td>≥65 y</td>
<td>0.0 (0.0 to 1.0)</td>
<td>–0.1 to 0.6</td>
<td>–0.1 to 0.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Environmental covariates included daily averages of temperature, relative humidity, dew point, and particulate matter with a diameter <2.5 μm.
†We could not discern between influenza A(H3N2) and influenza A(H1N1) because of testing limitations over the study period.
‡With covariates minus without covariates.
geographic area can increase confidence that the population studied was truly exposed to the environmental covariates used in our models, but this design choice limits our ability to evaluate rarer outcomes, such as critical illness or death. We used clinical and virologic surveillance data to model the incidence rates for severe influenza but did not have specific data relating to influenza vaccine, and we were not able to incorporate other respiratory viruses because we lacked robust surveillance data for the study period. For influenza and RSV, the use of percent positive rather than absolute numbers in the model corrects for changing surveillance intensity over time but may decrease estimates of disease incidence during intense seasons when testing volume also increases. We did not have subtype information for influenza or RSV available, which limited our ability to assess whether certain circulating strains were more affected by environmental covariates. Of the meteorological and pollution factors, we did not assess absolute humidity, wind velocity, sunshine duration, ozone, or other measures of pollution, and it is possible that one or more of these factors either independently or in addition may have modified the effect on influenza- or RSV-associated RC hospitalizations. Finally, this is an ecologic study, and the results may not necessarily be representative of patient-level associations. Regardless, this comprehensive study spans over a decade of data using expanded standard ecologic models to assess the relationships of respiratory virus-associated hospitalizations and meteorological and pollution variables in a large population comprising children and adults.

In conclusion, our population-based study in western Washington state over 12 years assessed how incorporation of environmental and air pollution covariates can influence influenza- and RSV-associated disease burden estimates. Our modeled estimates for influenza and RSV hospitalization rates were similar to national rates and changed little with incorporation of seasonal environmental covariates. Our study should strengthen confidence in the traditional ecologic models used to estimate influenza illness. In addition to continued efforts to reduce the extent of vaccine-preventable respiratory viral disease, future work should assess the role environmental parameters have on severe influenza and RSV outcomes in regions with more extreme pollution and meteorological exposures. Finally, there is ample evidence that environmental pollution is deleterious to health, irrespective of its impact on influenza, and more needs to be done to improve the quality of air we breathe.

Acknowledgments

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Data availability: The study code is available by request to the corresponding author. In accordance with Washington law, the Comprehensive Hospital Abstract Reporting System dataset can only be distributed by Washington State Department of Health, and we are unable to publish an aggregated dataset due to state guidelines regarding publication of aggregated data that include cells with small numbers.

About the Author

Dr. Somayaji is an infectious disease physician and an assistant professor in the Departments of Medicine and Microbiology, Immunology and Infectious Disease at the University of Calgary. Her research interests include understanding the rates, risk factors, and effects of viral and bacterial infections on populations.

References


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Epidemiologic and Clinical Progression of Lobomycosis among Kaiabi Indians, Brazil, 1965–2019

Marcos C. Florian, Douglas A. Rodrigues, Sofia B.M. de Mendonça, Arnaldo L. Colombo, Jane Tomimori

Lobomycosis is a rare granulomatous skin disease with a high prevalence in the Amazon region. The Kaiabi Indians are an especially affected group. We studied the current epidemiologic and clinical progression of lobomycosis among the Kaiabi in Brazil, from initial case reports in 1965 through 2019. A total of 60 lobomycosis cases had been reported among the Kaiabi, and we identified 3 new cases in our review. Of 550 cases of lobomycosis ever reported worldwide, 11.5% were among the Kaiabi. We note a high incidence among female Kaiabi and a precocious onset of disease in this indigenous population. Male Kaiabi frequently are infected with the multicentric form and women more frequently exhibit the localized form. Ulcerated lesions are observed more often in the multicentric form. The prevalence among this indigenous group could be explained by genetic susceptibility and lifestyle, which exposes them to a particular agent in the habitats in which they live.

Lobomycosis is a chronic and granulomatous fungal disease that affects the skin and subcutaneous tissue. Lobomycosis is classified as a neglected mycosis and is endemic to Latin America, especially the Amazon region (1,2). It has been reported in travelers who have visited that area but is rarely reported outside this region (3,4). In 1931, Jorge Lobo published a report of this disease in a nonindigenous man in Recife, Pernambuco state, Brazil (5). Since then, the disease has been given many different names, including lobomycosis, Jorge Lobo’s disease, keloidal blastomycosis, and lacaziosis. Of 550 cases reported worldwide, 332 (58.5%) have occurred in Brazil (6).

The etiologic agent of lobomycosis is Lacazia loboii, an as-yet uncultured fungus that has phylogenetic and antigenic similarity to Paracoccidioides brasiliensis, a dimorphic fungus. L. loboii also could be a dimorphic fungus (1,7). Cutaneous lesions associated with this disease are polymorphic and often clinically appear as a keloid-like nodule. Other manifestations include ulcers, atrophy, tumors, macules, plaques, gummas, scleroderma, infiltrations, or scars; patients can have >1 type of lesion. Lobomycosis can be classified into localized or multicentric forms, depending on the extent of skin lesions. The disease does not usually affect general health, but ulceration or secondary infection can impair quality of life for those affected (8). No mucosal or systemic involvement has been reported, but the lymphatic system may be affected, and 1 case involving the testicles has been reported (9).

Many dermatologic diseases occur in indigenous people (10), but lobomycosis is particularly prevalent among the Kaiabi Indians, an ethnic population that lives in central Brazil. During 1961–1966, most Kaiabi migrated 400 km west from regions where they originally lived (7.3502°S, 58.1383°W) to the Xingu Indigenous Park (XIP; 11.2320°S, 53.1850°W). The migration changed their habits and brought them in contact with other indigenous groups. Most Kaiabi now live in the XIP, an indigenous reserve, providing an opportunity for long-term medical follow-up (11).

At different times, 60 cases of lobomycosis have been reported among the Kaiabi (8,11,12). We provide a clinical review and epidemiologic update of all registered cases of lobomycosis among the Kaiabi. In addition, we conducted field visits to the various Kaiabi indigenous villages and identified the current state of lobomycosis in each village, including 3 newly identified cases. We compared the new cases with other reported cases to determine whether other particularities are associated with lobomycosis among this population.

Materials and Methods

Study Design and Area
We conducted a clinical-epidemiologic analysis of reported cases of lobomycosis in members of the Kaiabi ethnic group indexed during 1965–2019.
We used a previous extensive review of published lobomycosis cases among the Kaíabi conducted in 1986 (12) as the basis of this study (12,11,13). We analyzed medical records obtained during 1965–2019 by the Xingu Project of the Department of Preventive Medicine, Escola Paulista de Medicina, Federal University of São Paulo, São Paulo, Brazil. Each Kaíabi person included in the project had a medical record, and 56 had lobomycosis. We obtained additional unpublished information from the Xingu Project archives and field visits to indigenous villages. We performed an observational follow-up study with information from different time points during 1965–2019. We conducted 2 field visits to reexamine case-patients and identify new cases and analyzed 3 areas in which the Kaíabi have lived (Figure 1). When we did not have access to a medical record, we consulted previous literature reports (8,11,12).

Case Definition for Lobomycosis
We used a standard case definition for lobomycosis, which included presence of skin lesions and histopathologic evidence of the disease. Skin lesions related to lobomycosis include keloid-like nodules, plaques, papules, ulcerations, or atrophic lesions. Histopathologic evidence includes the presence of an inflammatory reaction, especially with histiocytic cell infiltrate, and detection of L. loboi fungal cells by hematoxylin-eosin and Grocott’s methenamine silver staining.

Parameters Analyzed
We analyzed the following clinical and epidemiologic parameters: the number of lobomycosis cases among the Kaíabi; the regions in which the Kaíabi currently live and previously lived; sex distribution; age of onset; extent of the lesions; anatomic location of lesions; data concerning the progression of skin lesions; and treatment attempts. We classified cases as multicentric, skin lesions in >1 anatomic area (Figure 2); or localized, lesions restricted to 1 anatomic area (Figure 3).

Data Analysis
Initial data analysis was descriptive. We also analyzed the prevalence at different times over a 54-year period. For some quantitative samples, we calculated the mean, median, minimum, and maximum.
values and SDs. We analyzed qualitative variables by calculating absolute and relative frequencies as percentages. We performed inferential analysis by using \( \chi^2 \) test for 2 independent qualitative samples, Student t-test for independent samples, and analysis of variance for analyzing clinical signs and symptoms and immunologic data. We considered \( p<0.05 \) statistically significant.

**Ethics Considerations**
This project was approved by Kaiabi indigenous leaders, the Indian National Foundation, and the Brazilian National Research Ethics Committee (registration no. 12776). All participants provided written informed consent before the start of the project.

**Results**

**Number of Cases among the Kaiabi**
We identified 63 lobomycosis cases among the Kaiabi from 1965–2019. Of these, 60 were reported previously (13–15), and we identified 3 new cases. All cases were associated with the case-patients’ original habitat, and none of the reported case-patients were born at the XIP. A temporal series of cross-sectional studies of lobomycosis prevalence reported 51 cases among 400 (12.7%) Kaiabi in 1981. In 2019, a total of 26 cases of lobomycosis were known among 2,242 Kaiabi, a prevalence of 1.16% (Table 1).

**Regions Where the Kaiabi Live and Have Lived**
We obtained information about the regions in north Mato Grosso state in which 41 case-patients originally resided. Twenty-two (53.7%) case-patients were from the Arinos River region, 17 (41.5%) were from the Teles Pires River region, and 2 (4.9%) had lived in both regions.

**Sex Distribution and Age of Onset**
Among the 63 case-patients, 39 (61.9%) were male and 24 (38.1%) were female. We obtained information about the age of onset for 38 case-patients. In 24 (63.2%) case-patients, lesions developed before the patient was 21 years of age; in 12 (31.6%), lesions developed at 21–40 years of age; and in 2 (5.2%), the lesions developed at >40 years of age. The earliest age of onset was 1 year of age, and the oldest was 63 years of age. The median age of onset was 18.5 years. We did not obtain data regarding patient age for 25 (39.6%) cases.

**Extent and Location of Lesions**
We obtained information on the extent and location of lobomycosis lesions in 62 case-patients, 38 males and 24 females. We noted 34 (54.8%) cases of the multicentric form (Figure 2) and 28 (45.2%) cases of the localized form (Figure 3). The multicentric form more frequently was associated with male sex (Table 932).
2); 27 (71.1%) male patients had the multicentric form and 11 (28.9%) had the localized form. Among female patients, 7 (29.2%) had the multicentric form and 17 (70.8%) had the localized form. For 61 case-patients for whom information on lesion location was available, we noted lesions on the lower limbs in 38 cases, on the upper limbs in 32 cases, on the trunk in 24 cases, on the head and neck in 2 cases, and on the prepuce in 1 case. All patients with the multicentric form had lesions at >1 anatomic site.

### Progression of Cutaneous Lesions
During clinical follow-up, 8/18 (44.1%) patients with the multicentric form seen after 20.2 years had new lesions and 2/17 (11.8%) patients with the localized form seen after 28.3 years had new lesions. In addition, 15/19 (78.9%) patients with the multicentric form seen after 12.3 years had lesions with periods of ulcerations and 3/8 (37.5%) patients with the localized form seen after 27.4 years had lesions with periods of ulcerations. We could not perform statistical analysis of these data because of variations in follow-up times.

### Treatment Attempts
Some case-patients received systemic treatment with ketoconazole, itraconazole, or clofazimine, which were not successful. Among 15 cases of localized lobomycosis that received surgical treatment, 6 (40%) cases followed for 30 years had recurrence of lesions. In addition, 2 patients had carcinomatous degeneration in the form of cutaneous squamous cell carcinoma, and both died from metastasis (8).

### Discussion
Pereira Filho reported a case of lobomycosis among the Kaiabi in 1957 (12,16), and Nery-Guimarães reported another in 1964 (12,17). Since then, many cases in have been reported among this population in the medical literature. The high prevalence of lobomycosis in this population is a notable epidemiologic finding in medical ethnography (18). The disease is so prevalent that, since 1915, references have been made to a Kaiabi-associated skin disease, called pirai'p in the Kaiabi language, a branch of the Tupi-Guarani linguistic family.

The prevalence of lobomycosis in the Kaiabi has been declining over time. Changes in the geographic region in which they live after many Kaiabi moved 400 km east to the XIP, changes in cultural behavior, a lack of fungal agents in the new ecosystem, and changes in the immunity of this population are some hypotheses that could explain the reduction in this prevalence. Before moving to the XIP, all Kaiabi reported with lobomycosis lived in the Arinos River or Teles Pires River regions. No new cases have occurred in Kaiabi who have only lived in XIP. The environment of the XIP is similar to Arinos River or Teles Pires River regions, but the regions have distinct watersheds.

Lobomycosis also occurs in some species of estuarine, inshore cetaceans (Tursiops truncatus) and offshore cetaceans (Sotalia guianensis) (19–21). Clinical and phenotypic features of the uncultivated agent of the disease in dolphins suggested that this pathogen was the same organism, *L. loboi*. However, molecular data suggest that the cause of cutaneous lesions in dolphins is a novel strain of *P. brasiliensis*, *P. brasiliensis* var. *ceti*.
(22,23). Moreover, a lobomycosis-like disease among bottlenose dolphins has been reported (24). Bermudez et al. (25) hypothesized that the marine environment could be a source of the fungus in a human case they reported. Some cetaceous species inhabit the rivers of the Amazon region, including Sotalia fluviatilis dolphins in the Orino River in Venezuela and Inia geoffrensis dolphins, known as boto, in the Amazonas River in Brazil. However, these animals do not inhabit the XIP rivers, and lobomycosis has never been identified in these animals (19). Curiously, 1 case in Africa and 1 in Greece were reported in persons who had never traveled to South or Central America (15,26). These cases might support water as the environmental source of this fungus, but evidence of dolphin–human transmission has been weak.

Of the 63 cases of lobomycosis among the Kaiabi, most patients were male, but female Kaiabi also had a high prevalence of the disease. Early reports indicated that 32% of lobomycosis case-patients among the Kaiabi were female. Among nonindigenous women and girls, the disease occurrence ranged from 10%–12% (27). In addition, the increased prevalence in female Kaiabi might be because they work in close contact with the environment during activities such as small-scale farming.

Medical reports have shown that the age of onset of the disease is typically 20–40 years of age. However, most Kaiabi case-patients began to exhibit the disease before 21 years of age. The earliest onset we noted was at 1 year of age. The susceptibility and early onset of lobomycosis among Kaiabi children can be explained by their precocious contact with wood and soil.

Some researchers have proposed different clinical classifications for lobomycosis. For their analysis, Baruzzi et al. (11) adopted a modified classification, involving a localized form and multicentric form. The multicentric form is unusual and infrequent among nonindigenous populations. Other authors reported 41 cases with multicentric forms among 249 cases in nonindigenous populations (27). However, among the Kaiabi, the multicentric form was more common than the localized form. In analyzing the classification for sex, we noted that manifestations of the disease are quite distinct in male and female Kaiabi (Table 2). The multicentric form was more frequently seen in male case-patients and the localized form was more frequent in female case-patients.

Cellular immunodeficiency is possible in cases of lobomycosis (28). Different host immune responses against the fungus or relatively high exposure to the fungal agent are possible explanations for the high incidence of lobomycosis among female case-patients. However, hormonal factors could be responsible for the protection against disease dissemination in females.

When reviewing anatomic locations of lesions, we noted that nonindigenous people more frequently have lobomycosis lesions on the outer ear. However, among the Kaiabi population, only 2 case-patients had lesions on the outer ear and many more lesions were noted on the limbs and truck. A possible reason is a variation in behavior. Nonindigenous groups tend to carry heavy burdens on their shoulders, but the Kaiabi tend to carry wood and other materials on their heads and backs. In addition, the Kaiabi do not wear shirts, so the skin of the upper body is exposed and could be exposed to the causative agent in materials they carry. The prepuce lesion was a rare localization found in 1 case.

We based our analysis on data collected by physicians who visited the XIP for >45 years. Among the Kaiabi, most of the patients with localized lobomycosis did not develop new lesions over time, but 44.1% of patients with the multicentric form had new lesions over time. Patients reported that recurrent ulcerations impaired their quality of life, and patients with the multicentric form had periods of ulcerations more frequently than those with the localized form. We did not observe any change in ulcer healing or worsening related to weather or seasons of the year. Among all lobomycosis cases worldwide, 4 reports of progression to squamous cell carcinoma have occurred; 2 were in the Kaiabi population, and both died from metastasis (8). Outside of the Kaiabi population, no deaths from complications of lobomycosis have been reported. In addition, among indigenous populations living in the XIP, lobomycosis greatly affects quality of life; those who have it are stigmatized, and the disease has been known as Kaiabi leprosy in the past.

Treatment is a challenge, and systemic drugs are not effective (29). However, many treatment attempts have been reported in some case-patients. The medical literature reports some isolated positive results with itraconazole, clofazimine, leprosy multidrug therapy, or posaconazole (27,30). Other approaches, such as surgical excision, cryosurgery, and electrosurgery, should be considered in addition to drug treatment. However, no known effective treatment for lobomycosis, especially the multicentric form, has been found (30). Among the Kaiabi, treatment by surgery was successful in some localized cases without recurrence in a 30-year follow-up.

In conclusion, lobomycosis is a rare disease that affects certain geographic regions, especially
in countries with low socioeconomic status (31), but its prevalence among the Kaiabi population in Brazil is exceptionally high. The 63 cases we identified among this population represent 11.5% of all cases of lobomycosis known in the world since the disease was identified. Our clinical and epidemiologic analysis shows cases among the Kaiabi have particular characteristics, including a high incidence of female case-patients, early onset in persons <21 years of age, a high incidence of the multicentric form in male case-patients, and a high incidence of the localized form in female case-patients. Little is known about the prevalence of lobomycosis, and effective treatment remains a challenge, especially for the multicentric form. Phylogenetic analysis of \textit{L. loboi} could help in the development of treatments for this fungal infection.

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Address for correspondence: Marcos Cesar Florian, Escola Paulista de Medicina, Universidade Federal de São Paulo, Rua Borges Lagoa, 508, São Paulo (SP), 04038-000, Brazil; email: mcflorian@unifesp.br
Mucormycosis is a debilitating fungal infection; the mortality rate among persons with predisposing factors such as skin trauma (e.g., surgery), diabetes mellitus, or organ transplant is high. The fungus can be directly inoculated into a wound or inhaled (1–3). Rhizopus spp. are the Mucorales fungi that most commonly cause mucormycosis (1,2,4) and are the most common non-Aspergillus cause of invasive filamentous fungal infections (5). However, although Mucorales fungi are ubiquitous in the environment, mucormycosis is relatively uncommon.

Rhizopus microsporus has been shown to be a cause of serious infections after anterior cruciate ligament reconstruction surgeries in Argentina (6,7). A recent review of 40 Rhizopus-associated cases of osteomyelitis that developed after these surgeries from 2005 through 2017 in several regions across Argentina identified 3 species—R. microsporus var. rhizopodiformis, R. microsporus var. microsporus, and R. arrhizus [syn. R. oryzae]—and implicated healthcare practices and facility shortcomings in the infections (8). Limited molecular analyses of osteomyelitis-associated R. microsporus infections identified commonalities among isolated strains (7); however, no genomic epidemiologic analyses have been performed on this nosocomial cluster. In this study, we analyzed the genomes of R. microsporus var. rhizopodiformis isolates from patients from multiple facilities in Argentina in the context of unrelated controls from outside the geographic area to empirically establish the relationships among them and determine whether infections may have originated from a common source.

Materials and Methods

During 2006–2014, we collected 24 R. microsporus isolates from patients at 14 healthcare facilities in 10 provinces in Argentina (8). For unrelated control isolates, used to establish genomic context for the nosocomial cluster in Argentina, we selected 13 isolates from the US Centers for Disease Control and Prevention (Atlanta, GA, USA), collected from 2003 through 2015 (Table). We extracted DNA from the 37 isolates by using a DNeasy Blood and Tissue Kit (QIAGEN, https://www.qiagen.com), according to the manufacturer’s
Table. Characteristics for controls and patients with Rhizopus microsporus infection associated with surgical procedures in Argentina, 2006–2014*  

<table>
<thead>
<tr>
<th>Isolate</th>
<th>R. microsporus variety</th>
<th>Genome assembly size, Mbp</th>
<th>GC content, %</th>
<th>Year</th>
<th>Surgery</th>
<th>Isolate site</th>
<th>Patient age, y</th>
<th>US state/Argentina province</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control†</td>
<td><strong>Rhizopodiformis</strong> 25.1</td>
<td>37.2</td>
<td>NA</td>
<td>2011</td>
<td>Other (chest)</td>
<td>Sternum muscle</td>
<td>&lt;18</td>
<td>Caba</td>
</tr>
<tr>
<td>B05459</td>
<td><em>Oligosporus</em> 45.3</td>
<td>37.1</td>
<td>2003</td>
<td>NA</td>
<td>Knee</td>
<td>Bone, soft tissue</td>
<td>18–35</td>
<td>Mendoza</td>
</tr>
<tr>
<td>B06590</td>
<td><em>Oligosporus</em> 46.5</td>
<td>36.6</td>
<td>2003</td>
<td>NA</td>
<td>Knee</td>
<td>Bone, soft tissue</td>
<td>18–35</td>
<td>Mendoza</td>
</tr>
<tr>
<td>B07367</td>
<td><strong>Rhizopodiformis</strong> 25.0</td>
<td>37.3</td>
<td>2008</td>
<td>NA</td>
<td>Skin</td>
<td>NA</td>
<td>&lt;18</td>
<td>Caba</td>
</tr>
<tr>
<td>B07386</td>
<td><em>Microsporus</em> 30.2</td>
<td>39.1</td>
<td>2008</td>
<td>NA</td>
<td>Knee</td>
<td>NA</td>
<td>&lt;18</td>
<td>Caba</td>
</tr>
<tr>
<td>B07585</td>
<td><em>Microsporus</em> 29.2</td>
<td>39.5</td>
<td>2009</td>
<td>NA</td>
<td>Knee</td>
<td>NA</td>
<td>&lt;18</td>
<td>Caba</td>
</tr>
<tr>
<td>B07643</td>
<td><strong>Rhizopodiformis</strong> 25.1</td>
<td>37.3</td>
<td>2009</td>
<td>NA</td>
<td>Knee</td>
<td>NA</td>
<td>&lt;18</td>
<td>Caba</td>
</tr>
<tr>
<td>B07675</td>
<td><strong>Rhizopodiformis</strong> 25.1</td>
<td>37.3</td>
<td>2009</td>
<td>NA</td>
<td>Chest tissue</td>
<td>NA</td>
<td>18–35</td>
<td>Ente Rios</td>
</tr>
<tr>
<td>B08956</td>
<td><em>Microsporus</em> 28.7</td>
<td>40.3</td>
<td>2010</td>
<td>NA</td>
<td>Respiratory</td>
<td>NA</td>
<td>&lt;18</td>
<td>Georgia</td>
</tr>
<tr>
<td>B11147</td>
<td><strong>Rhizopodiformis</strong> 25.1</td>
<td>37.3</td>
<td>2015</td>
<td>NA</td>
<td>BAL</td>
<td>NA</td>
<td>35–65</td>
<td>Colorado</td>
</tr>
</tbody>
</table>

Patient†  
| B11523   | **Rhizopodiformis** 25.5 | 37.3 | 2011 | Other (chest) | Sternum muscle | <18 | Caba |
| B11526   | **Rhizopodiformis** 25.1 | 37.3 | 2010 | Knee | Bone, soft tissue | 18–35 | Mendoza |
| B11529   | **Rhizopodiformis** 25.1 | 37.3 | 2011 | Knee | Knee | 18–35 | Ente Rios |
| B11531   | **Rhizopodiformis** 25.1 | 37.3 | 2006 | Knee | Bone | 18–35 | Santa Fé |
| B11532   | **Rhizopodiformis** 25.1 | 37.3 | 2011 | Renal transplant | Transplant | 35–65 | Tucuman |
| B11533   | **Rhizopodiformis** 27.7 | 37.3 | 2011 | Transplant | Renal transplant | 35–65 | Tucuman |
| B11534   | **Rhizopodiformis** 25.1 | 37.3 | 2010 | Knee | ACL | 18–35 | Mendoza |
| B11535   | **Rhizopodiformis** 25.1 | 37.3 | 2011 | Unknown | Surgical site | <18 | Caba |
| B11538   | **Rhizopodiformis** 25.1 | 37.3 | 2011 | Other (chest) | Surgical site | <18 | Caba |
| B11539   | **Rhizopodiformis** 25.1 | 37.3 | 2011 | Renal transplant | Transplant | 35–65 | Ente Rios |
| B11540   | **Rhizopodiformis** 25.1 | 37.3 | 2011 | Renal transplant | Transplant | 35–65 | Tucuman |
| B11541   | *Microsporus* 50.1 | 37.3 | 2011 | Renal transplant | Transplant | 35–65 | Ente Rios |
| B11542   | **Rhizopodiformis** 25.1 | 37.3 | 2011 | Other (hip replacement) | Hip | >65 | Corrientes |
| B11547   | **Rhizopodiformis** 25.1 | 37.3 | 2006 | Knee | Environmenatal surface | 18–35 | San Juan |
| B11549   | **Rhizopodiformis** 25.0 | 37.3 | 2011 | Environmental surface | Femur | 18–35 | Ente Rios |
| B11550   | **Rhizopodiformis** 25.0 | 37.3 | 2008 | Knee | Knee | 18–35 | Gran Buenos Aires |
| B11551   | **Rhizopodiformis** 25.1 | 37.3 | 2010 | Other (unknown) | Abdominal cavity | <18 | Salta |
| B11552   | **Rhizopodiformis** 25.1 | 37.3 | 2010 | Renal transplant | Transplant | 35–65 | Ente Rios |
| B11553   | **Rhizopodiformis** 25.2 | 37.3 | 2011 | Knee | Knee | 18–35 | Cordoba |
| B11554   | **Rhizopodiformis** 45.7 | 37.1 | 2014 | Knee | Bone | 18–35 | Santa Fe |
| B11555   | **Rhizopodiformis** 25.5 | 37.3 | 2011 | Other (chest) | Pericardial fluid | <18 | Caba |
| B11556   | **Microsporus** 25.1 | 37.3 | 2009 | Knee | Knee | <18 | Gran Buenos Aires |
| B11557   | **Microsporus** 43.7 | 37.2 | 2013 | Renal transplant | Transplant | 35–65 | Salta |

*ACL, anterior cruciate ligament; BAL, broncho-alveolar lavage sample; NA, not available.†Controls from the Centers for Disease Control and Prevention, 2003–2015; patients from Argentina with R. microsporus infection associated with surgical procedures, 2006–2014.‡These genomes include sequence for Burkholderia rhizoxinica, a known symbiont of Rhizopus.

recommends. Genomic DNA was fragmented to ≈500 bp by using a QSonica Q800R2 Sonicator (https://www.sonicator.com), genome libraries were prepared for paired-end sequencing and quantified by using a KAPA Hyper Prep Kit and KAPA Library Quantification Kit (KAPA Biosystems, https://sequencing.roche.com), and 33 samples were sequenced on the Illumina NextSeq at 150 × 150-bp reads and 4 samples on the Illumina MiSeq at 300 × 300-bp reads (both https://www.illumina.com). We deposited Illumina read data in the National Center for Biotechnology Information Sequence read archive.
We assembled short read data by using UGAP (https://github.com/jasonsahl/UGAP), which uses the SPAdes genome assembler (10), and assembled the PacBio long reads of sample B11533 by using Canu (11); we performed error correction by using the Illumina short reads in 6 rounds of Pilon (12). Whole-genome single-nucleotide polymorphism (SNP) typing (WGST) included only the 32 genomes (of 37 total) that assembled to ≈25 Mbp. For WGST, we generated SNP matrices to identify point mutations among the isolates (and thus infer strain relatedness) with NASP (13), in which reads were aligned to the assembly of sample B11533 by using the Burrows-Wheeler Alignment tool (14). We called SNPs with the Genome Analysis Toolkit (15) and included them in further analyses only if they were present in all samples, covered by $\geq 10\times$ depth with $\geq 90\%$ consensus in each sample and not in any duplicated regions in the reference genome as identified by NUCmer (16). The resulting SNP matrix comprised the core genome common to all samples in the analysis. We performed maximum-likelihood phylogenetic analyses with IQ-TREE (17) and maximum-parsimony analyses with MEGA version 7.0 (18), and we constructed phylogenetic trees in iTOL version 3 (19).

We assessed the spatial distribution of SNPs among the Rhizopus genomes by using RecomboMamba, which is part of the RECAP toolbox (https://github.com/TGenNorth/RECAP). RecomboMamba was designed to easily detect regions of relatively high SNP density that may indicate recombination or regions under selection that may confound phylogenetic inference. It uses output from an SNP analysis pipeline and a sliding window to tally the numbers of SNPs for each sample by reference genome position to build a graphic display of SNP density, read depth, and pairwise homoplasy index (20).

Results
Read lengths from the PacBio sequencing of B11533 averaged 2,175 bp. The assembly of PacBio and Illumina data of this genome resulted in a genome size of 27.7 Mbp. Approximately 22.6% of the genome was identified as repeat regions according to NUCmer (16) in the NASP analysis, which is consistent with the size and repeat region variation characteristic of Rhizopus (4). We uploaded this assembly into GenBank (accession no. SMRR0000000).

Genomic Relationships among Isolates
Using the whole-genome sequencing (WGS) data, we confirmed that most (22 of 24) of the isolates from the Argentina cluster were R. microsporus var. rhizopodiformis by WGST and by 18S, internal transcribed spacer, 28S, and act1 genetic typing (21,22). A total of 21 isolates fell into a single clade that also included 3 controls and the publicly available genome of the American Type Culture Collection (ATCC) 11559 strain, first described in 1935 in the USSR (23), with 3,170 SNPs among them (Figure). The isolates were collected from patients who had undergone various types of surgeries, encompassing a wide geographic range across multiple years (Figure), and from patients of various ages (Table). Most of the Argentina cluster isolates (n = 17) formed a well-supported inner clade consisting of 1,235 SNPs (Figure). Although 2 sets of epidemiologically related isolates were separated by $\leq 20$ SNPs, the closest relationship between any other 2 isolates in the tree was 60 SNPs (range 60–912, mean 430), a considerable evolutionary distance, not indicative of a recent transference. A set of 3 samples from the same patient (B11523, B11538, and B11555) were appropriately closely related; the first 2 isolates were identical (i.e., 0 SNPs) and the third was separated by 17 SNPs. One pair of isolates outside the large cluster clade (B11529 and B11543) were separated by 6 SNPs and were collected from 2 patients from the same facility, whose surgeries were 3 weeks apart. This low number of SNPs is characteristic of recent direct transmission or indirect transmission from a common source. The SNP-based phylogenetic analysis included 21.3 Mbp, which covers 99% of the 21.5 Mbp of the unduplicated reference genome of B11533, derived from the 27.7-Mbp full assembly minus the 22.6% of the genome identified as repeat...
The 21.3-Mbp finding indicates that insertions/deletions may have played a small, if any, role in the evolutionary history of this sample set. We found no apparent evidence of recombination or selective force in the SNP distribution that could potentially skew phylogenetic inference across the 25 genomes (Appendix Figure 1, https://wwwnc.cdc.gov/EID/article/26/5/19-1045-App1.pdf). The even distribution of SNPs is characteristic of neutral point mutations.

**Genome Variability**

Five isolates had assembled genome sizes of 43 to 51 Mbp; all other assemblies were ≈25 Mbp (Table). The 5 isolates included 3 from the Argentina cluster, of which 2 were typed as *R. microsporus* var. *microsporus* and 1 as *R. microsporus* var. *rhizopodiformis*, and 2 controls, which were typed as *R. microsporus* var. *oligosporus* by 18S, 28S, ITS, act1, EF-1α sequences (21,22). NASP analysis showed that these samples had multiple SNP states, indicating heterozygosity at several of the SNP loci identified by NASP. Heterozygosity may result from genome expansion and aneuploidy or sample mixtures. Including these 5 samples in the phylogenetic analyses made results inconclusive; therefore, we removed them from the analyses. Sequence data from 3 control samples (all *R. microsporus microsporus* isolated from skin, respiratory tract, and an unknown source) also contained sequences from *Burkholderia rhizoxinica* (Table), a known endosymbiont of some *R. microsporus* strains (24).

An analysis of all samples with ≈25-Mbp genomes (which included 11 control isolates, 3 genomes from public databases, and 21 Argentina cluster isolates) illustrated large genomic distances among different isolates of *R. microsporus*, even within a variety (Ap-
SNP analysis identified 1.2 million SNPs, compared with 3,170 SNPs identified within the cluster. The phylogeny shows that the public genomes for ATCC 52813 and ATCC 52814 differ from the closest control isolates by >800k SNPs, despite all having been identified as *R. microsporus* var. *microsporus*, which is remarkable considering that *R. microsporus* var. *rhizopodiformis* differs from *R. microsporus* var. *microsporus* control isolates by >500k SNPs. Overall, the *R. microsporus* var. *rhizopodiformis* group is a relatively tight genomic cluster compared with *R. microsporus* var. *microsporus*, possibly because of sampling bias or differential rates of evolution (Appendix Figure 2).

**Discussion**

The genomic diversity among isolates from the Argentina cluster of *R. microsporus* infections is not consistent with a point-source outbreak (25–27). We identified no associations between isolate phylogenetic placement and patient metadata, which included facility, procedure type, and province. Given the extent of genomic differences among the isolates from the cluster and the lack of associations between genotypes and epidemiologic factors, we found no evidence to support the hypotheses of patient-to-patient transmission or a common source. However, our data do not rule out the possibility of a common source because different strains or even species may come from a common source (28). In our case series, the most likely source of infection was environmental contamination at the facilities or during hospital practices (8); contamination of the operating room with unfiltered ambient air might be the common source. During our previous epidemiologic investigation (8), the only common factor among the patients studied was the use of an operating room (for arthroscopy of the knee for anterior cruciate ligament repair, placement of an implantable central venous catheter, or organ transplantation). This speculation is supported by findings from our previous epidemiologic investigation: operating rooms used for case-patients had no HEPA filters; if used, HEPA filters were not used properly; or the operating room was contaminated with unfiltered external air (8).

WGST has become an essential tool for investigating outbreaks of fungal infections; however, defining levels of SNP identity among isolates to determine relatedness remains challenging. Recent WGST analyses of several fungal infection outbreaks help shape our understanding about the relatedness of isolates from point-source outbreaks (25–27,29,30). However, WGST data from clusters not linked to a common source are scarce, and information about the expected genomic diversity among strains from the same region that cannot be linked to a common source is lacking. On the basis of WGS from outbreaks with strong epidemiologic data implicating a common source, isolates that differ by <10 SNPs are considered to be nearly identical and to originate from the same source; isolates sharing tens or hundreds of SNPs are considered different. However, these thresholds are arbitrary, dependent on bioinformatics pipelines, and species specific. To address this issue, Chow et al. defined pairwise SNP distances among isolates of *Candida auris* from the same patient as an identity reference point (31). Specifically, outbreak isolates are considered to be of the same origin if the number of SNPs between them is the same or lower than the average number of SNPs between multiple isolates from the same patient or known source. Although developed specifically to determine transmission of *C. auris*, this approach can be adapted to other species and outbreak situations if multiple isolates from the same patients are collected. The number of SNPs separating genomes in the inner clade of the phylogeny generated in this study, which included most of the Argentina isolates from the cluster, was relatively low compared with the number of SNPs separating genomes of the control isolates. However, this number was higher than the differences among multiple isolates from a single patient. Specifically, 0–17 SNPs separated isolates from the same patient, and 60–762 SNPs separated strains from different patients and different facilities. One case of apparent nosocomial transmission was identified in which 2 isolates from 2 patients admitted to the same hospital within 3 weeks differed by 6 SNPs. Because *Rhizopus* spp. infection is not contagious, transmission probably occurred through the contaminated equipment or from the same environmental source.

Because fungal genomes are large and highly complex, thousands of SNPs separating conspecifics is not uncommon (25,26,29), which is illustrated here within the *R. microsporus* var. *microsporus* group. The relatively low numbers of SNPs separating genomes in the inner clade is consistent with a common geographic origin and suggest a relatively recent common ancestor for these 17 isolates. Such limited population diversity is similar to that found for recently emerged fungal populations that display years to decades of evolution in a restricted geographic locale, such as the emergent clones of *Cryptococcus gattii* in the Pacific Northwest (32) and the recently described clonal population of *Coccidioides immitis* in southeast-
ern Washington state (33).

Although the mutation rate within *R. microsporus* is not known, we found no association between genetic distance and sampling dates by using root-to-tip regression analysis, which suggests a lack of molecular clock–like behavior. Furthermore, we found no apparent evidence of recombination or mutation selection in the even SNP distribution across the 25 genomes, suggesting that most SNPs resulted from neutral point mutations and showing that these samples are separated by substantial amounts of evolution, which is not typical of patient-to-patient or point-source outbreaks. However, we cannot rule out the possibility of rapid mutations occurring within a common-source outbreak or a well-established but minimally diverse common-source population. The recent global expansion of *C. auris* has advanced our knowledge of the varying evolutionary rate of nosocomial fungi; a recent analysis established a within-hospital rate of $5.7 \times 10^{-5}$ nt substitutions/site/year (34). However, whether the mutation rate of *C. auris* is applicable to that of *Rhizopus* spp., a different taxonomic group with different ecology, remains unclear. Such a rate in *R. microsporus* would predict >1,425 SNPs between genomes separated by only a year. The inclusion of the ATCC 11559 control strain, isolated in 1935 in the Soviet Union, indicates that hypermutation is not occurring because this 84-year-old strain is separated from the Argentina cluster clade by <800 SNPs.

*Rhizopus* spp. are known to undergo chromosomal duplication events and potentially cross-species hybridization and to contain large proportions of inactive transposable elements (4), which may explain the vast differences in genome sizes and multiple SNP states (i.e., heterozygosity) detected at many genomic loci in the samples with 43–51 Mbp assembled genome sizes. The public genomes for *R. microsporus* in GenBank are also of various assembly sizes, ranging from 24.1 Mbp (GenBank accession no. GCA_002083735) to 75.1 Mbp (GCA_000697275). A substantial expanse of genome size variation could also result from suboptimal sequence data quality or read length, preventing proper contig formation during assembly and overestimation of genome size or pileup of repeat regions, thereby leading to underestimation of genome size (4). However, our data were of high quality and SNPs were filtered for high-confidence SNPs, although strain mixtures cannot be ruled out. In addition, hybridization between *Rhizopus* species or subspecies varieties has been described (4), which would confound phylogenetic analysis. Last, many fungi carry bacterial endosymbionts that alter the assembled genome sizes and GC content, including some strains of *R. microsporus* and *Burkholderia rhizoxinicus* (35); *B. rhizoxinicus* often requires obligate symbiosis with *R. microsporus* (36) and provides a toxin for plant pathogenesis to its host (36,37). In our sample set, 3 control isolates of *R. microsporus* var. *microsporus* from skin, respiratory tract, and an unknown source harbored *B. rhizoxinicus*. To our knowledge, whether the toxin or another factor from the symbiosis contributes to human infection has not been studied. Because many fungi are capable of these and other forms of genomic and chromosomal plasticity, phylogenetic analyses of fungal clusters, even in outbreak scenarios, need to account for these potentially confounding factors.

The cryptic diversity seen in this study might be missed by use of less discriminatory typing techniques, such as matrix-assisted laser desorption/ionization time-of-flight mass spectrometry or repetitive element palindromic PCR (7), possibly resulting in inappropriate point-source attribution. WGST has become the standard for molecular/genomic epidemiology, even (or especially) with understudied or rare pathogen events. However, despite the successful use of WGST to solve numerous medical and public health mysteries, the complexities of certain microbes and their resultant patient clusters are not always clarified, and without intensive sampling and routine genomic surveillance, causes of such clusters may remain hidden.

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EID Podcast: A Worm’s Eye View

Seeing a several-centimeters-long worm traversing the conjunctiva of an eye is often the moment when many people realize they are infected with Loa loa, commonly called the African eyeworm, a parasitic nematode that migrates throughout the subcutaneous and connective tissues of infected persons. Infection with this worm is called loiasis and is typically diagnosed either by the worm’s appearance in the eye or by a history of localized Calabar swellings, named for the coastal Nigerian town where that symptom was initially observed among infected persons. Endemic to a large region of the western and central African rainforests, the Loa loa microfilariae are passed to humans primarily from bites by flies from two species of the genus Chrysops, C. silacea and C. dimidiate. The more than 29 million people who live in affected areas of Central and West Africa are potentially at risk of loiasis.

Ben Taylor, cover artist for the August 2018 issue of EID, discusses how his personal experience with the Loa loa parasite influenced this painting.
Zika Virus Circulation in Mali


The circulation of Zika virus (ZIKV) in Mali has not been clearly characterized. Therefore, we conducted a serologic survey of 793 asymptomatic volunteers ≥15 years of age (2016), and 637 blood donors (2013) to assess the serorepivalence of ZIKV infection in 2 ecoclimatic regions of Mali, tropical savannah and warm semiarid region, using ELISA and seroneutralization assays. The overall seroreivalence was ≈12% and increased with age, with no statistical difference between male and female participants. In the warm semiarid study sites we detected immunological markers of an outbreak that occurred in the late 1990s in 18% (95% CI 13%–23%) of participants. In tropical savannah sites, we estimated a low rate of endemic transmission, with 2.5% (95% CI 2.0%–3.1%) of population infected by ZIKV annually. These data demonstrate the circulation of ZIKV in Mali and provide evidence of a previously unidentified outbreak that occurred in the late 1990s.

Zika virus (ZIKV) is an arbovirus (genus Flavivirus; enveloped positive-stranded RNA virus) (1). The isolation of ZIKV took place in 1947 from a caged sentinel rhesus monkey during a yellow fever virus survey conducted in Zika forest of Uganda. The first

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notable human epidemic was recorded on the Western Pacific Islands of Yap, Federated States of Micronesia, in 2007 (2,3). The Asian genotype of the virus spread in the Pacific Islands, then to Latin America and the Caribbean (4).

ZIKV is transmitted to humans primarily through the bite of Aedes spp. mosquitoes; however, sexual and maternofetal routes of transmission have been identified during recent outbreaks (5–7). ZIKV infections are believed to be frequently asymptomatic or pauci-symptomatic (common mild and self-limiting symptoms that include macular or papular rash, fever, arthritis, conjunctivitis and headache) (8). In Martinique, estimates of truly asymptomatic cases among blood donors infected by ZIKV were ≈45%, and cases that did not require medical attention were ≈80% (9). A range of 29%–82% for asymptomatic infections has been reported (10), possibly reflecting differences between human populations or viral strains but most likely because of the imprecise nature of the definition of asymptomatic cases. However, recent outbreaks in Polynesia and the Americas have shown rare but serious complications, such as eye lesions (11), neurologic conditions in adults (myelitis, encephalitis, Guillain-Barré syndrome) (12) and developmental abnormalities (including microcephaly) in fetuses (13).

Although the first identification of ZIKV was made in Africa, Zika virus disease did not draw much attention in Africa, possibly because no large outbreaks were detected that were caused by the African genotype of the virus, or because it was misdiagnosed as a generic arboviral febrile illness. Reports of ZIKV isolation from humans and mosquitoes, as well as several seroepidemiological surveys, showed that the virus has been circulating endemically in countries in Africa for decades (14,15). The reason is the sylvatic transmission cycle of the African genotype, which differs from the recently observed urban transmission cycle of the Asian genotype (16,17).

1These authors contributed equally to this article.
2Deceased.
The risk for epidemic spread of ZIKV in Africa is a public health issue. Strains belonging to the African genotype may over time become adapted to peridomestic mosquitoes circulating in Africa and cause large urban epidemics (18). Furthermore, recent epidemics in Cabo Verde (19) and Angola (20) are reminders that the importation of ZIKV strains belonging to the Asian genotype and adapted to urban mosquitoes may cause the spread of the virus in mostly naïve populations.

We present the results of a cross-sectional seroepidemiological study and report evidence of ZIKV circulation in Mali. Our work is part of a global effort to characterize more accurately the circulation of ZIKV in Africa, estimate the level of immunity of local populations, and identify populations vulnerable to future epidemics.

Methods

Study Design and Population
We conducted a cross-sectional study primarily dedicated to establishing the seroprevalence of arboviral and hemorrhagic viral infections in Mali during October–November 2016 in 7 sites. In Bamako only, we also used 637 serum samples collected in 2013 from eligible volunteer blood donors for studying the seroprevalence of arboviral infections.

The Institutional Review Board of the Faculty of Medicine and Odonto-Stomatology, University of Sciences, Techniques and Technologies, Bamako, Mali, reviewed and approved this study (IRB letter no. 2016/113/CE/FMPOS). First, we visited all sites to meet health professionals, administrative authorities, and local community to explain the study context; after obtaining community permission, the study staff visited participant families. We conducted the study in alignment with institutional procedures and guidelines.

We selected 7 districts representing the different ecoclimatic areas of Mali: Diéma, Kita, Bougouni, Kadiolo, Niono, Bandiagara, and Commune IV of Bamako (Figure 1). Those districts are spread over the different administrative regions of Mali, excluding the northern region, which could not be investigated for security reasons. The selected districts are also those used by the Ministry of Health for infectious diseases surveillance.

We selected villages or city areas randomly from an exhaustive list in each district. We randomly
selected families and recruited volunteers with the objective of including 100–150 participants per site (Table 1), which corresponds to the estimate of the recruitment capacity by field teams for each site investigated. This sample size allows establishing seroprevalence in each site and globally. For significance level $\alpha = 0.05$ and seroprevalence values 5%–30%, the precision varies from $\approx$4% to 9% (sample size = 100), and from $\approx$1.5% to 3% (sample size = 800).

We enrolled only healthy, nonfebrile male and female volunteers $\geq$15 years of age. We provided study information in the most familiar language of the volunteer and in the presence of an observer designated by the village authorities who ensured that volunteers understood the information completely and helped obtain answers to any question. All volunteers signed the informed consent form before enrollment and received a copy of the signed form.

We used the Open Data Kit (ODK) platform on tablets to collect data, including sociodemographic information, weight, and history of travels. The same day, we drew venous blood (5 mL) in SST vacutainer tubes (Becton Dickinson, https://www.bd.com), kept them at 2°C–8°C, and centrifuged (1,500–2,000 × g) within 12 h before aliquoting and storing at −80°C.

Eligibility criteria for blood donors in Bamako included acceptance and signature of the consent form, age (18–55 y), a normal blood pressure, a bodyweight $>$50 kg, the absence of recent fever or history of fever, and negative testing for HIV, hepatitis B and C, and syphilis. We excluded persons with known chronic disease or sensitivity to blood collection or those who were pregnant or breastfeeding. We heat inactivated samples collected in Mali, packed them at the Malaria Research and Training Center in Bamako, and sent them to the National Reference Centre for Arboviruses (Unité des Virus Émergents, Marseille, France) for serologic investigations, in compliance with national regulations in both countries.

### Serologic Analysis

Before the analysis, we inactivated serum specimens at 56°C for 30 min. We detected ZIKV IgG using a primary ELISA screening (Euroimmun anti-NS1 IgG ELISA kit, Medizinische Labordiagnostika, https://www.euroimmun.com); the sensitivity of the ELISA appears to be high in these studies (21). We further investigated serum samples that yielded a positive or equivocal ELISA result using a cytopathic effect (CPE)–based virus neutralization test (VNT) and a strategy previously described and validated for seroprevalence studies. Serum samples with a neutralizing titer $\geq$40 were considered positive. This strategy enables us to reach sensitivity of 98.1% and specificity of 98.8% in a population with high exposure to dengue virus infection (22).

### Statistical Analyses

We used IBM SPSS Statistics 24 (http://www.ibm.com) to perform statistical analyses. We evaluated associations between sociodemographic variables (age and sex) and ZIKV seroprevalence by logistic regression and Pearson $\chi^2$ test.

The analysis of seroprevalence stratified by age can provide insight on the history and mode of circulation of a pathogen (23). For example, a slow continuous rise of seroprevalence with age may indicate endemic circulation, whereas a sudden increase in seroprevalence for persons $>$20 years of age suggests that an outbreak took place 20 years ago. We used serocatalytic statistical models to reconstruct the force of infection, defined as the per capita rate of infection of susceptible persons, from such data (23).

We considered different statistical models; in the constant model, we assumed that the force of infection $\lambda$ is constant with age and time. In that case, the seroprevalence of an individual is expected to increase with their age $a$: $Pa = 1 – \exp(–\lambda a)$. In the epidemic model, we assume that infections occurred during an epidemic. The force of infection was equal to $\lambda$ during the epidemic year and 0 at other times. Persons born after the epidemic were therefore all seronegative and older ones had a probability $p = 1 – \exp(–\lambda a)$ of being seropositive.

We fitted these statistical models to data with a Markov Chain Monte Carlo (MCMC) Metropolis-Hastings sampler implemented in the Rstan package (24). We chose flat priors for the parameters and simulated 4 independent chains of 5,000 runs (2,500 burn-ins) for each fit. For the constant model, we reported the mean and 95% credible interval of the annual probability of infection of a susceptible patient using the formula $Pi = 1 – \exp(–\lambda a)$. The deviance information criterion (DIC) was used to assess the

### Table 1. Precision of seroprevalence determination according to prevalence estimates and sample size in study of Zika virus, Mali

<table>
<thead>
<tr>
<th>Prevalence estimate</th>
<th>100</th>
<th>150</th>
<th>800</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.050</td>
<td>0.044</td>
<td>0.035</td>
<td>0.015</td>
</tr>
<tr>
<td>0.100</td>
<td>0.060</td>
<td>0.048</td>
<td>0.020</td>
</tr>
<tr>
<td>0.150</td>
<td>0.070</td>
<td>0.058</td>
<td>0.025</td>
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<tr>
<td>0.200</td>
<td>0.080</td>
<td>0.065</td>
<td>0.028</td>
</tr>
<tr>
<td>0.250</td>
<td>0.086</td>
<td>0.070</td>
<td>0.030</td>
</tr>
<tr>
<td>0.300</td>
<td>0.091</td>
<td>0.074</td>
<td>0.032</td>
</tr>
</tbody>
</table>

*a = 0.05.*
model fits (25). Lower DICs indicate better fits, with a DIC difference of 5 considered substantial.

We grouped the localities according to local climate areas from Köppen classification: the cities of Diéma, Bandiagara, and Niono comprised a semi-arid area, and the cities of Bamako, Kadiolo, Bougouni, and Kita comprised a tropical area. We fitted the models to each of these areas (Figure 1).

Results
We included a total of 1,430 healthy volunteers. Overall, 793 healthy participants ≥15 years of age were enrolled in October–November 2016 in 7 different sites in Mali: Niono (n = 65), Bamako (n = 129), Kadiolo (n = 136), Bougouni (n = 127), Kita (n = 40), Bandiagara (n = 187), and Diéma (n = 109). The sex ratio (M/F) was 0.44 (242/551) and median age was 33 years. We also included serum samples from 637 eligible blood donors from Bamako who provided samples in 2013. In this group, there were few women (sex ratio 8.8 (572/65) as previously observed in Mali blood donor populations (26), and the median age was 28.0 years (Table 2).

ZIKV Seroprevalence
Using the ELISA+CPE-based VNT strategy, we found IgG seropositivity for ZIKV of 11.98% among the 793 serum specimens collected from participants in 2016. Seroprevalence range was 3.1%–20.2% in different regions: 3.1% in Niono, 5.4% in Bamako, 10.3% in Kadiolo, 11.8% in Bougouni, 15% in Kita, 15.5% in Bandiagara, and 20.2% in Diéma (Table 3). The endpoint titer average was 26.32 ± 5.41 (range 10–320). In a global analysis, we did not detect a statistical difference between sexes (11.6% in male participants, 12.2% in female participants; Pearson p = 0.834); however, in Bougouni the seroprevalence was significantly higher in male participants (21.4% vs. 7.1% in female participants; p = 0.04).

In blood donors sampled in 2013, seroprevalence was 7.4% (47/637), and we found no statistical difference between male and female participants (7.9% versus 3.1%, Pearson p = 0.162). The seroprevalence values in blood donors sampled in 2013 and participants of commune IV of Bamako sampled in 2016 were similar: 7.4% (47/637) from 2013 versus 5.4% (7/129) from 2016 (Pearson p = 0.430). This finding suggests the absence of sustained ZIKV circulation in this area between 2013 and 2016, making it possible to pool results obtained in Bamako for the estimation of the force of infection.

When we categorized the 2016 cross-sectional study population into age groups, we found that ZIKV seropositivity increases with age (logistic regression p = 0.003, 95% CI 1.006–1.029): 7.7% for the 15–29-year age group, 12.8% for 30–44 years, 16.0% for 45–59 years, and 17.1% for >60 years (Figure 2). This trend was maintained when we included the blood donors population (7.3% for the 15–29-year age group, 9.6% for 30–44 years, 14.6% for 45–59 years, and 17.1% for >60 years; p = 0.000, 95% CI 1.011–1.031). Seventy of the 793 participants of 2016 reported travels during the 6 months preceding blood collection, including 7 who VNT-ZIKV positives, and 88 VNT-ZIKV positives of the remaining 723 participants did not report travel (no statistical difference; Pearson p = 0.593).

ZIKV Transmission Dynamics
We found that the continuous increase of seroprevalence with age in the tropical savannah area was better explained by a model assuming constant low-level circulation of ZIKV (Figure 3, panel A) than one assuming a single outbreak occurred in the past (Figure 3, panel B). From the best fitting model with a constant force of infection, we estimated that 2.5% (95% CI 2.0%–3.1%) of the susceptible population is infected by ZIKV annually (Figure 3, panel C). In the semi-arid areas, we saw seroprevalence sharply increase for participants >20 years of age and then stabilize (Figure 3, panels D, E). This age profile was best explained by the epidemic model, with an epidemic expected to have occurred in the late 1990s (Figure 3, panel F). The proportion of the population infected at that time was estimated to be 18% (95% CI 13%–23%).

Discussion
We conducted a Zika seroepidemiological study in Mali and found evidence for the circulation of ZIKV.

---

**Table 2. Demographic characteristics of the population in study of Zika virus, Mali**

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>9</td>
<td>50</td>
<td>572</td>
<td>40</td>
<td>42</td>
<td>16</td>
<td>58</td>
<td>27</td>
<td>814</td>
</tr>
<tr>
<td>F</td>
<td>56</td>
<td>79</td>
<td>65</td>
<td>96</td>
<td>85</td>
<td>24</td>
<td>129</td>
<td>82</td>
<td>616</td>
</tr>
<tr>
<td>M/F ratio</td>
<td>0.2</td>
<td>0.6</td>
<td>8.8</td>
<td>0.4</td>
<td>0.5</td>
<td>0.7</td>
<td>0.4</td>
<td>0.3</td>
<td>1.3</td>
</tr>
<tr>
<td>Median age, y</td>
<td>35</td>
<td>32</td>
<td>28</td>
<td>30</td>
<td>45</td>
<td>23</td>
<td>35</td>
<td>28</td>
<td>30</td>
</tr>
</tbody>
</table>

*Volunteer blood donors.
We found an average ZIKV seroprevalence of ≈12%, ranging from 3.1% to 20.2% depending on the study site. Seroprevalence was higher than expected because recent surveys conducted in several Central and West Africa countries reported lower values: ≈5% in Cameroon (17), 0.1% in the Democratic Republic of the Congo (27) and 3.4% in Nigeria (28). Those countries have ecoclimatic conditions that have been typically associated with the circulation of ZIKV and its implication in the transmission to humans by peridomestic mosquitoes (i.e., tropical forested areas with nonhuman primates and sylvatic Aedes spp. mosquitoes).

The situation is different in Mali, where this sort of ecologic environment is uncommon and can be found only in some sites located in the southwestern part of the country. Mali includes a tropical savannah belt in the south (containing the study sites Kita, Bamako, Bougouni, and Kadiolo), and a warm semiarid region (Diéma, Niono, and Bandiagara). Further north are vast Sahelian regions that we could not investigate for security reasons. Of note, a single ZIKV serosurvey was conducted previously in Mali in 1964–1967 and reported prevalence as high as 52% with hemagglutination inhibition (HI) assay, a method which is highly susceptible to cross-reaction with related flaviviruses that cause high false positive rates (29,30).

Of interest, when modeling our data we concluded that the most probable mode of transmission in the tropical savannah region was nonepidermic and associated with low seroprevalence values, which is reminiscent of the low-rate endemic transmission that has been reported in Central Africa (Cameroon and Congo) (17,27) and West Africa (28,31). This transmission may correspond in part to sylvatic exposure. In contrast, our best model points to the occurrence of a large ZIKV epidemic in the warm semiarid regions in the late 1990s (Figure 3), with seroprevalence values reaching 20.2% in Diéma. It is likely that transmission was facilitated by a peridomestic mosquito species (most probably Aedes aegypti), which raises the possibility that the cause was an imported Asian genotype strain or an African genotype strain with an improved competence for peridomestic mosquitoes.

The identification of 2 different transmission dynamic patterns of ZIKV, combined with the high percentage of neutralizing antibodies in several study sites, deserves further investigation. No strain of ZIKV has been isolated or characterized by molecular methods in Mali to date, and direct detection of ZIKV in the samples we studied was not possible due to systematic heat inactivation. Implementing entomological surveys and the study of nonmalarial febrile patients in Mali is necessary to isolate, sequence, and genetically characterize the circulating ZIKV strains in Mali and characterize the putative enzootic maintenance cycle of ZIKV in the tropical savannah region. In addition, the recent report of an association between ZIKV IgG and microcephaly in Guinea-Bissau (32) suggests that comprehensive case-control studies of pregnant women and their infants with congenital

### Table 3. Results of seroepidemiological investigations for Zika virus according to study sites and time of sampling, Mali

<table>
<thead>
<tr>
<th>Study site and year</th>
<th>Total no.</th>
<th>IgG* doubtful, no. (%)</th>
<th>IgG* positive, no. (%)</th>
<th>VNT† positive, no. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Niono 2016</td>
<td>65</td>
<td>4 (6.0)</td>
<td>4 (6.2)</td>
<td>2 (3.1)</td>
</tr>
<tr>
<td>Bamako 2016</td>
<td>129</td>
<td>0 (0.0)</td>
<td>11 (8.5)</td>
<td>7 (5.4)</td>
</tr>
<tr>
<td>Bamako 2013‡</td>
<td>637</td>
<td>18 (2.8)</td>
<td>67 (10.5)</td>
<td>47 (7.4)</td>
</tr>
<tr>
<td>Kadiolo 2016</td>
<td>136</td>
<td>14 (10.3)</td>
<td>22 (16.2)</td>
<td>14 (10.3)</td>
</tr>
<tr>
<td>Bougouni 2016</td>
<td>127</td>
<td>3 (2.4)</td>
<td>22 (17.3)</td>
<td>15 (11.8)</td>
</tr>
<tr>
<td>Kita 2016</td>
<td>40</td>
<td>5 (12.5)</td>
<td>7 (17.5)</td>
<td>6 (15.0)</td>
</tr>
<tr>
<td>Bandiagara 2016</td>
<td>187</td>
<td>24 (12.8)</td>
<td>81 (43.3)</td>
<td>29 (15.5)</td>
</tr>
<tr>
<td>Diéma 2016</td>
<td>109</td>
<td>14 (12.8)</td>
<td>39 (35.8)</td>
<td>22 (20.2)</td>
</tr>
<tr>
<td>Total</td>
<td>1,430</td>
<td>82 (5.7)</td>
<td>253 (17.7)</td>
<td>142 (9.9)</td>
</tr>
</tbody>
</table>

*Result by Euroimmun IgG ELISA assay.
†Result by cytopathic effect-based virus neutralization test.
‡Volunteer blood donors.

![Figure 2. Zika virus seroprevalence by age group, Mali, 2016.](link)
neurologic abnormalities should be performed in Mali to clarify the possible implication of ZIKV. This study had several limitations. First, the size of the population tested and the number of sites included in the study were limited by severe logistical and security constraints. Accordingly, the population studied is not fully representative of the Mali general population. However, our results provide a novel and credible picture of the circulation of ZIKV according to the sites and age classes. Second, the circulation of potentially cross-reacting flaviviruses in the region raises questions of detection specificity. Nevertheless, previous investigations conducted in a region where the population is heavily exposed to dengue virus showed that the testing strategy we used in our study is robust and highly specific (22). Third, the detection of neutralizing antibodies may underestimate the actual proportion of the population that was infected by ZIKV if a fraction of the infected persons lose antibodies to ZIKV over time (33). This possibility requires further investigation to better understand the potential effects on seroprevalence studies.

In conclusion, we demonstrated the circulation of ZIKV in Mali, and observed seroprevalence rates that most probably are insufficient to create protective herd immunity against potential future outbreaks. The identification of a previously unknown ZIKV outbreak in the semiarid regions of Mali in the late 1990s emphasizes the need for improving the detection of emerging infections in Africa (34). Additional research revealing the dynamics of transmission, prevalence of symptomatic and asymptomatic ZIKV infections, and the frequency and severity of ZIKV-related congenital anomalies and other neurologic complications should be implemented.

Acknowledgments
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This article is dedicated to the memory of Ogobara K. Doumbo, who initiated the corresponding research work.
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About the Author
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Tuberculosis caused by concurrent infection with multiple Mycobacterium tuberculosis strains (i.e., mixed infection) challenges clinical and epidemiologic paradigms. We explored possible transmission mechanisms of mixed infection in a population-based, molecular epidemiology study in Botswana during 2012–2016. We defined mixed infection as multiple repeats of alleles at ≥2 loci within a discrete mycobacterial interspersed repetitive unit–variable-number tandem-repeat (MIRU-VNTR) result. We compared mixed infection MIRU-VNTR results with all study MIRU-VNTR results by considering all permutations at each multiple allele locus; matched MIRU-VNTR results were considered evidence of recently acquired strains and nonmatched to any other results were considered evidence of remotely acquired strains. Among 2,051 patients, 34 (1.7%) had mixed infection, of which 23 (68%) had recently and remotely acquired strains. This finding might support the mixed infection mechanism of recent transmission and simultaneous remote reactivation. Further exploration is needed to determine proportions of transmission mechanisms in settings where mixed infections are prevalent.

Tuberculosis (TB) caused by concurrent infection with multiple Mycobacterium tuberculosis during 1 episode is commonly referred to as mixed infection. In 1972, Canetti et al. suggested the concept of mixed infection of exogenous reinfection of nonprimary TB among elderly patients in France (1). Their observation was followed by phage typing of cultured isolates from patients with concurrent disease in multiple organ sites observed during clinical practice in North America, mixed cultures among Eskimo patients during the mid-1970s (2,3), and cultures collected during outbreak investigations in the 1980s and 1990s (4,5). However, more recent applications of advanced molecular tools suggest mixed infection might occur more frequently than initially expected (6,7). This possibility led to many research studies of mixed infection, which found that mixed infection is associated with poor treatment outcomes (6,8), including acquisition of multidrug-resistant TB (7,8). Mixed infection research contributed to the discovery that exogenous reinfection was responsible for a substantial portion of incident TB, implying incomplete protection from a primary infection in subsequent infections (9,10).

Despite the clinical importance of mixed infection, its potential leading mechanisms of transmission have not been examined using empirical data. Infections caused by multiple M. tuberculosis strains can occur after simultaneous transmission of multiple strains during a single transmission episode (i.e., the index patient transmits multiple strains) or by sequential infections of ≥2 strains acquired at different times, resulting in superinfection (10). So far, transmission mechanisms of mixed infection and its population-level effect have been explored only hypothetically (9,11). Research on the transmission mechanisms for mixed infection with empirical data might improve understanding of M. tuberculosis dynamics and designing effective TB control interventions (12). Our objective was to explore possible transmission mechanisms leading to mixed infection.
M. tuberculosis infections by comparing genotypes and spatial proximity of all detected M. tuberculosis strains.

Methods

Study Setting
This analysis was part of a population-based, molecular epidemiology study in Botswana (the Kopanyo Study). The study design and methods were previously described (13). In brief, the study recruited and enrolled patients with newly diagnosed TB at 30 TB and HIV clinics during 2012–2016. Behavioral, clinical, and demographic information (including residential address at enrolment) were collected during medical record abstraction and standardized patient interview. Sputum collected from participants underwent smear-microscopy, culture, drug-susceptibility testing, and 24-locus mycobacterial interspersed repetitive unit–variable-number tandem-repeat (MIRU-VNTR) genotyping using a standard international protocol (14), when applicable.

Definition of Mixed Infection
MIRU-VNTR genotyping counts the numbers of tandem repeats at the selected loci, which are unique in different strains of M. tuberculosis. We defined mixed infection as multiple allele repeat numbers (e.g., double allele) at ≥2 loci within a discrete MIRU-VNTR result (10). We defined possible mixed infection as multiple allele repeat numbers at 1 locus within a discrete MIRU-VNTR result and single infection as a discrete MIRU-VNTR result with single alleles at all 24 loci (Figure 1; Appendix Tables 1, 2, https://wwwnc.cdc.gov/EID/article/26/5/19-1638-App1.xlsx).

Definition of Genotype Cluster
We defined TB genotype clusters as ≥2 patient isolates with exact match 24-loci results, suggesting recently acquired strains (12,15). We considered genotype results that matched no other patient isolate results in the dataset nonclustered, suggesting remotely acquired strains (12,15). To identify putative mixed infection M. tuberculosis genotype clusters, we compared MIRU-VNTR results for each mixed infection patient to MIRU-VNTR results of all other M. tuberculosis strains, considering permutations of each repeat number at multiple allele loci. We also considered 24-loci results to be nonclustered if no permutation of the mixed MIRU-VNTR result matched any other study strain; if ≥1 permutation of the mixed
MIRU-VNTR result matched any other study strain, we considered it to be clustered. When we considered all permutations at each double allele locus, if ≥1 permutation accounting for each repeat number at each locus matched another study strain but no permutation accounting for the alternate repeat number at each locus matched another study strain, we considered it to be evidence of simultaneously clustered and nonclustered strains. For example, if the patient isolate results had repeat numbers 4 and 5 at the third locus, the matched M. tuberculosis strain’s MIRU-VNTR results should include repeat numbers 4, 5, or both at the same locus (Figure 1). We excluded patients with isolates that had missing or incomplete MIRU-VNTR results. We reviewed all laboratory procedures (i.e., sputum collection and processing, culture isolation and storage, DNA abstraction and storage, and MIRU-VNTR batching processes) to identify potential points of cross-contamination or mishandling. We reviewed all laboratory registries and electronic databases to record processing and reporting dates for all patient isolates.

Classification of Mixed Infection Mechanisms
On the basis of the genotype cluster analysis, we classified patients with mixed infection into 1 of 3 categories: 1) simultaneous reactivation of ≥2 remotely acquired strains if no mixed infection MIRU-VNTR permutations accounting for multiple different repeat numbers at each locus matched any other study strain; 2) infection from a recently acquired strain and simultaneous reactivation of a remotely acquired strain if ≥1 permutation accounting for 1 repeat number at each locus matched another study strain but no permutation accounting for the other repeat number at each locus matched any other study strain; and 3) rapid progression of ≥2 recently acquired strains if ≥1 permutation accounting for each repeat number at each double allele locus matched another study strain.

Statistical Analyses
For each mixed infection MIRU-VNTR result, we wrote a loop function using SAS (SAS Institute Inc., https://www.sas.com) to compare tandem numbers from the first locus to 24th locus with all other MIRU-VNTR results locus by locus. When discrepancies existed between tandem numbers at a locus, the locus was flagged. We counted the number of flags after 24 loci were compared. If all numbers matched, the number of flags was 0; if no loci matched, the number was 24. We used the number of flags to classify the degree to which the MIRU-VNTR pattern matched that of the mixed infection MIRU-VNTR result. At the end of the loop function, we created a subset dataset with all MIRU-VNTR results by descending order of the number of exactly matched loci (from 0 for exactly matched at all 24 loci), for each mixed infection MIRU-VNTR result.

We calculated simple frequencies and proportions for the main outcomes (mixed infection, possible mixed infection, and single infection) stratified by patient sex, HIV status, and residential address. Primary residential address of each patient was geocoded and mapped using ArcGIS (ESRI, https://www.esri.com). We showed the distribution of M. tuberculosis genotype clusters if found within 1 km of one another to add epidemiologic plausibility. We excluded patients with missing residential geocoding from the spatial analysis.

Sensitivity Analysis
To assess potential variation within genotype relatedness, we explored an alternative clustering definition to include 1 locus difference. For this sensitivity analysis, potential near matches (i.e., matched on all other loci results but with a nonmatched tandem number at the locus of interest) were considered genotype clusters. We excluded patients with isolates with missing or incomplete MIRU-VNTR results from the sensitivity analysis.

Ethics Approval
This study was approved by the Institutional Review Boards of the US Centers for Disease Control and Prevention (#6291; Atlanta, GA, USA); Health Research and Development Committee, Botswana Ministry of Health and Wellness (Gaborone, Botswana); University of Pennsylvania (Philadelphia, PA, USA); and University of California, Irvine (Irvine, CA, USA). Participants provided written informed consent.

Results
A total of 2,137 patients were enrolled, of whom 1,130 (53%) were HIV positive (Table 1). After excluding patients with missing or incomplete MIRU-VNTR results (including 3 patients with mixed infection), we included 2,051 patients in the analyses (Figure 2). A total of 862 discrete genotyping MIRU-VNTR results were obtained (a more detailed strain analysis is available elsewhere [15]). We detected no evidence of laboratory cross-contamination events within sputum processing, culturing, DNA abstraction, or genotyping processing. All mixed infection patient isolates were processed on different days from isolates from other purported patients in the cluster.
Thirty-four (2%) patients had mixed infection, and 88 (4%) patients had possible mixed infection. Overall, we classified mixed infection in 23 (68%) patients as infection from a recently acquired strain and simultaneous reactivation of a remotely acquired strain, 7 (21%) as simultaneous reactivation of ≥2 remotely acquired strains, and 4 (12%) as ≥2 recently acquired strains (Table 2). Mixed infection in 27 (79%) patients involved recently acquired strains (Appendix Tables 1–3). The MIRU-VNTR results of 34 patients with mixed infection had a median of 7.5 loci (interquartile range 3–11) of multiple tandem repeats. The most prevalent MIRU-VNTR result in the population, MIRU identification no. [ID] 644 (n = 147 isolates), was not included in any genotype clusters with potential mixed infection transmission events. The second most prevalent strain, MIRU ID 382 (n = 81), matched with 2 genotype clusters involving
patients with mixed infection (MIRU ID 838 and MIRU ID 970) (Appendix Table 1).

After excluding additional 137 patients with no residential address (including 3 patients with mixed infection and 6 with possible mixed infection), we explored spatiotemporal transmission among 1,914 patients (Figure 2). We found 4 genotype clusters of mixed infection within 1 km of the location of patient with mixed infection as the center: 3 in Gaborone (Figure 3) and 1 in Ghanzi (Figure 4).

In sensitivity analysis, we allowed MIRU-VNTR patterns to differ by 1 locus, which changed the transmission category for 7 patients with mixed infection. Our main finding that the highest proportion (19 [51%]) of mixed infection occurred through a combination of genotype clustered and nonclustered strains did not change. The second highest proportion (10 [27%]) of mixed infection was a combination of multiple genotype clustered strains.

Discussion
We describe genotype patterns consistent with hypothesized mixed infection transmission mechanisms, using a multiyear, population-based TB cohort. In our study, most patients with mixed infection (68%) had both recently and remotely acquired strains, suggesting recent transmission and simultaneous remote reactivation. Recent infection that progresses to disease might further compromise the immune system, leading to reactivation. A previous case study described a patient with mixed infection with an apparent triggering of a remote multidrug-resistant M. tuberculosis strain after recent exposure to a drug-sensitive strain (16). A similar phenomenon has been described for relapse of Plasmodium vivax malaria triggered by infection with P. falciparum (17).

Table 2. Characteristics of 34 patients with mixed-strain Mycobacterium tuberculosis infection (the Kopanyo Study), Botswana, 2012–2016

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary residential site</td>
<td></td>
</tr>
<tr>
<td>Gaborone</td>
<td>22 (65)</td>
</tr>
<tr>
<td>Ghanzi District</td>
<td>7 (21)</td>
</tr>
<tr>
<td>Other Botswana</td>
<td>2 (6)</td>
</tr>
<tr>
<td>Missing residential address</td>
<td>3 (9)</td>
</tr>
<tr>
<td>HIV infection status</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>18 (53)</td>
</tr>
<tr>
<td>Negative</td>
<td>14 (41)</td>
</tr>
<tr>
<td>Unknown</td>
<td>2 (6)</td>
</tr>
<tr>
<td>Transmission mechanism</td>
<td></td>
</tr>
<tr>
<td>Recently acquired + recently acquired</td>
<td>23 (68)</td>
</tr>
<tr>
<td>Recently acquired + remotely acquired</td>
<td>7 (21)</td>
</tr>
<tr>
<td>Remotely acquired + remotely acquired</td>
<td>4 (12)</td>
</tr>
<tr>
<td>HIV infection &amp; transmission mechanism</td>
<td></td>
</tr>
<tr>
<td>Recently acquired + recently acquired</td>
<td></td>
</tr>
<tr>
<td>Positive: 1 (6); negative: 2 (14)</td>
<td></td>
</tr>
<tr>
<td>Recently acquired + remotely acquired</td>
<td></td>
</tr>
<tr>
<td>Positive: 12 (66); negative: 10 (72)</td>
<td></td>
</tr>
<tr>
<td>Remotely acquired + remotely acquired</td>
<td></td>
</tr>
<tr>
<td>Positive: 5 (28); negative: 2 (14)</td>
<td></td>
</tr>
</tbody>
</table>

Figure 3. Potential spatial relationships (residence within 1 km of another patient) between patients with mixed-strain infection and with other genotype-clustered strains, Gaborone, Botswana, 2012–2016. Shown are location of patients with mixed Mycobacterium tuberculosis infection and other genotype-clustered cases in Gaborone. Each color represents each genotype cluster. The 1-km radius blue-shaded area from each mixed infection patient shows the neighborhood boundary. Three patients with mixed infection had potential spatial relationships with 3–6 other patients within the neighborhood.
Similarly, our findings suggest that most mixed infection transmission events included reactivation of remotely acquired strains triggered by recently acquired strains, implying that mixed infection may be affected by the force of infection in communities (10, 11). We estimated the prevalence of each discrete MIRU-VNTR result as a proxy measure of force of infection in our study population. Contrary to our expectation, the 2 most prevalent strains (MIRU IDs 644 and 382) appeared in only 1 mixed infection transmission event. The dominate strain in the mixed infection was MIRU ID 838, which appeared 3 times. Further studies can show whether less transmissible strains outcompete other strains within the host to establish long-term persistence (11).

Our results add to the complexity of TB transmission dynamics in high TB prevalence settings (7). Current TB prevention strategies primarily focus on interrupting recent TB transmission through early detection and treatment of sputum smear–positive patients (18). Although interventions to interrupt transmission can reduce opportunities of exogenous re-infection and hence reduce the prevalence of mixed infection (10), our findings also imply the importance of treating latent TB infection to reduce the risk for mixed infection (19). No statistical association between HIV status—a proxy for reduced latency—and mixed infection (data not shown [odds ratio 1.15 (95% CI 0.79–1.68)]) also further supports the influence of remotely acquired strains in polyclonal transmission events. Our study alone might not be sufficient to generalize the results and emphasize reactivation. However, we envision further exploration of our suggested 3 transmission mechanisms in a setting where the transmission intensity is expected to be higher (e.g., high population density or dense slum area) and the role of reactivation is accounted for accordingly.

We added the spatial information to provide epidemiologic evidence of possible *M. tuberculosis* transmission. If patients whose isolates are in the same genotype cluster are spatially close to each other, it suggests possible transmission. The map shows the locations of patients with mixed infection and other genotype-clustered cases. Each color represents each genotype cluster. The 1-km radius blue-shaded area from each mixed infection patient shows the neighborhood boundary. Two patients with mixed infection were genotype-clustered and had a potential spatial relationship. (Their mycobacterial interspersed repetitive unit–variable-number tandem-repeat results were not exactly matched.)
other (i.e., within 1 km), they might be more likely to be in a transmission network than otherwise. This interpretation may be limited as we accounted only for the patients’ residential address as the spatial information, the close proximity set as 1 km was arbitrary, and the few TB clusters (and number of patients therein) may be missed if mixed infection is not included in transmission network reconstructions. However, our finding reconfirmed that TB transmission was ongoing in the community. A comprehensive molecular characterization of within-host M. tuberculosis diversity, as well as an attempt to temporally identify the primary source or index of transmission by comparing diagnosis times and the times of symptom onset (20), might be needed to fully capture TB transmission chains and accurately infer TB transmission (21,22).

Our results should be interpreted with caution. The prevalence of mixed infection was lower than in other studies (9,23) because of the method of molecular analyses. Although 24-loci MIRU-VNTR is a standardized molecular characterization tool and offers simple results that can be readily used to identify mixed infection (7,24), it has limited resolution to distinguish mixed infection from clonal heterogeneity or within-host bacterial microevolution (9,25,26). Different tools, such as whole-genome sequencing and 2 lineage-specific PCRs, might identify M. tuberculosis strains more sensitively and lead to a different dominating transmission mechanism if more patients with mixed infection were detected (23,27).

In the meantime, we defined and analyzed possible mixed infection and mixed infection separately in an attempt to more conservatively differentiate mixed infection from within-host heterogeneity. Another limitation involves misclassification bias from detection sensitivity (28); that is, all potential genotyping matches depend on the sensitivity of the characterization method. The 24-loci MIRU-VNTR method is relatively sensitive and has high discriminatory power; however, it characterizes only part of the M. tuberculosis genome (7). Hence, we might have missed genetic heterogeneity present in loci not covered by this method (12). The prevalence of each M. tuberculosis strain also depended on the degree to which we captured all M. tuberculosis strains present in the community. Although our study was multiyear and covered a broad geographic area, some important patients in the transmission network could have been missed (e.g., their TB was diagnosed before the study period, they resided in areas not covered by the study, or they refused enrolment), leading to clustering misclassification. We recruited TB patients through both passive and active case finding (13) to increase coverage, but not every patient produced sputum, and not all sputum samples led to M. tuberculosis isolation or valid genotype results (15). This limitation might lead to missed transmission links (18,21). Given generally low bacillary load among children, the transmission mechanism would have been affected in a way that the role of reacti-

ated strains was reduced if missing sputum samples had been successfully identified. On the other hand, by enabling multiple permutations of possible MIRU-VNTR results for mixed infection and possible mixed infection cases, MIRU-VNTR results with multiple alleles had more possible combinations and higher chance of matching with other genotypes. This finding may imply an imbalanced chance of being a member of a genotype cluster.

Future studies to investigate molecular profiles of M. tuberculosis with serial sputum collection, including nonrespiratory samples, and use of more sensitive and specific genome sequencing technologies, will be of interest to thoroughly assess possible transmission events leading to mixed infection. Despite the lower prevalence of mixed infection in the population in this study, the proposed mixed infection transmission mechanisms can be useful to characterize how similar or different mixed-infection transmission mechanisms would be across different settings with different burden of mixed infection.

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International travel–related nonpharmaceutical interventions (NPIs), which can include traveler screening, travel restrictions, and border closures, often are included in national influenza pandemic preparedness plans. We performed systematic reviews to identify evidence for their effectiveness. We found 15 studies in total. Some studies reported that NPIs could delay the introduction of influenza virus. However, no available evidence indicated that screening of inbound travelers would have a substantial effect on preventing spread of pandemic influenza, and no studies examining exit screening were found. Some studies reported that travel restrictions could delay the start of local transmission and slow international spread, and 1 study indicated that small Pacific islands were able to prevent importation of pandemic influenza during 1918–19 through complete border closure. This limited evidence base indicates that international travel-related NPIs would have limited effectiveness in controlling pandemic influenza and that these measures require considerable resources to implement.

From time to time, novel influenza A virus strains emerge and cause global influenza pandemics (1). Pandemics occurred 3 times in the 20th century and 1 time so far in the 21st century (2). The recognition that influenza pandemics can have substantial social and economic effects in addition to the impact on public health, along with the emergence of highly pathogenic strains of avian influenza virus in the past 20 years, has stimulated greater attention in preparing for future influenza pandemics (3,4). Given the delays in the availability of specific vaccines and limited supplies of antiviral drugs, nonpharmaceutical interventions (NPIs) form a major part of pandemic plans (2).

A range of NPIs can be applied at international, national, and local levels, with the objectives of delaying the arrival of infected persons, slowing the spread of infection, delaying the epidemic peak, and reducing the size of the peak (5). This article focuses on the use of measures related to international travel, including entry and exit screening of travelers for infection, travel restrictions, and border closures (Table 1). We aimed to review the evidence base assessing the effectiveness of these travel-related NPIs against pandemic influenza and to identify the barriers to implementation of these interventions.

Methods and Results
We searched for literature reporting or estimating the effectiveness of NPIs related to international travel and movement, including entry and exit screening travelers, travel restrictions, and border closures on pandemic or interpandemic influenza. We conducted literature searches on PubMed, Medline, Embase, and Cochrane Library for peer-reviewed articles published from January 1, 1946, through April 28, 2019. The search terms used were identified from relevant systematic reviews and research reports (8,9). We collected additional studies from secondary references from included studies or other relevant searches. Articles were eligible for inclusion if they reported or estimated the effectiveness of international travel–related NPIs for pandemic influenza using quantitative indicators such as delaying the introduction of infection, delaying the epidemic peak, or reducing the size of the peak. We excluded
articles if they did not investigate the quantitative effectiveness of international travel–related NPIs or were editorials, reviews, or commentaries without primary data. Furthermore, we restricted articles to those published in English. Two independent reviewers (S.R. and H.G.) screened titles and abstracts and assessed full-text articles for eligibility. A third reviewer (B.J.C.) adjudicated any disagreements between the 2 reviewers.

We extracted the information on the effectiveness of NPIs from included studies by using a structured data-extraction form. Information of interest included the study setting, specific measures implemented, timing of intervention implementation, study results regarding effectiveness indicators, and potential barriers to implementation. The assessment of quality of evidence considered study design and assigned generally higher quality to randomized trials, lower quality to observational studies, and lowest quality to simulation studies. We provide full search terms, search strategies, selection of articles, and summaries of the selected articles (Appendix, https://wwwnc.cdc.gov/EID/article/26/5/19-0993-App1.pdf).

Screening Travelers for Infection
We identified 4 relevant studies that considered the effect of screening on influenza transmission, including 2 epidemiologic studies from the 2009 pandemic (10,11) and 2 simulation studies (12,13). The epidemiologic studies estimated that entry screening delayed the arrival of influenza A(H1N1)pdm09 virus to previously unaffected areas by an average of 7–12 days (11) and delayed the epidemic in China by 4 days by reducing imported cases by 37% from border entry screening (10). The simulation studies predicted that entry screening would delay the arrival of infection into a country by a few days or 1–2 weeks at most (12,13). We did not identify any studies on exit screening; in the 2009 influenza pandemic, exit screening was not implemented by Mexico (14), nor by most other countries.

We did not systematically review studies of the technical performance of various screening tools (e.g., screening case definitions and thermal scanners) but identified in an informal search 4 studies that discussed the challenges of screening travelers for infection, which include limited screening sensitivity (10,11,13), an incubation period of 1–7 days for influenza A(H1N1)pdm09 virus (meaning some infected travelers might not show symptoms until after arrival at their destination) (10,12,13), limited local capacity of influenza surveillance (10,11), and limited public health resources, such as laboratory capacity and funding (10,11,13).

Screening inbound travelers for infection is a very visible public health intervention and can reduce the number of infectious persons entering the country (15). Infrared thermometers are currently used in many ports of entry in Asia because of the instantaneous and noninvasive nature of their use. Several simulation studies (10–13) included in this review estimated that this intervention helped to delay the introduction of infected persons. However, the sensitivity of screening travelers has been largely reliant on the sensitivity of detection of fever. Epidemiologic studies (16,17) conducted during the 2009 influenza pandemic demonstrated the low detection rate of entry screening that used the infrared thermal scanner and health declaration form at the airport; the sensitivity of screening travelers for infection was 5.8% in New Zealand and 6.6% in Japan. In addition to the lack of sensitivity for detecting febrile travelers (e.g., some travelers with febrile illness might take antipyretic medicine and evade detection), some infected travelers might travel during the incubation period, which is typically 1–2 days, and thus would not be identified as infected at departure or arrival (10,12). Once infection begins spreading in a local community, identifying additional inbound travelers with infection will do little to limit local spread. In addition, entry screening consumes considerable public health resources, including trained staff, screening devices, and laboratory resources, and thus might not be justifiable (18).

Travel Restrictions
We identified 1 epidemiologic study and 9 simulation studies that estimated or predicted the effectiveness of international travel restrictions (19–28) (Table 2). An epidemiologic study estimated that the peak
in the number of influenza-associated deaths was delayed by 2 weeks when international flight volume was reduced by 27% (28). Simulation studies predicted that 90%–99% of travel restrictions could delay international spread of cases by 2–19 weeks (20), delay the importation of the first case-patients by 1–8 weeks (23–26), and delay the epidemic peak by 1–12 weeks (19,23,24,26,27).

A simulation study predicted that selectively restricting the travel of children could delay the spread of infection by 35 days ($R_0 = 1.2–2.0$) (22), and another simulation study assessing the probability of escaping 1918–19 influenza pandemic among 17 Pacific Island countries and territories estimated that 4–5 countries avoided influenza pandemic ($R_0 = 1.5–3.0$) by strict limitation (79% or 99% restriction) of incoming travelers (21). Three studies explored the barriers to travel restrictions, which included the threat of economic loss (21,26) and lack of compliance among the public (20).

### Table 2. Overall summary of effectiveness international travel-related non-pharmaceutical interventions for reducing influenza transmission

<table>
<thead>
<tr>
<th>Objective</th>
<th>Screening travelers</th>
<th>Travel restriction</th>
<th>Border closure</th>
</tr>
</thead>
</table>
| Delaying introduction of case       | • Likely delay by 4 d with detection rate of 37% travelers identified from the port of entry at the border (10)*  
• Associated with mean additional delay of case importation (7–12 d, 95% CI 0–30 days, 2009 H1N1 pandemic) (11)*  
• Might delay 3 d reaching 20 infected cases at risk-country ($R_0 = 1.5$ with 400 travelers/day) (12)  
• Might delay importation of infected case-patientss (21–1555 d, 2009 H1N1 pandemic) (13) | • The mean time delays for exporting the infected case is 5.3 d (80% restriction), 11.7 d (90%), and 131.7 d (99%) ($R_0 = 1.8$ with implementation of 20 d from first case occurred) (20)*  
• Among 17 Pacific Island countries and territories, with 99% restriction, 6 countries (with $R_0 = 1.5$) and 4–5 countries (with $R_0 > 2.25$) would likely escape the pandemic influenza with >90% probability (implemented at very beginning of pandemic) (21)  
• Full children-selective travel restriction might delay an epidemic by 19–35 d ($R_0 = 1.2$), and less than 15 d ($R_0 = 1.6$ and 2.0, implemented after pandemic declared) (22)  
• Mean delay of the first imported case in influenza-unaffected countries was estimated <3 d (40% restriction), and ≥2 weeks (90% restriction) with $R_0 = 1.7$ and implementation after pandemic declared (23)  
• Likely delay interval between first global case and the importation of the first cases by 7–37 d ($R_0 = 1.4$, 1.7, or ≥2: 90% or 99% restriction; implemented 30 d after first global case occurrence) (24)  
• Might delay the first passage time of infected case-patient from 18 d to 31 d (outbreak originated from Hong Kong) and from 7 d to 27 d (from Sydney) with $R_0 = 1.7$ (25)  
• A 99% restriction of air-only, both air and land, and all modes of transportation might delay the interval between the first imported case and 100 infected case-patients passed the border by a week, 1–2 weeks, and 2 mo, respectively ($R_0 = 1.4$; implemented on the day after the first global case reported) (26) | • Arrival of influenza pandemic was significantly delayed and reduced compare with the other Pacific Island Jurisdictions (29)* |
| Delaying the epidemic peak          | • Not available                                                                      | • Imported infections might delay the epidemic peak of the United States by 1.5 wks (90% restriction), 3 wks (99%), or 6 wks (99.9%) with $R_0 = 1.4–2.0$ (implemented 30 d into global pandemic) (19)  
• Might delay pandemic peak by 6–39 d ($R_0 = 1.4$, 1.7, or ≥2: 90% or 99% restriction; implemented 30 d after first global case occurrence) (24)  
• Might delay epidemic peak by 2 wks (99% air travel restriction), 3.5 wks (99% air and land travel restriction), and 12 wks (99% all mode of transportation) with $R_0 = 1.4$ (26)  
• Might delay median epidemic peak by 7–102 d ($R_0 = 1.8–5$; 50%–99.9% restriction) (27)  
• Peak of influenza mortality delayed by 2 wks (27% international flight volume reduction) (28) | • Not available |
| Reducing the size of the peak       | • Not available                                                                      | • Not available                                                                      | • Not available |

*Epidemiology study.
Because the volume of transportation is associated with the spread of influenza (28,30), travel restrictions have been considered as a measure to reduce international spread (31). Although previous expert survey and reviews suggested that travel restrictions are less likely to be effective (8,9,32), international travel restrictions are still included in some national pandemic plans (33). Several of the studies we reviewed (19,20,22-28) predicted that international travel restrictions might delay the importation of new infected persons from other affected areas, slow the international spread of the epidemic, and delay the epidemic peak (25). However, simulation studies estimated that travel restrictions after 5 months of the international arrival of the first infected persons would not be effective (26) and that only strict travel restrictions was likely to be effective (19); thus, the time of implementation of this measure should be considered with strict travel restrictions at the early stage of a pandemic. Some barriers exist to implementation of travel restrictions against pandemic influenza, most notably the potential economic consequences of restricting business travelers, as well as legal and ethical issues regarding mobility restrictions (34), discrimination of persons from influenza-affected area (35), and lack of public compliance.

Border Closures
One study investigated the effectiveness of border closures in 11 South Pacific Island jurisdictions during the 1918–19 influenza pandemic. We identified 4 islands where strict border control, including 5–7 days of maritime quarantine, substantially delayed the importation of influenza from 3 to 30 months and reduced the mortality rate compared with the other islands that had not implemented border control (36).

Because travel can drive cross-border transmission of infectious diseases, complete border closure could in theory prevent or delay the spread of influenza or its introduction in previously unaffected countries (21,36). However, in practice, complete border closure is likely to be unfeasible, even on isolated islands, because of the need to import food and medical supplies (21), and would result in substantial economic and social disruption (34).

Discussion
We reviewed the effectiveness of each international travel–related NPI and the barriers to its implementation to provide scientific evidence to public health authorities. Our review found that the effect of screening travelers on entry to a country or region is very limited and unlikely to be a rational use of resources. However, this intervention has a potential role to inform travelers about the risk for infection and provide travel advice on avoiding travel to certain regions after departure or how to seek treatment after arrival (16). Furthermore, such screening can be seen by policy makers and politicians as a visible public health measure to help assure the public that action is being taken (16).

Our review identified the potential threat of economic consequences as a major barrier to implementation of travel restrictions. A simulation study demonstrated that children-selective travel restriction during a pandemic is less likely to affect economic impact compared with nonselective travel restrictions (22). A more structured epidemiologic study is needed to examine the cost and benefit of travel restriction by different risk groups of influenza transmission. A previous study demonstrated that successful border closure for 6 months in an island country provided a net societal benefit of USD 7.3 billion (36). However, this extreme measure is unlikely to be implemented unless required by national law in extraordinary circumstances during a very severe pandemic. The literature on border closure included in our review was based on the historical scenario of the 1918–19 influenza pandemic in isolated islands; this research might have limited relevance given the current and ever increasing levels of globalization.

Although international travel–related NPIs are not likely to be able to prevent importation of pandemic influenza to a country or region, NPIs implemented at the early phase might delay the start of a local epidemic by a few days or weeks (37), which is important if such delay can contribute to reducing the effect of the epidemic (e.g., by buying time to prepare healthcare providers and the public before the arrival of the epidemic, to plan and coordinate social distancing measures, and to purchase additional pharmaceuticals such as antiviral drugs or vaccines) (38). Once an epidemic has started, travel restrictions might also be used to delay the peak of the epidemic in an isolated location where heavy seeding by incoming infected persons could accelerate local transmission. International Health Regulations could play a role in decisions on whether to implement certain international measures (39).

We identified several knowledge gaps that could be filled by further research. Most fundamentally, information is still lacking on some aspects of the basic epidemiology of influenza, including the dynamics of person-to-person transmission (e.g., Can a person be infectious before the onset of symptoms? Can transmission occur from an asymptomatic or
pauci-symptomatic case-patient? What fraction of infections are asymptomatic?). In terms of specific research on the effectiveness of travel-related NPIs, it is difficult to envisage how intervention studies could be done, but epidemiologic studies could be planned in advance of influenza pandemics or perhaps severe influenza epidemics. Studies could answer questions such as how many infections are imported from overseas or whether travel advisories might encourage infected persons not to travel.

Our review needs to be interpreted in light of some limitations. First, although international travel or trade of infected animals might have a role in the international spread of influenza, the study that assessed the movement restriction of animals was not included in this review. Second, mathematical models are useful tools for investigating the advantages and disadvantages of different interventions, but the results often depend on key modeling assumptions that are difficult to verify (19). The assessment of the quality of evidence was considered weak overall, given that most of the epidemiologic studies included in our review were ecologic studies. Third, only a few studies on the ethical and economic considerations regarding travel-related measures during influenza epidemics and pandemics were available (26,40).

Many countries continue to update their influenza pandemic plans on the basis of the latest available evidence. We found that international travel-related NPIs could delay the introduction of influenza and delay the start of local transmission; however, limited evidence exists to inform the use of these NPIs for controlling pandemic influenza. The evidence that we identified in our review does not support entry screening as an efficient or effective measure, and travel restrictions and border closures are likely to be too disruptive to consider. Additional prospective research on the effectiveness of travel-related NPIs would be valuable to support evidence-based decisions for future influenza pandemics.

This work was conducted in preparation for the development of guidelines by the World Health Organization on the use of nonpharmaceutical interventions for pandemic influenza in nonmedical settings and was financially supported by the World Health Organization.

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Influenza pandemics occur at irregular intervals when new strains of influenza A virus spread in humans (1). Influenza pandemics cause considerable health and social impact that exceeds that of typical seasonal ( interpandemic) influenza epidemics. One of the characteristics of influenza pandemics is the high incidence of infections in all age groups because of the lack of population immunity. Although influenza vaccines are the cornerstone of seasonal influenza control, specific vaccines for a novel pandemic strain are not expected to be available for the first 5–6 months of the next pandemic. Antiviral drugs will be available in some locations to treat more severe infections but are unlikely to be available in the quantities that might be required to control transmission in the general community. Thus, efforts to control the next pandemic will rely largely on nonpharmaceutical interventions.

Most influenza virus infections cause mild and self-limiting disease; only a small fraction of case-patients require hospitalization. Therefore, influenza virus infections spread mainly in the community. Influenza virus is believed to be transmitted predominantly by respiratory droplets, but the size distribution of particles responsible for transmission remains unclear, and in particular, there is a lack of consensus on the role of fine particle aerosols in transmission (2,3). In healthcare settings, droplet precautions are recommended in addition to standard precautions for healthcare personnel when interacting with influenza patients and for all visitors during influenza seasons (4). Outside healthcare settings, hand hygiene is recommended in most national pandemic plans (5), and medical face masks were a common sight during the influenza pandemic in 2009. Hand hygiene has been proven to prevent many infectious diseases and might be considered a major component in influenza pandemic plans, whether or not it has proven effectiveness against influenza virus transmission, specifically because of its potential to reduce other infections and thereby reduce pressure on healthcare services.

In this article, we review the evidence base for personal protective measures and environmental hygiene measures, and specifically the evidence for the effectiveness of these measures in reducing transmission of laboratory-confirmed influenza in the community. We also discuss the implications of the evidence base for inclusion of these measures in pandemic plans.
Methods and Results
We conducted systematic reviews to evaluate the effectiveness of personal protective measures on influenza virus transmission, including hand hygiene, respiratory etiquette, and face masks, and a systematic review of surface and object cleaning as an environmental measure (Table 1). We searched 4 databases (Medline, PubMed, EMBASE, and CENTRAL) for literature in all languages. We aimed to identify randomized controlled trials (RCTs) of each measure for laboratory-confirmed influenza outcomes for each of the measures because RCTs provide the highest quality of evidence. For respiratory etiquette and surface and object cleaning, because of a lack of RCTs for laboratory-confirmed influenza, we also searched for RCTs reporting effects of these interventions on influenza-like illness (ILI) and respiratory illness outcomes and then for observational studies on laboratory-confirmed influenza, ILI, and respiratory illness outcomes. For each review, 2 authors (E.Y.C.S. and J.X.) screened titles and abstracts and reviewed full texts independently.

We performed meta-analysis for hand hygiene and face mask interventions and estimated the effect of these measures on laboratory-confirmed influenza prevention by risk ratios (RRs). We used a fixed-effects model to estimate the overall effect in a pooled analysis or subgroup analysis. No overall effect would be generated if there was considerable heterogeneity on the basis of $I^2$ statistic $\geq 75\%$ (6). We performed quality assessment of evidence on hand hygiene and face mask interventions by using the GRADE (Grading of Recommendations Assessment, Development and Evaluation) approach (7). We provide additional details of the search strategies, selection of articles, summaries of the selected articles, and quality assessment (Appendix, https://wwwnc.cdc.gov/EID/article/26/5/19-0994-App1.pdf).

Personal Protective Measures

Hand Hygiene
We identified a recent systematic review by Wong et al. on RCTs designed to assess the efficacy of hand hygiene interventions against transmission of laboratory-confirmed influenza (8). We used this review as a starting point and then searched for additional literature published after 2013; we found 3 additional eligible articles published during the search period of January 1, 2013–August 13, 2018. In total, we identified 12 articles (9–20), of which 3 articles were from the updated search and 9 articles from Wong et al. (8). Two articles relied on the same underlying dataset (16,19); therefore, we counted these 2 articles as 1 study, which resulted in 11 RCTs. We further selected 10 studies with $>10,000$ participants for inclusion in the meta-analysis (Figure 1). We excluded 1 study from the meta-analysis because it provided estimates of infection risks only at the household level, not the individual level (20). We did not generate an overall pooled effect of hand hygiene only or of hand hygiene with or without face mask because of high heterogeneity in individual estimates ($I^2$ 87 and 82%, respectively). The effect of hand hygiene combined with face masks on laboratory-confirmed influenza was not statistically significant (RR 0.91, 95% CI 0.73–1.13; $I^2$ = 35%, $p = 0.39$). Some studies reported being underpowered because of limited sample size, and low adherence to hand hygiene interventions was observed in some studies.

We further analyzed the effect of hand hygiene by setting because transmission routes might vary...
in different settings. We found 6 studies in household settings examining the effect of hand hygiene with or without face masks, but the overall pooled effect was not statistically significant (RR 1.05, 95% CI 0.86–1.27; $I^2 = 57\%$, $p = 0.65$) (Appendix Figure 4) (11–15,17). The findings of 2 studies in school settings were different (Appendix Figure 5). A study conducted in the United States (16) showed no major effect of hand hygiene, whereas a study in Egypt (18) reported that hand hygiene reduced the risk for influenza by >50%. A pooled analysis of 2 studies in university residential halls reported a marginally significant protective effect of a combination of hand hygiene plus face masks worn by all residents (RR 0.48, 95% CI 0.21–1.08; $I^2 = 0\%$, $p = 0.08$) (Appendix Figure 6) (9,10).

In support of hand hygiene as an effective measure, experimental studies have reported that influenza virus could survive on human hands for a short time and could transmit between hands and contaminated surfaces (2,21). Some field studies reported that influenza A(H1N1)pdm09 and influenza A(H3N2) virus RNA and viable influenza virus could be detected on the hands of persons with laboratory-confirmed influenza (22,23), supporting the potential of direct and indirect contact transmission to play a role in the spread of influenza. Other experimental studies also demonstrated that hand hygiene could reduce or remove infectious influenza virus from human hands (24,25). However, results from our meta-analysis on RCTs did not provide evidence to support a protective effect of hand hygiene against transmission of laboratory-confirmed influenza. One study did report a major effect, but in this trial of hand hygiene in schools in Egypt, running water had to be installed and soap and hand-drying

### Table 1. Meta-analysis of risk ratios for the effect of hand hygiene with or without face mask use on laboratory-confirmed influenza from 10 randomized controlled trials with >11,000 participants.

<table>
<thead>
<tr>
<th>Author (reference)</th>
<th>Hand hygiene Events Total</th>
<th>Control Events Total</th>
<th>Weight</th>
<th>Risk ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cowling et al. 2008 (12)</td>
<td>5 84</td>
<td>12 205</td>
<td>1.5%</td>
<td>1.02 (0.37–2.80)</td>
</tr>
<tr>
<td>Cowling et al. 2009 (11)</td>
<td>14 257</td>
<td>28 279</td>
<td>5.9%</td>
<td>0.54 (0.29–1.01)</td>
</tr>
<tr>
<td>Larson et al. 2010 (13)</td>
<td>29 946</td>
<td>24 904</td>
<td>5.4%</td>
<td>1.15 (0.68–1.97)</td>
</tr>
<tr>
<td>Ram et al. 2015 (14)</td>
<td>17 177</td>
<td>10 250</td>
<td>1.8%</td>
<td>2.40 (1.13–5.12)</td>
</tr>
<tr>
<td>Simmerman et al. 2011 (15)</td>
<td>66 292</td>
<td>58 302</td>
<td>12.6%</td>
<td>1.18 (0.86–1.61)</td>
</tr>
<tr>
<td>Stebbins et al. 2011 (16)</td>
<td>51 1695</td>
<td>53 1665</td>
<td>11.8%</td>
<td>0.95 (0.65–1.38)</td>
</tr>
<tr>
<td>Talaat et al. 2011 (18)</td>
<td>125 808</td>
<td>281 848</td>
<td>80.8%</td>
<td>0.47 (0.39–0.56)</td>
</tr>
</tbody>
</table>

Heterogeneity: $I^2 = 87\%$, $r^2 = 0.2837$, $p<0.01$
material had to be introduced into the intervention schools as part of the project (18). Therefore, the impact of hand hygiene might also be a reflection of the introduction of soap and running water into primary schools in a lower-income setting. If one considers all of the evidence from RCTs together, it is useful to note that some studies might have underestimated the true effect of hand hygiene because of the complexity of implementing these intervention studies. For instance, the control group would not typically have zero knowledge or use of hand hygiene, and the intervention group might not adhere to optimal hand hygiene practices (11,13,15).

Hand hygiene is also effective in preventing other infectious diseases, including diarrheal diseases and some respiratory diseases (8,26). The need for hand hygiene in disease prevention is well recognized among most communities. Hand hygiene has been accepted as a personal protective measure in >50% of national preparedness plans for pandemic influenza (5). Hand hygiene practice is commonly performed with soap and water, alcohol-based hand rub, or other waterless hand disinfectants, all of which are easily accessible, available, affordable, and well accepted in most communities. However, resource limitations in some areas are a concern when clean running water or alcohol-based hand rub are not available. There are few adverse effects of hand hygiene except for skin irritation caused by some hand hygiene products (27). However, because of certain social or religious practices, alcohol-based hand sanitizers might not be permitted in some locations (28). Compliance with proper hand hygiene practice tends to be low because habitual behaviors are difficult to change (29). Therefore, hand hygiene promotion programs are needed to advocate and encourage proper and effective hand hygiene.

Respiratory Etiquette
Respiratory etiquette is defined as covering the nose and mouth with a tissue or a mask (but not a hand) when coughing or sneezing, followed by proper disposal of used tissues, and proper hand hygiene after contact with respiratory secretions (30). Other descriptions of this measure have included turning the head and covering the mouth when coughing and coughing or sneezing into a sleeve or elbow, rather than a hand. The rationale for not coughing into hands is to prevent subsequent contamination of other surfaces or objects (31). We conducted a search on November 6, 2018, and identified literature that was available in the databases during 1946–November 5, 2018. We did not identify any published research on the effectiveness of respiratory etiquette in reducing the risk for laboratory-confirmed influenza or ILI. One observational study reported a similar incidence rate of self-reported respiratory illness (defined by >1 symptoms: cough, congestion, sore throat, sneezing, or breathing problems) among US pilgrims with or without practicing respiratory etiquette during the Hajj (32). The authors did not specify the type of respiratory etiquette used by participants in the study. A laboratory-based study reported that common respiratory etiquette, including covering the mouth by hands, tissue, or sleeve/arm, was fairly ineffective in blocking the release and dispersion of droplets into the surrounding environment on the basis of measurement of emitted droplets with a laser diffraction system (31).

Respiratory etiquette is often listed as a preventive measure for respiratory infections. However, there is a lack of scientific evidence to support this measure. Whether respiratory etiquette is an effective nonpharmaceutical intervention in preventing influenza virus transmission remains questionable, and worthy of further research.

Face Masks
In our systematic review, we identified 10 RCTs that reported estimates of the effectiveness of face masks in reducing laboratory-confirmed influenza virus infections in the community from literature published during 1946–July 27, 2018. In pooled analysis, we found no significant reduction in influenza transmission with the use of face masks (RR 0.78, 95% CI 0.51–1.20; P = 30%, p = 0.25) (Figure 2). One study evaluated the use of masks among pilgrims from Australia during the Hajj pilgrimage and reported no major difference in the risk for laboratory-confirmed influenza virus infection in the control or mask group (33). Two studies in university settings assessed the effectiveness of face masks for primary protection by monitoring the incidence of laboratory-confirmed influenza among student hall residents for 5 months (9,10). The overall reduction in ILI or laboratory-confirmed influenza cases in the face mask group was not significant in either studies (9,10). Study designs in the 7 household studies were slightly different: 1 study provided face masks and P2 respirators for household contacts only (34), another study evaluated face mask use as a source control for infected persons only (35), and the remaining studies provided masks for the infected persons as well as their close contacts (11–13,15,17). None of the household studies reported a significant reduction in secondary laboratory-confirmed influenza virus infections in the face
Pandemic Influenza—Personal Protective Measures

Disposable medical masks (also known as surgical masks) are loose-fitting devices that were designed to be worn by medical personnel to protect accidental contamination of patient wounds, and to protect the wearer against splashes or sprays of bodily fluids (36). There is limited evidence for their effectiveness in preventing influenza virus transmission either when worn by the infected person for source control or when worn by uninfected persons to reduce exposure. Our systematic review found no significant effect of face masks on transmission of laboratory-confirmed influenza.

We did not consider the use of respirators in the community. Respirators are tight-fitting masks that can protect the wearer from fine particles (37) and should provide better protection against influenza virus exposures when properly worn because of higher filtration efficiency. However, respirators, such as N95 and P2 masks, work best when they are fit-tested, and these masks will be in limited supply during the next pandemic. These specialist devices should be reserved for use in healthcare settings or in special subpopulations such as immunocompromised persons in the community, first responders, and those performing other critical community functions, as supplies permit.

In lower-income settings, it is more likely that reusable cloth masks will be used rather than cost-effective medical masks.
disposable medical masks because of cost and availability (38). There are still few uncertainties in the practice of face mask use, such as who should wear the mask and how long it should be used for. In theory, transmission should be reduced the most if both infected members and other contacts wear masks, but compliance in uninfected close contacts could be a problem (12,34). Proper use of face masks is essential because improper use might increase the risk for transmission (39). Thus, education on the proper use and disposal of used face masks, including hand hygiene, is also needed.

Environmental Measures

Surface and Object Cleaning
For the search period from 1946 through October 14, 2018, we identified 2 RCTs and 1 observational study about surface and object cleaning measures for inclusion in our systematic review (40–42). One RCT conducted in day care nurseries found that biweekly cleaning and disinfection of toys and linen reduced the detection of multiple viruses, including adenovirus, rhinovirus, and respiratory syncytial virus in the environment, but this intervention was not significant in reducing detection of influenza virus, and it had no major protective effect on acute respiratory illness (41). Another RCT found that hand hygiene with hand sanitizer together with surface disinfection reduced absenteeism related to gastrointestinal illness in elementary schools, but there was no major reduction in absenteeism related to respiratory illness (42). A cross-sectional study found that passive contact with bleach was associated with a major increase in self-reported influenza (40).

Given that influenza virus can survive on some surfaces for prolonged periods (43), and that cleaning or disinfection procedures can effectively reduce or inactivate influenza virus from surfaces and objects in experimental studies (44), there is a theoretical basis to believe that environmental cleaning could reduce influenza transmission. As an illustration of this proposal, a modeling study estimated that cleaning of extensively touched surfaces could reduce influenza A infection by 2% (45). However, most studies of influenza virus in the environment are based on detection of virus RNA by PCR, and few studies reported detection of viable virus.

Although we found no evidence that surface and object cleaning could reduce influenza transmission, this measure does have an established impact on prevention of other infectious diseases (42). It should be feasible to implement this measure in most settings, subject to the availability of water and cleaning products. Although irritation caused by cleaning products is limited, safety remains a concern because some cleaning products can be toxic or cause allergies (40).

Discussion
In this review, we did not find evidence to support a protective effect of personal protective measures or environmental measures in reducing influenza transmission. Although these measures have mechanistic support based on our knowledge of how influenza is transmitted from person to person, randomized trials of hand hygiene and face masks have not demonstrated protection against laboratory-confirmed influenza, with 1 exception (18). We identified only 2 RCTs on environmental cleaning and no RCTs on cough etiquette.

Hand hygiene is a widely used intervention and has been shown to effectively reduce the transmission of gastrointestinal infections and respiratory infections (26). However, in our systematic review, updating the findings of Wong et al. (8), we did not find evidence of a major effect of hand hygiene on laboratory-confirmed influenza virus transmission (Figure 1). Nevertheless, hand hygiene might be included in influenza pandemic plans as part of general hygiene and infection prevention.

We did not find evidence that surgical-type face masks are effective in reducing laboratory-confirmed influenza transmission, either when worn by infected persons (source control) or by persons in the general community to reduce their susceptibility (Figure 2). However, as with hand hygiene, face masks might be able to reduce the transmission of other infections and therefore have value in an influenza pandemic when healthcare resources are stretched.

It is essential to note that the mechanisms of person-to-person transmission in the community have not been fully determined. Controversy remains over the role of transmission through fine-particle aerosols (3,46). Transmission by indirect contact requires transfer of viable virus from respiratory mucosa onto hands and other surfaces, survival on those surfaces, and successful inoculation into the respiratory mucosa of another person. All of these components of the transmission route have not been studied extensively. The impact of environmental factors, such as temperature and humidity, on influenza transmission is also uncertain (47). These uncertainties over basic transmission modes and mechanisms hinder the optimization of control measures.
Table 2. Knowledge gaps for personal protective and environmental nonpharmaceutical interventions for pandemic influenza*

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Knowledge gaps</th>
<th>Suggested studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hand hygiene</td>
<td>There are major gaps in our knowledge of the mechanisms of person-to-person</td>
<td>Additional high-quality RCTs of efficacy of hand hygiene against laboratory-confirmed influenza in other nonhealthcare settings, except households and university residential halls, would be valuable. In particular, studies in school settings are needed to solve the discrepancy between the two studies from the United States and Egypt.</td>
</tr>
<tr>
<td></td>
<td>transmission of influenza, including the role of direct and indirect contact,</td>
<td></td>
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<tr>
<td></td>
<td>the degree of viral contamination on hands and various types of surfaces in</td>
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<tr>
<td></td>
<td>different settings, and the potential for contact transmission to occur in</td>
<td></td>
</tr>
<tr>
<td></td>
<td>different locations and under different environmental conditions. There is</td>
<td></td>
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<tr>
<td></td>
<td>little information on whether greater reductions in transmission could be</td>
<td></td>
</tr>
<tr>
<td></td>
<td>possible with combinations of personal intervention (e.g., isolation away</td>
<td></td>
</tr>
<tr>
<td></td>
<td>from family members as much as possible, plus using face masks and enhancing</td>
<td></td>
</tr>
<tr>
<td></td>
<td>hand hygiene).</td>
<td></td>
</tr>
<tr>
<td>Respiratory etiquette</td>
<td>There is no evidence about the quantitative effectiveness of respiratory</td>
<td>RCTs of interventions to demonstrate the effectiveness of respiratory etiquette</td>
</tr>
<tr>
<td></td>
<td>etiquette against influenza virus.</td>
<td>in reducing influenza transmission would be valuable.</td>
</tr>
<tr>
<td>Face mask</td>
<td>There are major gaps in our knowledge of the mechanisms of person-to-person</td>
<td>Additional high-quality RCTs of efficacy of face masks against laboratory-confirmed influenza would be valuable. Effectiveness of face masks or respirator use to prevent influenza prevention in special subpopulation, such as immunocompromised persons, would be valuable.</td>
</tr>
<tr>
<td></td>
<td>transmission of influenza, including the importance of transmission through</td>
<td></td>
</tr>
<tr>
<td></td>
<td>droplets of different sizes including small particle aerosols, and the</td>
<td></td>
</tr>
<tr>
<td></td>
<td>potential for droplet and aerosol transmission to occur in different locations</td>
<td></td>
</tr>
<tr>
<td></td>
<td>and with environmental conditions.</td>
<td></td>
</tr>
<tr>
<td>Surface and object cleaning</td>
<td>The effectiveness of different cleaning products in preventing influenza</td>
<td>RCTs of interventions to demonstrate the effectiveness of surface and object</td>
</tr>
<tr>
<td></td>
<td>transmission—in terms of cleaning frequency, cleaning dosage, cleaning</td>
<td>cleaning in reducing influenza transmission would be valuable. Studies that can demonstrate the reduction of environmental detection of influenza virus through cleaning of surfaces and objects would also be valuable.</td>
</tr>
<tr>
<td></td>
<td>time point, and cleaning targeted surface and object material—remains unknown.</td>
<td></td>
</tr>
</tbody>
</table>

*RCT, randomized control trial.

In this review, we focused on 3 personal protective measures and 1 environmental measure. Other potential environmental measures include humidification in dry environments (48), increasing ventilation (49), and use of upper-room UV light (50), but there is limited evidence to support these measures. Further investigations on the effectiveness of respiratory etiquette and surface cleaning through conducting RCTs would be helpful to provide evidence with higher quality; evaluation of the effectiveness of these measures targeting specific population groups, such as immunocompromised persons, would also be beneficial (Table 2). Future cost-effectiveness evaluations could provide more support for the potential use of these measures. Further research on transmission modes and alternative interventions to reduce influenza transmission would be valuable in improving pandemic preparedness. Finally, although our review focused on nonpharmaceutical measures to be taken during influenza pandemics, the findings could also apply to severe seasonal influenza epidemics. Evidence from RCTs of hand hygiene or face masks did not support a substantial effect on transmission of laboratory-confirmed influenza, and limited evidence was available on other environmental measures.

This study was conducted in preparation for the development of guidelines by the World Health Organization on the use of nonpharmaceutical interventions for pandemic influenza in nonmedical settings.

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References


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Experiences from previous influenza pandemics, in particular the 2009–10 pandemic, have demonstrated that we cannot expect to contain geographically the next influenza pandemic in the location it emerges, nor can we expect to prevent international spread of infection for more than a short period. Vaccines are not expected to be available during the early stage of the next pandemic (1), and stockpiles of antiviral drugs will be limited, mostly reserved for treating more severe illnesses and for patients at higher risk for influenza complications. Therefore, nonpharmaceutical interventions (NPIs), such as social distancing (2), will be heavily relied on by health authorities to slow influenza transmission in the community, with 3 desired outcomes (Figure). The first outcome would be to delay the timing of the peak of infections to buy time for preparations in the healthcare system, the second to reduce the size of the epidemic peak so that the healthcare system is not overwhelmed, and the third to spread infections over a longer time period, enabling better management of those cases and the potential for vaccines to be used at least later in the epidemic to reduce impact.

Influenza virus infections are believed to spread mainly through close contact in the community (e.g., homes, workplaces, preschool and day care centers, schools, public places), and more frequent and intense contact among children has a particularly major role in transmission (5). Social distancing measures aim to reduce the frequency of contact and increase physical distance between persons, thereby reducing the risks of person-to-person transmission. These measures have played a role in mitigating previous pandemics, including the 1918–19 pandemic (6,7), and are a key part of current pandemic preparedness plans (3,4). Although a clear biological and epidemiologic rationale supports the potential effectiveness of social distancing measures, there are few opportunities for rigorous controlled trials of community interventions against influenza. Our objective was to review the evidence base for social distancing measures, focusing on the evidence supporting the effectiveness of these measures in reducing influenza transmission in the community.

Methods and Results
We conducted separate systematic reviews to gather available evidence on the effectiveness of 6 measures in reducing influenza transmission in the community: isolating ill persons; contact tracing; quarantining exposed persons; school dismissals or closures; workplace measures, including workplace closures; and

Nonpharmaceutical Measures for Pandemic Influenza in Nonhealthcare Settings—Social Distancing Measures

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1These first authors contributed equally to this article.
avoiding crowding (Table 1). We retrieved literature from the Cochrane Library, Embase, Medline, and PubMed. Two authors (M.W.F. and H.G.) reviewed the retrieved literature independently for inclusion and synthesis of evidence, and a third author (J.Y.W.) resolved any discrepancies. We were unable to identify randomized controlled trials for the listed social distancing measures. Therefore, we included observational studies (contemporary as well as analysis of archival data from the 1918 pandemic) and simulation studies. We gave greater weight to observational studies than to simulation studies when we inferred the effectiveness of each measure, because assumptions and parameters in simulation studies are more difficult to assess and validate.

**Isolating Ill Persons**

We focused on the measure of isolating ill persons at home, but not in medical facilities, because it is unlikely that medical facilities would have the capacity for isolating persons with mild symptoms beyond the early stages of the next pandemic. We reviewed 4 observational studies (6,8–10) and 11 simulation studies (Appendix Tables 3, 4, https://wwwnc.cdc.gov/EID/article/26/5/19-0995-App1.pdf). Outbreaks of influenza A(H1N1)pdm09 during 2009 in various settings, including a navy ship from Peru and a physical training camp in China, have provided evidence that isolating case-patients, together with other personal protective, social distancing, and environmental measures, had substantial effect on reducing attack rates of outbreaks (8,10). During the 1918–19 pandemic, excess death rates caused by pneumonia and influenza decreased in some cities in the United States after a mixture of interventions were implemented, including isolation or quarantine, school closure, banning of public gatherings, and staggered business hours (6).

Although simulation studies were conducted on the basis of a wide range of assumptions, most of these studies suggested that isolation would reduce transmission, including reducing the epidemic size and delaying the epidemic peak. However, Fraser et al. (11) discussed the difficulty in controlling influenza transmission, even with high level of isolation combined with contact tracing and quarantine, because of the potentially high proportion of influenza transmission that occurs from mild or asymptomatic infections.

Given that influenza is believed to spread from person to person mostly through close contact, there is a clear rationale for preventing contact between infectious and susceptible persons. However, we found limited scientific evidence to support the effectiveness of this intervention in the community. The observational studies included in this review were conducted in atypical settings, and the effectiveness of isolation in these settings might not be generalizable to the community-at-large. Nonetheless, with the rationale discussed, and assuming that a high level of compliance with home isolation is possible for symptomatic persons, voluntary home isolation could be a preferable strategy to prevent onward transmission compared with other personal protective measures, which have not shown effectiveness in multiple randomized controlled trials.

One area in which there is a lack of evidence is the duration of infectivity, which has implications for the period of voluntary isolation. Current recommendations include voluntary isolation until cessation of fever or until 5–7 days after illness onset (4,12). The second recommendation would be a better trigger for uncomplicated cases without concurrent conditions, benchmarking the duration of viral shedding (13). Another area of uncertainty is the...
degree to which transmission occurs before illness onset (presymptomatic transmission) and the degree to which mild or asymptomatic cases are infectious. If there is a substantial fraction of asymptomatic transmission (14), this fraction would reduce the impact of isolation.

Contact Tracing
We reviewed 4 simulation studies, all of which found contact tracing to be effective when used in combination with other interventions, including isolation, quarantine, and prophylactic treatment with antiviral drugs (11,15–17). However, Wu et al. (15) estimated that the addition of contact tracing to an existing combination of quarantine, isolation, and antiviral prophylaxis measures would only provide modest benefit, while increasing considerably the proportion of population in quarantine and the consequent costs.

Contact tracing requires substantial resources to sustain after the early phases of a pandemic because the number of case-patients and contacts grows exponentially within a short generation time. Therefore, there is no obvious rationale for the routine use of contact tracing in the general population for control of pandemic influenza. However, contact tracing might be implemented for other purposes, such as identification of case-patients in high-risk groups to enable early treatment. There are some specific circumstances in which contact tracing might be more feasible and justified, such as to enable short delay of widespread transmission in small, isolated communities, or within aircraft settings to prevent importation of cases.

Quarantine of Exposed Persons
We reviewed 1 intervention study (18), 5 observational studies (6,19–22), and 10 simulation studies (Appendix Tables 9, 10). Miyaki et al. (18) conducted an intervention study in Japan during 2009–2010 involving 2 companies. One company was used as a control; in the other company, a change was introduced in which employees could voluntarily stay at home on receiving full pay when a household member showed development of influenza-like illness (ILI) until days after the symptoms subside. The authors reported a significant reduced rate of infections among members of the intervention cluster (18). However, when comparing persons who had an ill household member in the 2 clusters, significantly more infections were reported in the intervention group, suggesting that quarantine might increase risk for infection among quarantined persons (18).

Among the observational studies, Li et al. (20) estimated that the mandatory quarantine policy in Beijing during the influenza A(H1N1)pdm09 pandemic reduced the number of cases at the peak of the epidemic by a factor of 5 compared with a projected scenario without the intervention, and also delayed the epidemic peak, albeit at high economic and social costs (20). Similar to the intervention study in Japan, van Gemert et al. (21) reported an increased risk for infection among household contacts who were concurrently quarantined with an isolated person and estimated that the risk for infection increased with a longer duration of quarantine. The evidence base from simulation studies supplemented these

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**Table 1. Summary of results for systematic review of literature on nonpharmaceutical interventions for pandemic influenza**

<table>
<thead>
<tr>
<th>Type of NPI</th>
<th>No. studies identified</th>
<th>Study designs included</th>
<th>Main findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolation</td>
<td>15</td>
<td>Observational, simulation</td>
<td>Isolation has moderate impact in reducing influenza transmission and impact.</td>
</tr>
<tr>
<td>Quarantine</td>
<td>16</td>
<td>Intervention study, observational, simulation</td>
<td>Quarantine has in general moderate impact in reducing influenza transmission and impact.</td>
</tr>
<tr>
<td>Contact tracing</td>
<td>4</td>
<td>Simulation</td>
<td>Combination of contact tracing with other measures (e.g., isolation and quarantine) can reduce influenza transmission and impact; the addition of contact tracing to existing measures might provide only modest benefit but will need substantial resources.</td>
</tr>
<tr>
<td>School closure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Planned holiday</td>
<td>28</td>
<td>Observational</td>
<td>The transmission of influenza decreases during routine school holidays but might increase after schools reopen.</td>
</tr>
<tr>
<td>Reactive closures</td>
<td>16</td>
<td>Observational</td>
<td>The effectiveness of reactive school closure varies.</td>
</tr>
<tr>
<td>Preemptive closures</td>
<td>13</td>
<td>Observational</td>
<td>Preemptive school closure has moderate impact in reducing influenza transmission.</td>
</tr>
<tr>
<td>Workplace measures</td>
<td>18</td>
<td>Intervention study, observational, simulation</td>
<td>Workplace measures are effective; combination with other interventions will further strengthen the effect.</td>
</tr>
<tr>
<td>Workplace closures</td>
<td>10</td>
<td>Simulation</td>
<td>Workplace closures might have modest impact in reducing influenza transmission.</td>
</tr>
<tr>
<td>Avoiding crowding</td>
<td>3</td>
<td>Observational</td>
<td>Timely and sustained application of measures to avoid crowding might reduce influenza transmission.</td>
</tr>
</tbody>
</table>

*Details of literature review are described in the Appendix (https://wwwnc.cdc.gov/EID/article/26/5/190995-App1.pdf)
findings, and in general, quarantine is suggested to be able to reduce transmission.

In addition, we found some observational evidence for maritime and onboard quarantine. McLeod et al. (22) analyzed archival data for the 1918–19 pandemic from the South Pacific jurisdictions and found that strict maritime quarantine delayed or prevented arrival of the pandemic, indirectly reducing the mortality rate compared with that for islands that practiced partial or no maritime quarantine. However, the applicability of these findings is uncertain because maritime travel is uncommon in the 21st century. Conversely, Fujita et al. (19) reviewed the onboard quarantine experience at Narita International Airport in Tokyo, Japan, during the influenza A(H1N1)pdm09 pandemic, and reported that the intervention detected few cases and was ineffective in preventing virus entry into the country (19).

Overall, we found that the evidence base was weak for home quarantine. In general, the intervention is estimated to be effective. However, being able to identify case-patients and their close contacts in a timely manner can be challenging during the early phase of a pandemic, and impossible for health authorities after the early phase. Quarantine also raises major ethical concerns regarding freedom of movement because the evidence on the effectiveness is limited, providing no solid rationale for the intervention, in addition to restricting movement of some uninfected and noninfected persons. The increased risks of infection among quarantined persons (18,21,23) further exacerbate the ethical concerns. Therefore, voluntary/self-quarantine is likely to be preferred over mandatory quarantine in most scenarios (24).

No evidence-based insights or discussions have addressed the optimal duration of quarantine or deactivating trigger. Theoretically, a quarantine duration of 4 days might be sufficient, covering 2 incubation periods of influenza (25). If necessary, the duration could be adjusted once the incubation period distribution of the pandemic virus strain is established. Prolonged quarantine can cause substantial burden to social services and working persons (26). Some measures can be taken to minimize the possible harms, such as pairing quarantine with antiviral prophylaxis provision for the household (23).

School Dismissals or Closures
School dismissal refers to the situation where a school campus remains open with administrative staff and teachers present but most children stay at home. Schools can then continue to provide meals for children from low-income families or look after children of essential workers. School closure is a stricter intervention in which a school campus is closed to all children and all staff. Although most of the currently available studies on the impact of school dismissals or closures on influenza transmission are presented as studies of school closures, we found that the interventions applied were in some instances school dismissals. Because it was not always possible to identify whether a scenario involved closure or dismissal, and because we expected the effects of closure and dismissal on transmission to be roughly similar, we did not distinguish between the 2 scenarios in our systematic review.

Jackson et al. (27) published a systematic review in 2013 that included 79 epidemiologic studies on school closures and found compelling evidence that school closures could reduce influenza transmission, especially among school-age children. However, the duration and the optimal timing of closure were not clear because of the heterogeneity in the available data, and transmission tended to increase when schools reopened (27). To update the evidence base presented by Jackson et al., we identified 22 additional studies published since 2013 and included 101 epidemiologic studies in total (Appendix Tables 14–17). Most of these studies were conducted in primary and secondary schools; only a few studies were conducted in universities. Overall, findings from the updated systematic review supported the conclusions by Jackson et al.

Thirteen studies investigated preemptive school closures, in which schools are closed with the aim of slowing transmission in the community (28). A correlation analysis between weekly mortality rates and interventions (which included school closure) during the 1918–19 pandemic in cities in the United States estimated that early and sustained interventions reduced mortality rates by ≤25% (29). Two studies conducted in Hong Kong as a public health response to influenza A(H1N1)pdm09 estimated that school closures, followed by planned school holidays, reduced influenza transmission (30,31).

We found 16 studies reporting the effectiveness of reactive school closures, in which individual schools or groups of schools were closed after substantial ILI outbreaks in those schools (28). Two studies conducted in Japan estimated that the peak number of cases and the cumulative number of cases were reduced by ≈24% (32) and 20% (33). However, some studies estimated that reactive school closures had no effect in reducing the total attack rate and duration of school outbreaks, and the spread of influenza (34–36).
The effect of routine school holidays in reducing influenza transmission was investigated in 28 studies. Planned school holidays were estimated to reduce influenza transmission and delay the time to epidemic peak occurrence for >1 week (37,38). In some instances, transmission resurged after schools reopened (39). It is well established that school children play a major role in spreading influenza virus because of higher person-to-person contact rates, higher susceptibility to infection, and greater infectiousness than adults (40,41). Therefore, school closures or dismissals are a common-sense intervention to suppress transmission in the community, and several observational studies have confirmed that overall transmission of influenza in the community is reduced when schools are closed. However, major caveats are noted in the literature, primarily that transmission will only be reduced when schools are closed. In some past epidemics, closing of schools after the epidemic peak showed little impact on the overall attack rate and none on the timing of the peak or the size of the epidemic peak because it has already passed (27). In other past epidemics, transmission resurges after schools reopen, so that the closures delayed the epidemic peak but might not necessarily have reduced the size of the epidemic peak or the overall attack rate (27). Although these points seem obvious, the appropriate timing and duration of school closures can be difficult to discern in the heat of an epidemic with delays in information and difficulties in interpreting surveillance data.

School closures can also have adverse impacts on ethical and social equity, particularly among vulnerable groups (e.g., low-income families), which could be ameliorated by dismissing classes, but allowing some children to attend school for free school meals or to enable parents to go to work. Extended school closures might increase domestic travel and contact rates in households and other social gatherings (e.g., malls, theaters), with the potential to increase transmission in the community. The optimum combination of timing, geographic scale, and duration of school closure might differ for the control of different epidemic/pandemic scenarios (42). A useful area for further research would be providing validated tools to enable real-time estimation of not only how an epidemic or pandemic is progressing (43), but also what the public health impact of an intervention, such as school closure, would be with alternative choices of timing and duration.

Workplace Measures and Closures

Workplace measures and closures aim to reduce influenza transmission in workplaces or during the commute to and from work. Teleworking at home, staggered shifts, and extended holidays are some common workplace measures considered for mitigating influenza pandemics. A systematic review of workplace measures by Ahmed et al. (2) concluded that there was evidence, albeit weak, to indicate that these measures could slow transmission, reduce overall attack rates or peak attack rates, and delay the epidemic peak. We updated the evidence base with 3 additional recently published studies and obtained similar results (Appendix Table 20). Paid sick leave could improve compliance with a recommendation to stay away from work while ill (44,45).

We conducted a separate search for evidence on the effectiveness of workplace closures in influenza pandemics and identified 10 studies, all of which were simulation studies (Appendix Table 21). In general, the simulation studies predicted that workplace closures would be able to reduce transmission somewhat in the community, but probably would have a smaller effect on transmission than school closures.

We found limited evidence that workplace measures and closures would be effective in reducing influenza transmission. Two recent studies not included in our systematic review have contrasting findings on the effect of having paid sick leave and taking a day off from work because of ILI (46,47). As with school closures, the timing and duration of workplace interventions would be a critical issue affecting their impact in mitigating a pandemic. This scenario is an area with rich potential for intervention studies to contribute higher quality evidence (e.g., teleworking policies or staggered shifts). However, workplace measures and closures could have considerable economic consequences, and inclusion in pandemic plans would need careful deliberations over which workplaces might be suitable for application of interventions, whether to compensate employees or companies for any loss in income or productivity, and how to avoid social inequities in lower income workers, including persons working on an ad hoc basis.

Avoiding Crowding

We reviewed 3 observational studies (6,48,49). Timely bans on public gatherings and closure of public places, including theaters and churches, were suggested to have had a positive effect on reducing the excess death rate during the 1918 pandemic in the United States (6,48). During an influenza outbreak that occurred during World Youth Day 2008, a higher attack rate was reported among a group of pilgrims accommodated in 1 large hall than in pilgrims sleeping in smaller groups (49).

The evidence for avoiding crowding is limited. The implementation of measures to avoid crowding...
might require a large amount of resources (e.g., financial and trained personnel), which might be less feasible in low-income and middle-income countries. Measures to avoid crowding might also be difficult to implement in some settings because of cultural and religious reasons (e.g., Hajj).

Discussion

Overall, our systematic reviews suggested that social distancing measures could be effective interventions to reduce transmission and mitigate the impact of an influenza pandemic. However, the evidence base for these measures was derived largely from observational studies and simulation studies; thus, the overall quality of evidence is relatively low. Natural experiments or controlled studies of single or combined interventions are needed to clarify the use of social distancing measures; improve knowledge on basic transmission dynamics of influenza, including the role of presymptomatic contagiousness and the fraction of infections that are asymptomatic (50); determine the optimal timing and duration for implementation of these measures, and school closures in particular; and provide cost-benefit assessment for implementation of these measures (Table 2).

Table 2. Knowledge gaps on social distancing measures as nonpharmaceutical interventions for pandemic influenza and suggested areas for future study

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Knowledge gaps</th>
<th>Suggested studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolation of sick persons</td>
<td>Few observational studies use laboratory-confirmed influenza as outcome and study isolation as a single intervention; most observational studies were in atypical settings; transmission dynamics of influenza: role of presymptomatic contagiousness, fraction of infections that are asymptomatic, duration of infectivity; optimal strategy for symptomatic persons, trigger to stop isolation</td>
<td>Randomized trials in community settings to evaluate the effectiveness of voluntary isolation against transmission of laboratory-confirmed influenza; epidemiologic studies to understand transmission dynamics of influenza, including symptomatic profiles and duration of infectiousness; compliance of the public with voluntary isolation at home</td>
</tr>
<tr>
<td>Contact tracing</td>
<td>Value of adding contact tracing on top of other existing interventions remain unclear; strategy for feasible implementation</td>
<td>Might not be a research priority for pandemic preparedness because of the lack of feasibility of this intervention</td>
</tr>
<tr>
<td>Quarantine of exposed persons</td>
<td>Few observational studies use laboratory-confirmed influenza as outcome and provide evidence on the effect of quarantine as a single intervention or the value quarantine adds to existing interventions; transmission dynamics of influenza: fraction of infections that are asymptomatic, possibility of superspreaders; optimal duration of quarantine</td>
<td>Randomized trials in community settings to evaluate the effectiveness of quarantine against transmission of laboratory-confirmed influenza; epidemiologic studies to understand transmission dynamics of influenza including the incubation period and the asymptomatic fraction</td>
</tr>
<tr>
<td>School closures</td>
<td>Triggers to close and reopen schools; optimal timing and duration of school closures, taking into account the possible disruptions to the public; compliance of persons of different socioeconomic status; alternative school-based measures, such as staggering lunch breaks and increasing spacing between desks: feasibility and effectiveness</td>
<td>Observational studies on optimal closure triggers and duration, taking into account the possible disturbances brought by school closures; comprehensive review of the acceptance and compliance of the interventions by different subgroups of the population; develop tools to enable real-time estimation of epidemic or pandemic growth, and the effect of implementing closures at different time points of the epidemic/pandemic; while school-based measures were not specifically covered in our systematic review, it would be useful to examine randomized trials of measures to prevent influenza transmission in schools, such as increasing spacing between desks during influenza seasons</td>
</tr>
<tr>
<td>Workplace measures and closures</td>
<td>Triggers to close and reopen workplaces; optimal timing and duration of workplace closure, taking into account the possible disruption to the public; alternative workplace measures (e.g., improving teleworking infrastructure, or providing segregated working areas for persons with mild symptoms): feasibility and effectiveness, cost-benefit</td>
<td>Randomized control trials to evaluate the effectiveness of workplace measures (e.g., telework from home, staggered shifts, weekend extension and paid-leave policies) against laboratory-confirmed influenza transmission; studies on optimal triggers, timing and duration for workplace measures and closures, taking into account the possible disruptions caused by workplace measures; cost-benefit analyses of alternative workplace measures</td>
</tr>
<tr>
<td>Avoiding crowding</td>
<td>Methods to reduce population density in different settings (e.g., transport hub, mass events, and public places); feasibility and effectiveness</td>
<td>More observational or simulation studies on the alternative methods to avoid crowding in different settings.</td>
</tr>
<tr>
<td>Combined interventions</td>
<td>Limited evidence on synergy of alternative interventions or the best combinations of interventions</td>
<td>Policy studies to identify feasible interventions that would complement each other when combined</td>
</tr>
</tbody>
</table>
Although we reviewed the evidence for each NPI individually, it is common for social distancing measures to be implemented in combination. For example, during the 1918 pandemic, multiple NPIs were implemented simultaneously in some cities in the United States, including school closures and public gathering bans (6). Although simulation studies have estimated progressively increasing effectiveness as more NPIs are added, we believe that some thought should be given to identifying interventions that would complement each other when combined. Social distancing measures such as school closures and mall closures could be implemented simultaneously to prevent an increase in social contact rates outside schools. School closures could also be paired with teleworking policies to provide opportunities for parents to take care of school-age children at home.

Despite the limitations and uncertainties, social distancing measures will be useful components of the public health response to the next pandemic. Careful consideration of these measures is required when composing pandemic plans, particularly in terms of public compliance and resource planning and distribution. Recommending that ill persons stay at home is probably the most straightforward social distancing measure, and pandemic plans should consider how to enable ill children and employees to stay at home from school or work. For example, health authorities might recommend suspending the usual requirement for doctors’ notes to support absence from school or work. Finally, although our review focused on nonpharmaceutical measures to be taken during influenza pandemics, the findings could also apply to severe seasonal influenza epidemics.

In conclusion, our review found some evidence from observational and simulation studies to support the effectiveness of social distancing measures during influenza pandemics. Timely implementation and high compliance in the community would be useful factors for the success of these interventions. Additional research on transmission dynamics, and research on the optimal timing and duration of school and workplace closures would be useful. This study was conducted in preparation for the development of guidelines by the World Health Organization on the use of nonpharmaceutical interventions for pandemic influenza in nonmedical settings. This study was supported by the World Health Organization. M.W.F. and J.X. were supported by the Collaborative Research Fund from the University Grants Committee of Hong Kong (project no. C7025-16G).

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Candidatus Rickettsia xinyangensis as Cause of Spotted Fever Group Rickettsiosis, Xinyang, China, 2015

Hao Li,1 Xiao-Mei Li,1 Juan Du, Xiao-Ai Zhang, Ning Cui, Zhen-Dong Yang, Xiao-Jia Xue, Pan-He Zhang, Wu-Chun Cao, Wei Liu

In 2015, we evaluated 221 patients with undifferentiated fever and tick bite or animal exposure in Xinyang, China, for Rickettsia infection. Three with mild disease were infected with Candidatus R. xinyangensis, which clustered with R. fournieri and R. vini in phylogenetic analyses. Field investigations suggest Haemaphysalis longicornis ticks might be involved in transmission.

Spotted fever group (SFG) rickettsiae (SFGR) are obligate intracellular bacteria of the genus Rickettsia and family Rickettsiaceae and comprise >20 species identified as human pathogens (1). Most SFGR are transmitted by ticks (1), and flea-transmitted R. felis and mite-transmitted R. akari are recognized as members of the transitional group rickettsiae (2). In China, 4 different species and 1 new genotype of SFGR have been identified in association with human diseases (3,4).

Clinical symptoms of SFG rickettsioses are often simply fever and rash, although several other features, such as eschar and lymphadenopathy, are also commonly described (1). Diverse manifestations of diseases can make their clinical diagnoses rather difficult. Moreover, with the aid of molecular techniques, many new pathogenic SFGR are being discovered globally with increasing frequency. This increased discovery calls for researchers to intensify their efforts investigating patients with undifferentiated febrile illness. Here, we report a case series of 3 patients in China infected with the same novel SFG Rickettsia.

The Study
During March–November 2015, we recruited 221 patients with undifferentiated febrile illness and history of tick bite or animal contact within the past month to a study conducted at the People’s Liberation Army 154 Hospital in Xinyang, Henan Province, China. We excluded patients with severe fever with thrombocytopenia syndrome virus infection (Appendix, https://wwwnc.cdc.gov/EID/article/26/5/17-0294-App1.pdf) and then tested for infection with SFGR.

We collected peripheral blood samples (using EDTA tubes) from patients at hospital admission and extracted DNA using the QIAmp DNA Blood Mini Kit (QIAGEN, https://www.qiagen.com). We concurrently performed nested PCRs specific for the conserved citrate synthase gene (gltA) and SFGR-restricted outer membrane protein A gene (ompA) (Appendix) (4). We then purified samples positive for amplicons and sequenced in both directions.

Three patients were found to be infected with a novel SFGR genotype with identical gltA and ompA gene sequences, which we designated Rickettsia sp. XY118. The gltA of XY118 (GenBank accession no. KU853023) had 99.6% (1,088/1,092) similarity with that of R. vini (accession no. KJ626330) and 99.6% (1,145/1,150) similarity with that of R. heilongjiangensis (accession no. CP002912) and R. fournieri (accession no. KF666471). The ompA gene sequence of XY118 (accession no. KU853021) was identical to those of undetermined Rickettsia species from ticks in China (accession no. AF169629) and Japan (accession no. AB516963) and rodents in South Korea (accession no. DQ402485). Moreover, the sequence of the ompA gene obtained in our patients had 96.1% (299/311) similarity with the corresponding gene in R. vini (accession no. KX159442) and 96.5% (335/347) similarity with that of R. fournieri (accession no. KF666477).

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1These first authors contributed equally to this work.
We collected serum samples from patients during the acute and convalescent phases of illness and tested for IgG against R. rickettsii by using an indirect immunofluorescence assay (Rickettsia IFA IgG; Focus Diagnostics, https://www.focusdx.com). Results showed that 2 patients had seroconverted and 1 had a 4-fold increased IgG titer (Appendix Table 2). In addition, we tested patients for acute infection with Anaplasma phagocytophilum, Ehrlichia chaffeensis, Borrelia burgdorferi, and Babesia microti by PCR and indirect immunofluorescence assay (5), and all blood samples were negative for both DNA of and specific IgG against these pathogens.

Two of 3 patients had reported history of tick bite, and 1 had reported animal contact (Table). All 3 patients had fever, asthenia, and anorexia. Two patients had eschar, 1 had lymphadenopathy, and none had rash. None of the 3 patients had any severe complications (i.e., hemorrhagic or neurologic signs or symptoms). Laboratory test results showed that 3 patients had elevated levels of hepatic aminotransferase, lactate dehydrogenase, and creatine kinase when admitted to the hospital (Appendix Figure 3). Clinical signs resolved and laboratory test findings were null (except for 1 patient with elevated hepatic aminotransferase levels) after 4–9 days’ hospitalization.

To identify local natural foci of SFGR, we performed a field investigation for infections among ticks captured around the 3 patients’ residences. We collected 232 host-seeking Haemaphysalis longicornis ticks and subjected each tick separately to DNA extraction with the DNeasy Blood & Tissue Kit (QIAGEN). Rickettsia sp. XY118 was detected in 2 (0.9%) ticks, and the nucleotide sequences of the gltA (GenBank accession no. KY617774) and ompA (accession no. KY617775) genes from these ticks were identical to those found in our patients. To further describe the genetic characteristics of this new genotype, we amplified the 16S rRNA gene (rrs; accession no. KY617772), 120-kDa genus common antigen gene (ompB; accession no. KY617776), PS120 protein-encoding gene (sc4; accession no. KY617777), and 17-kDa antigen gene (htrA; accession no. KY617773). The nucleotide sequence (1,320 bp) of rrs of XY118 had 99.7% (1,316/1,320) similarity with that of R. japonica (accession no. AP017602) and 99.6% (1,315/1,320) similarity with that of R. heilongjiangensis (accession no. CP002912; Appendix Figure 1). The nucleotide sequences of htrA (99.5%), gltA (99.6%), ompA (96.1%), and ompB (99.8%) from XY118 had the highest identity with the corresponding genes from R. vinii. Compared with the partial sc4 sequence of R. fournieri, the corresponding sequence of XY118 contained 5 variable base pairs sites and an 18-bp deletion (Appendix Figure 2). A phylogenetic tree that we constructed using the 2,546-bp nucleotide sequence of these 5 genes concatenated showed that Rickettsia sp. XY118, R. fournieri, and R. vinii comprise a separate cluster that appears most closely related to R. japonica and R. heilongjiangensis (Figure). According to the gene sequence–based criteria for taxonomic classification of new Rickettsia isolates (6,7), a Candidatus status could be assigned to XY118, so we named this species Candidatus Rickettsia xinyangensis.

**Conclusions**

We found a novel SFG Rickettsia in human patients and ticks in China and propose the name Candidatus R. xinyangensis for this species. Our phylogenetic analyses involving comparisons with 5 different rickettsial genes showed that this newly identified SFG Rickettsia was most closely related to R. fournieri, a strain first isolated from Argas lagenoplois ticks in Australia in 2013 (8) that has unknown pathogenicity in humans.

Our finding of Candidatus R. xinyangensis in 0.9% of H. longicornis ticks suggests a natural foci of this bacterium in Xinyang. However, extended field surveys and tick surveillance are required to understand the distribution of this agent and to identify specific tick vectors.

For Candidatus R. xinyangensis, a causal relationship between infection and clinical disease may be inferred by the serologic evidence, although only 3 patients infected with this pathogen have been reported. On the other hand, considering that isolates with identical (311-bp) SFGR-restricted ompA gene sequences have been detected in H. yeni and H. longicornis ticks.
in China (9,10), H. longicornis ticks in Japan (11), H. bispinosa ticks in Bangladesh (12), and Apodemus arvalis rodents in Korea (13), Candidatus R. xinyangensis could be a tickborne infection of immense clinical relevance in humans.

In our study, patients with Candidatus R. xinyangensis infection had similar relatively mild febrile illnesses, as well as leukopenia and elevated hepatic enzyme levels, both of which are features of other SFG rickettsioses. In contrast, unlike patients with many other SFG rickettsioses, including Rocky Mountain spotted fever, our patients had eschars, not rashes (1,14). However, the patients described in this report were few in number and from a single hospital, and the true disease presentation of Candidatus R. xinyangensis infection might be more variable. Future investigations to further assess the disease spectrum of this pathogen and its contribution to clinical cases are needed.

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Pretreatment Out-of-Pocket Expenses for Presumptive Multidrug-Resistant Tuberculosis Patients, India, 2016–2017

Priya Rathi, Kalpita Shringarpure, Bhaskaran Unnikrishnan, Vineet Kumar Chadha, Vishak Acharya, Abirami Nair, Karuna D. Sagili, Suresh Shastri

In India, the annual economic loss resulting from tuberculosis (TB) is US $3 billion (1). Those in the economically productive age group (15–54 years) account for >70% of the total burden (1). Incidence of multidrug-resistant TB (MDR TB) is higher in India than anywhere else in the world; ≈99,000 new cases of MDR TB occur in India each year (1). Treatment of MDR TB is more complex, challenging, and costly to manage than that of drug-sensitive TB (2–4). In India, MDR TB is treated free of cost through programmatic management of drug-resistant TB (PMDT) under the National Tuberculosis Elimination Programme (5). However, most patients seek healthcare from the private sector and some resort to alternative forms of medicine, often preferring self-medication and consulting quacks over visiting the PMDT center (6,7). This behavior not only results in delayed diagnosis but also increases prediagnostic expenses (7). Increased expenses accompanied with loss of wages can compel patients and their families affected by TB to borrow money, take loans, or even sell their assets, thereby accentuating any existing financial crises in the family (6–9). Hence, we estimated the direct and indirect out-of-pocket expenses incurred for diagnosis and pretreatment evaluation by presumptive MDR TB patients in Mangalore, India.

The Study
Mangalore is a coastal city in the state of Karnataka, India. The state has 6 PMDT centers. Presumptive MDR TB patients, when referred to PMDT centers, are subjected to drug sensitivity testing, preferably by use of a rapid molecular test (cartridge-based nucleic acid amplification assay), line probe assay, or culture, per PMDT guidelines (10). Those with an MDR TB diagnosis are admitted to the center for a week for pretreatment evaluation. All services provided under PMDT are free of cost to the patient (10).

We included in our study all adults (>15 years of age) with MDR TB who were registered under PMDT during August 2016–April 2017. By using a valid, pretested, semistructured tool, we interviewed patients about various costs incurred by themselves, their families, or both, from the time they became a presumptive MDR TB patient until they underwent pretreatment evaluation at PMDT. Information about various costs reported by patients was validated with bills, if available. We used the following cost categories: direct medical, direct nonmedical, indirect, and coping. Direct medical costs are expenses incurred during diagnosis and treatment of illness; direct nonmedical costs are costs of food, accommodations, and additional nutrition/supplements; indirect costs are the loss of wages because of illness;
and coping costs are the costs of coping mechanisms (assets sold, school dropouts, loans, and money borrowed) (Appendix, https://wwwnc.cdc.gov/EID/article/26/5/18-1992-App1.pdf). Of the 40 MDR TB patients, the 16 who were admitted during the study period were interviewed in person and the 24 who continued home-based treatment were interviewed by telephone (Figure 1). Ethics approval was obtained from the Institutional Ethics Committee of Kasturba Medical College, Mangalore, and the Ethics Advisory Group of The International Union against Tuberculosis and Lung Disease, Paris, France.

Data were double entered in EpiData version 3.1 software (https://www.epidata.dk) and analyzed by using SPSS Statistics 25.0 (https://www.ibm.com) and EpiData analysis 2.2.2.183 software. Direct and indirect costs were summarized as median and interquartile ranges (IQRs). Categorical variables were expressed in proportions. Costs were collected by using Indian rupees (INR) converted to United States dollars (USD) based on the 2016 conversion rate (1 USD = 66.3731 INR). To compare the costs across different countries, we first converted the reported costs (USD) from other studies to local currency for the reported year, then adjusted them for inflation year by year until 2016 (11). Then we converted the costs back to USD by using the 2016 conversion rate (Appendix).

We included 40 of the 63 registered patients in the study. Median (IQR) age of participants was 39 (29–50) years. Most patients were male (28, 70%), and most lived in rural areas (28, 70%). Median (IQR) reported patient family income was $608 ($228–$912)/year. Of the 40 patients, 39 (97%) had pulmonary MDR TB and 24 (60%) had approached the private healthcare sector for their first clinical encounter (Table 1; Figure 2). The median total pretreatment out-of-pocket expenses incurred by patients were $171 ($72–$432) total, $105 ($49–$306) direct, and $51 ($2–$306) indirect. Within direct costs, direct nonmedical costs ($51) were more than direct medical costs ($37). Of the direct nonmedical costs, most was spent on food ($35). Most of the direct medical costs were for diagnostic investigation ($18) and treatment ($15) (Table 2). The median total pretreatment out-of-pocket expense incurred by patients in our study is similar to

---

**Table 1.** Sociodemographic characteristics of 40 MDR TB patients treated at PMDT Centre, Mangalore, India, August 2016–April 2017*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>28 (70)</td>
</tr>
<tr>
<td>F</td>
<td>12 (30)</td>
</tr>
<tr>
<td><strong>Education</strong></td>
<td></td>
</tr>
<tr>
<td>Illiterate</td>
<td>5 (12.5)</td>
</tr>
<tr>
<td>Primary school</td>
<td>15 (37.5)</td>
</tr>
<tr>
<td>Secondary school</td>
<td>14 (35.0)</td>
</tr>
<tr>
<td>Graduation/professional course</td>
<td>6 (15.0)</td>
</tr>
<tr>
<td><strong>Type of occupation</strong></td>
<td></td>
</tr>
<tr>
<td>Salaried job</td>
<td>13 (32.5)</td>
</tr>
<tr>
<td>Daily wage</td>
<td>9 (22.5)</td>
</tr>
<tr>
<td>Business owner</td>
<td>5 (12.5)</td>
</tr>
<tr>
<td>Homemaker</td>
<td>8 (20.0)</td>
</tr>
<tr>
<td>Other</td>
<td>5 (12.5)</td>
</tr>
<tr>
<td><strong>Place of residence</strong></td>
<td></td>
</tr>
<tr>
<td>Urban</td>
<td>12 (30.0)</td>
</tr>
<tr>
<td>Rural</td>
<td>28 (70.0)</td>
</tr>
<tr>
<td><strong>Socioeconomic status†</strong></td>
<td></td>
</tr>
<tr>
<td>Upper class</td>
<td>6 (15.0)</td>
</tr>
<tr>
<td>Upper-middle class</td>
<td>14 (35.0)</td>
</tr>
<tr>
<td>Middle class</td>
<td>8 (20.0)</td>
</tr>
<tr>
<td>Lower-middle class</td>
<td>7 (17.5)</td>
</tr>
<tr>
<td>Lower-class</td>
<td>5 (12.5)</td>
</tr>
<tr>
<td><strong>Health facility sequence where MDR TB diagnosis made</strong></td>
<td></td>
</tr>
<tr>
<td>First</td>
<td>23 (57.5)</td>
</tr>
<tr>
<td>Second</td>
<td>14 (35.0)</td>
</tr>
<tr>
<td>Third</td>
<td>3 (7.5)</td>
</tr>
<tr>
<td>Fourth</td>
<td>0</td>
</tr>
<tr>
<td><strong>Type of healthcare facility visited by patients before PMDT</strong></td>
<td></td>
</tr>
<tr>
<td>Private</td>
<td>24 (60)</td>
</tr>
<tr>
<td>Public</td>
<td>16 (40)</td>
</tr>
</tbody>
</table>

*MDR TB, multidrug-resistant TB; PMDT, programmatic management of drug-resistant TB; TB, tuberculosis.
that found in a study in Peru ($210) after adjusting for inflation rate and cost conversion (12). The median direct out-of-pocket expenses are higher than the adjusted cost values found in previous comparable studies conducted in Ethiopia ($87), Indonesia ($47), and Peru ($67) and lower than that reported from Cambodia ($144) (12–15).

The median indirect out-of-pocket expense incurred by patients in India was $51 ($2–$306). This finding contrasts with those of studies in Ethiopia and Indonesia, where indirect pretreatment costs after adjustment for annual inflation were substantially lower (Ethiopia $9, Indonesia $8) (15).

In contrast, for patients in Ecuador, the adjusted direct out-of-pocket expenses were 5 times greater than those for patients in India ($105 vs. $549). The adjusted indirect expenses were 10 times greater ($51 vs. $578) (12) (Appendix Table).

In addition, 18 (45%) patients in the study lost their job because of the disease and had to borrow money for disease management and daily household needs before receiving accurate diagnosis and appropriate treatment. The percentages of persons with job losses were substantially lower than those reported for Peru (90%) and Ethiopia (72%) but similar to those for Indonesia (53%) (12,15).

Median coping cost incurred by patients in the study was $640 ($324–$1,360). Wingfield et al. reported a median debt of $435 and a loss of income of $2,450 before diagnosis for patients in Peru (12). In the study cohort, total median cost was $171 ($72–$432), which amounted to 28% of median total family income ($608). This expense, when combined with a coping cost of $640, resulted in a financial burden that was 1.25 times greater than the median total family income of the cohort ($608). Also, the cost of disease was $811 (sum of total median cost and median coping cost), and coping costs accounted for 79% of the total. Coping cost in a study conducted in Ecuador was as high as 7 times the average annual income (14).

In our study, no patients reported school dropouts or separation in families. None of the patients reported selling assets such as property, gold, and other valuables. A total of 27 (67.5%) of the patients, approximately two thirds, had already incurred catastrophic expenses before they were registered for MDR TB treatment.

Table 2. Median disaggregated costs incurred by 40 patients (households) from the stage of presumptive MDR TB to pre-MDR TB treatment evaluation, India, August 2016–April 2017*

<table>
<thead>
<tr>
<th>Cost category</th>
<th>Median (IQR), USD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total income</td>
<td>608.00 (228.00–912.00)</td>
</tr>
<tr>
<td>Total direct medical costs†</td>
<td>37.44 (7.10–198.24)</td>
</tr>
<tr>
<td>Total diagnosis, n = 38</td>
<td>01.58 (0.30–2.40)</td>
</tr>
<tr>
<td>Total investigation, n = 36</td>
<td>17.70 (3.19–60.27)</td>
</tr>
<tr>
<td>Total treatment, n = 26</td>
<td>15.07 (11.30–47.08)</td>
</tr>
<tr>
<td>Total admission, n = 15</td>
<td>45.20 (30.13–75.34)</td>
</tr>
<tr>
<td>Total direct nonmedical costs‡</td>
<td>51.20 (28.00–85.36)</td>
</tr>
<tr>
<td>Total food, n = 38</td>
<td>35.41 (18.08–64.97)</td>
</tr>
<tr>
<td>Total travel, n = 39</td>
<td>12.84 (5.73–12.84)</td>
</tr>
<tr>
<td>Total accommodations, n = 1</td>
<td>36.16 (36.16–36.16)</td>
</tr>
<tr>
<td>Additional nutrition, n = 38</td>
<td>01.51 (0.75–3.77)</td>
</tr>
<tr>
<td>Total direct costs§</td>
<td>105.12 (48.75–306)</td>
</tr>
<tr>
<td>Total indirect costs, n = 18¶</td>
<td>51.20 (1.60–306.00)</td>
</tr>
<tr>
<td>Total expenditures#</td>
<td>171.31 (72.00–432.00)</td>
</tr>
<tr>
<td>Total coping costs</td>
<td>640.00 (324.00–1,360)</td>
</tr>
</tbody>
</table>

*Because all patients did not incur all categories of costs, n differs for different categories. Median (IQR) is calculated only for those who incurred a given cost. IQR, interquartile range; MDR TB, multidrug-resistant TB; TB, tuberculosis; USD, US dollars.
†Direct medical costs = sum of diagnosis investigation (general investigation and disease-specific investigations), complete blood count, erythrocyte sedimentation rate, liver function testing, renal function testing, spirometry, computed tomography, magnetic resonance imaging. Disease specific cost = sputum-smear microscopy, culture, drug-susceptibility testing, radiography, drugs, and hospitalization.
‡Direct nonmedical costs = sum of food, accommodation, travel by both patient and attendant.
§Direct costs = sum of total direct medical costs and total direct nonmedical costs.
¶Indirect costs = loss of wages for patient and attendant during the visit.
#Total expenditure = sum of total direct and total indirect costs.
Conclusions

Our study appraised the costs expended by MDR TB patients from a single PMDT center. Determination of a complete estimate of costs borne by all MDR TB patients in India would require a comprehensive study conducted at the community level and inclusion of patients receiving treatment from the public and private healthcare sectors.

New strategies that systematically engage private providers are needed to reduce the cost burden surrounding diagnosis for vulnerable patients. The government of India may consider widening the spectrum of free services before patient enrollment in a government-monitored treatment program channeled through the private sector.

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About the Author

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References


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Capybara and Brush Cutter Involvement in Q Fever Outbreak in Remote Area of Amazon Rain Forest, French Guiana, 2014

Jacques-Robert Christen, Sophie Edouard, Thierry Lamour, Enguerrane Martinez, Claire Rousseau, Franck de Laval, François Catzeflis, Félix Djossou, Didier Raoult, Vincent Pommier de Santi, Loïc Epelboin

We investigated a Q fever outbreak that occurred in an isolated area of the Amazon Rain Forest in French Guiana in 2014. Capybara fecal samples were positive for *Coxiella burnetii* DNA. Being near brush cutters in use was associated with disease development. Capybaras are a putative reservoir for *C. burnetii*.

Q fever is a cosmopolitan zoonosis caused by *Coxiella burnetii*, a gram-negative coccobacillus. Transmission occurs mainly through inhalation of contaminated particles present in the environment. Cattle, sheep, and goats constitute the main reservoirs worldwide, and afterbirth from infected animals is highly contagious.

The incidence of Q fever is particularly high in French Guiana, an overseas entity of France located in the Amazon region of South America between Suriname and the state of Amapá in Brazil. Disease in French Guiana is caused by a unique genotype, *C. burnetii* multispacer sequence type 17 (MST17) (1–3). The disparities in incidence between French Guiana and its neighboring countries suggest that Q fever incidence is underestimated in that part of the world, potentially because of misdiagnosis or the unavailability of diagnostic tools (4). Cases occur mainly in Cayenne, the capital of French Guiana, and the surrounding areas (5). Outbreaks beyond the outskirts of Cayenne have not been described. Studies of multiple domestic and wild fauna in French Guiana have only revealed the 3-toed sloth (*Bradypus tridactylus*) as a potential *C. burnetii* reservoir (6–9). However, another wild animal reservoir is highly suspected for this bacterium (4), and the epidemiologic cycle in French Guiana remains incomplete. In this article, we describe an outbreak that occurred in 2014 among French Navy service members in a remote area of French Guiana and the outbreak investigation findings, which implicated another species as a potential *C. burnetii* reservoir.

The Study

During August–September 2014, a total of 5 Q fever cases were diagnosed among 12 French Navy service members. All had been deployed for 3 days in mid-August to a carbet (an open-sided wooden shelter surrounded by forest) located on the Comté River, 30 km south of Cayenne (4°37′57.44″N, 52°23′49.5″W). Q fever diagnostic tests (Q Fever IFA IgM and Q Fever IFA IgG; Focus Diagnostics, http://www.focusdx.com; 100% sensitivity and 99% specificity to *C. burnetii* Nine Mile strain) were performed at the Institut Pasteur in Cayenne.

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1These senior authors contributed equally to this article.
Patient interviews revealed that they had spent 3 days cleaning the carbet with brooms and clearing the surrounding area using a brush cutter (also known as a weed cutter or grass trimmer). No other common exposure was found. We performed a retrospective case–control study that included the 5 cases and 15 controls. Control participants had spent multiple days at the carbet during the 3-day cleanup (7 controls) or during the weeks just before or after the cleanup (8 controls) without developing any symptoms. We also administered a questionnaire to assess activities potentially associated with the *C. burnetti* exposure.

Given the unexpected location of this outbreak (i.e., in an isolated spot in the middle of the rain forest, far from Cayenne), we performed an environmental investigation for *C. burnetti* in late September. We sampled several possible sources in and around the carbet: dust from the storage area; soil from under the carbet; soil from burrows of small, nonflying mammals; mammal feces and bird and reptile droppings; and water from the sink, shower, shower water tank, and toilets (Figure 1). To sample small mammals around the carbet, we used 12 animal traps from BTT Mécanique (https://www.bttmecanique.fr), Tomahawk Live Trap (https://www.livetrap.com), and H.B. Sherman Traps, Inc. (https://www.shermantraps.com) per night for 5 nights. With all samples, we performed a quantitative real-time PCR (qPCR) targeting IS1111 as previously described (10). We confirmed all positive results (i.e., DNA samples with a cycle threshold <35) using a second qPCR targeting the IS30a repeat sequence. We used another qPCR specific for *C. burnetii* MST17 to genotype *C. burnetii* DNA–positive samples (11).

We confirmed the 5 cases of acute Q fever (in 4 men and 1 woman of median age 27 [range 20–40] years) by serology (Table 1); the attack rate was 40% (5/12). Symptoms began 12–23 days after the stay; 4 patients had elevated fever (>39°C) and pneumonia with >1 lobe involved (Table 1). Each Q fever patient received 200 mg of doxycycline daily for 3 weeks. Outcomes were favorable, and none progressed to persistent focalized Q fever. The only risk factor found in univariate analysis was being
close to the brush cutter during brush cutting (p = 0.03; Table 2).

We failed to capture any small mammals during the 5-day sampling period. All qPCR tests performed with dust, soil samples, water samples, bird and reptile droppings, and fecal samples taken from burrows (attributable to small mammals) were negative for C. burnetii DNA. However, fresh mammal fecal samples were positive for C. burnetii (cycle threshold 31) and later genotyped as MST17. These fecal samples were identified by 3 independent experts as originating from capybaras (Hydrochoerus hydrochaeris, the world’s largest rodent; Figure 2), which were often found laying a few meters from the carbet. We did not perform DNA testing to confirm species origin, but capybara feces are distinctive and easily identified by their descriptions in the literature (12,13).

To prevent further Q fever cases, the carbet was closed for 4 months. In late December 2014, eight service members were sent to clean up the grounds surrounding the carbet. Instructions were issued to wear personal protective equipment (coveralls, gloves, and an FFP [filtering face piece] 2) during the operation. Only 1 young woman did not comply with the instructions for wearing the mask because she did not use the brush cutter herself; 3 weeks later, she had Q fever pneumonia (Table 1). None of the other service members got sick.

Conclusions

In total, 5 of 6 Q fever patients had pneumonia, confirming the virulence of MST17, the sole genotype found in French Guiana (3). This Q fever outbreak occurred outside of Cayenne and suburban areas, in the Amazon Rain Forest. These results confirm wildlife exposure and a sylvatic transmission cycle for C. burnetii MST17. In this outbreak, the capybara appeared to excrete C. burnetii in its feces, which caused environmental contamination that persisted for several months; alternatively, the environment might have still been infectious months later because of recontamination. Other wild animals in the Amazon Rain Forest might also be able to excrete C. burnetii (14,15), so the sites of future outbreaks cannot be predicted. The hazard of a C. burnetii infection probably exists throughout the entire rain forest because no barriers limit animal mobility in this environment.

Overlap between the sylvatic cycle and risky human activities led to this Q fever outbreak, and the availability of diagnostic tools led to its detection. Diagnostic tool availability could explain the lack of detection elsewhere in the Amazon region. Our case-control study found that being close to the brush cutter during its operation was associated with disease development. The unfortunate incident responsible for the sixth case 4 months after the initial outbreak confirmed this association; this

<p>| Table 1. Characteristics of French Navy service members who had Q fever after visit to carbet along Comté River, Amazon Rain Forest, French Guiana, 2014–2015* |
|---|---|---|---|---|---|---|---|</p>
<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age, y/sex</th>
<th>Dates of stay at carbet</th>
<th>Date of symptom onset</th>
<th>Date of serology</th>
<th>Phase I</th>
<th>Phase II</th>
<th>Clinical presentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>21/M</td>
<td>2014 Aug 15–17</td>
<td>2014 Sep 2</td>
<td>2014 Sep 9</td>
<td>&lt;50</td>
<td>800</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>40/F</td>
<td>2014 Aug 15–17</td>
<td>2014 Aug 29</td>
<td>2014 Sep 1</td>
<td>&lt;50</td>
<td>°6,400</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>27/M</td>
<td>2014 Aug 15–17</td>
<td>2014 Aug 31</td>
<td>2014 Sep 5</td>
<td>&lt;50</td>
<td>&lt;50</td>
<td>&lt;50</td>
</tr>
<tr>
<td>4</td>
<td>20/M</td>
<td>2014 Aug 15–17</td>
<td>2014 Aug 31</td>
<td>2014 Sep 7</td>
<td>&lt;50</td>
<td>6,400</td>
<td>800</td>
</tr>
<tr>
<td>5</td>
<td>28/M</td>
<td>2014 Aug 15–17</td>
<td>2014 Sep 13</td>
<td>2014 Sep 19</td>
<td>&lt;50</td>
<td>&lt;50</td>
<td>&lt;50</td>
</tr>
<tr>
<td>6</td>
<td>29/F</td>
<td>2014 Dec 22</td>
<td>2015 Jan 14</td>
<td>2015 Jan 28</td>
<td>200</td>
<td>800</td>
<td>800</td>
</tr>
</tbody>
</table>

*We measured antibody concentrations against both antigenic variants (phase I and phase II) of Coxiella burnetii. High levels of phase II antibodies are found in acute Q fever, whereas high levels of IgG phase I antibodies are predominant in chronic Q fever.

<p>| Table 2. Univariate analysis of risk factors for acute Q fever development in outbreak near Comté River, Amazon Rain Forest, French Guiana, 2014* |
|---|---|---|---|---|---|</p>
<table>
<thead>
<tr>
<th>Exposure type</th>
<th>Cases, n = 5, no. (%)</th>
<th>Controls, n = 15, no. (%)</th>
<th>Crude OR (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Using brush cutter</td>
<td>2 (40)</td>
<td>3 (20)</td>
<td>2.52 (0.15–36.3)</td>
<td>0.56</td>
</tr>
<tr>
<td>Being close to brush cutter while in use</td>
<td>5 (100)</td>
<td>5 (33)</td>
<td>NA (1.20–NA)</td>
<td>0.03</td>
</tr>
<tr>
<td>Collecting or moving wood</td>
<td>4 (80)</td>
<td>11 (73)</td>
<td>1.43 (0.09–89.2)</td>
<td>1</td>
</tr>
<tr>
<td>Collecting freshly cut grass</td>
<td>0</td>
<td>2 (13)</td>
<td>0 (0–16.8)</td>
<td>1</td>
</tr>
<tr>
<td>Cleaning dust on furniture</td>
<td>2 (40)</td>
<td>11 (73)</td>
<td>0.26 (0.02–3.19)</td>
<td>0.29</td>
</tr>
<tr>
<td>Sweeping carbet</td>
<td>3 (60)</td>
<td>13 (87)</td>
<td>0.25 (0.01–4.79)</td>
<td>0.25</td>
</tr>
<tr>
<td>Using shower inside carbet</td>
<td>3 (60)</td>
<td>2 (17)</td>
<td>3.81 (0.31–62.5)</td>
<td>0.29</td>
</tr>
<tr>
<td>Walking around carbet</td>
<td>2 (40)</td>
<td>5 (33)</td>
<td>1.31 (0.08–16.0)</td>
<td>1</td>
</tr>
<tr>
<td>Stirring soil</td>
<td>1 (20)</td>
<td>1 (7)</td>
<td>3.24 (0.04–293)</td>
<td>0.45</td>
</tr>
<tr>
<td>Cleaning animal droppings</td>
<td>0</td>
<td>7 (47)</td>
<td>0 (0–1.81)</td>
<td>0.11</td>
</tr>
</tbody>
</table>

*NA, not applicable; OR, odds ratio.
incident demonstrated that the brush cutter, a tool commonly used for gardening in French Guiana, generated an infectious aerosol when used to cut vegetation near contaminated soil. This incident also demonstrated that wearing an FFP2 is effective protection against infection in cases of exposure to *C. burnetii* infectious aerosols. Outbreaks occur when human activities lead to aerosolization of elements in the environment contaminated during the sylvatic cycle. As shown by this report, we cannot control sylvatic cycle transmission, but protective measures implemented during risky activities can prevent infections in humans.

**Acknowledgments**
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Dr. Christen works at Laveran Military Teaching Hospital, Marseille, France, and has worked in French Guiana. His research interests include the epidemiology of tropical diseases.

**References**

**Figure 2.** Feces of capybara (*Hydrochoerus hydrochaeris*) (A) and image of capybara (B), French Guiana. The length of the middle fingernail, which is often used in the field for feces measurement, is 12 mm. Photographs by Nicolas Defaux, http://www.photographienature.com.


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Zika virus infection (ZIKV), transmitted primarily by the *Aedes aegypti* and *Ae. albopictus* mosquitoes, is a serious threat to pregnant women because of the risk of microcephaly and other birth defects in infants (1,2). In late 2015, an unprecedented ZIKV outbreak emerged in South America and spread rapidly into other parts of the Americas. Although the outbreak has subsided, and pregnant women residing in the United States were at risk for ZIKV infection primarily if they traveled to affected areas or had sexual contact with a partner who traveled to an affected area, information about the outbreak was publicized (3). In particular, travel advisories and guidance about ZIKV were published during the 2016 Zika outbreak (4–6), but the degree of awareness of ZIKV among pregnant women in the United States is unknown. We examined awareness of ZIKV, discussions about ZIKV with healthcare providers, and knowledge of ZIKV-related travel advisories during pregnancy among women who delivered a live infant during the outbreak.

We surveyed women with a recent live birth who resided in 16 US states and 1 city during the 2016 Zika outbreak. We found high awareness about the risk of Zika virus infection during pregnancy and about advisories to avoid travel to affected areas but moderate levels of discussions with healthcare providers.

**The Study**

The Pregnancy Risk Assessment Monitoring System (PRAMS) is a state-specific, population-based surveillance system implemented by the Centers for Disease Control and Prevention (CDC) and state and local health departments to collect information about experiences and behaviors before, during, and after pregnancy among women with a live birth. A stratified random sample is drawn from birth certificate records every month in each participating site. Women are surveyed by mail or telephone 2–6 months after a live birth. Data are weighted to account for the stratified sampling design and to adjust for differential nonresponse (7). We analyzed PRAMS data from 16 US states and 1 city, referred to here as sites (Alabama, Connecticut, Florida, Illinois, Maryland, Massachusetts, Missouri, New Jersey, New York, Pennsylvania, South Carolina, Tennessee, Vermont, Virginia, West Virginia, and Wisconsin, plus New York City), for women who gave birth during March 2016–February 2017.

CDC and participating sites developed supplemental questions on ZIKV in 2016 (8), which sites voluntarily included in their surveys. Once added to the survey, the questions were integrated into the regular PRAMS data collection system, including data processing and weighting, and were considered part of the annual dataset.

We calculated prevalence estimates and 95% CIs for 3 ZIKV-related outcomes during pregnancy, a subset of the information collected in the supplement: never having heard of ZIKV, talking to a healthcare provider about ZIKV, and having knowledge of ZIKV-related travel advisories during pregnancy. We examined these outcomes by maternal demographics, including age, race/ethnicity, education, marital status, source of payment for delivery, infant birth month, and state of residence.

1Members of the PRAMS Zika State Working Group are listed at the end of this article.
We used multivariable logistic regression to assess the relationships between maternal demographics and each outcome using adjusted prevalence ratios (aPRs) and 95% CIs. We adjusted models for all demographics examined, along with factors likely to influence access to healthcare and exposure to information about ZIKV. We completed our analyses using SAS version 9.4 (https://www.sas.com) and SAS-callable SUDAAN 11.0 (https://www.rti.org) software to account for PRAMS complex survey design.

Of 12,845 women sampled from the 17 sites during the study period, 8,711 (68%) women responded. Among respondents, most women were 25–34 years of age (59.7%), were non-Hispanic white (56.9%), had more than a high school education (65.1%), were married (61.3%), and reported private insurance as a source of payment for delivery (55.8%) (data not shown). Overall, 8.8% of women had never heard of ZIKV during their recent pregnancy. These women were more likely to be <35 years of age, be non-Hispanic black or of other race, have a high school education or less, be unmarried, and report Medicaid as a source of payment for delivery than women who had heard of ZIKV (Appendix, https://wwwnc.cdc.gov/EID/article/26/5/19-0727-App1.pdf).

Table 1. Frequency of talking with a healthcare provider about Zika virus and knowledge of CDC travel advisories among women who had heard of Zika virus during their pregnancy and delivered a live infant, 17 US PRAMS sites, March 2016–February 2017*

<table>
<thead>
<tr>
<th>Maternal characteristics</th>
<th>Women who had heard of Zika virus during pregnancy, n = 7,920</th>
<th>Talked with a healthcare provider about Zika virus</th>
<th>Had knowledge of CDC travel advisory</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total No.†</td>
<td>% (95% CI)‡</td>
<td>Total No.†</td>
</tr>
<tr>
<td>Age ≤24</td>
<td>801</td>
<td>58.1 (54.5–61.7)</td>
<td>600</td>
</tr>
<tr>
<td>25–34</td>
<td>2,707</td>
<td>58.2 (56.7–60.6)</td>
<td>1,663</td>
</tr>
<tr>
<td>≥35</td>
<td>993</td>
<td>60.3 (57.2–63.5)</td>
<td>622</td>
</tr>
<tr>
<td>Race/ethnicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White, non-Hispanic</td>
<td>2,314</td>
<td>59.1 (57.1–61.1)</td>
<td>1,365</td>
</tr>
<tr>
<td>Black, non-Hispanic</td>
<td>837</td>
<td>60.3 (56.4–64.1)</td>
<td>596</td>
</tr>
<tr>
<td>Hispanic</td>
<td>800</td>
<td>57.4 (53.6–61.1)</td>
<td>600</td>
</tr>
<tr>
<td>Other, non-Hispanic</td>
<td>525</td>
<td>57.9 (53.3–62.4)</td>
<td>313</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High school or below</td>
<td>1,229</td>
<td>53.1 (50.1–56.1)</td>
<td>921</td>
</tr>
<tr>
<td>Less than high school</td>
<td>3,228</td>
<td>61.5 (59.7–63.3)</td>
<td>1,936</td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>2,942</td>
<td>61.5 (59.7–63.3)</td>
<td>1,742</td>
</tr>
<tr>
<td>Other</td>
<td>1,558</td>
<td>54.3 (51.6–57.1)</td>
<td>1,142</td>
</tr>
<tr>
<td>Source of payment for delivery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Private</td>
<td>2,738</td>
<td>62.5 (60.6–64.4)</td>
<td>1,606</td>
</tr>
<tr>
<td>Medicaid</td>
<td>1,564</td>
<td>54.2 (51.6–56.8)</td>
<td>1,135</td>
</tr>
<tr>
<td>No insurance</td>
<td>113</td>
<td>46.7 (37.4–56.1)</td>
<td>91</td>
</tr>
</tbody>
</table>

†Unweighted.
‡Weighted.
Table 2. Factors associated with never having heard of Zika virus, talking with a healthcare provider, and knowledge of CDC travel advisories among women who delivered a live infant, 17 US PRAMS sites, March 2016–February 2017*

<table>
<thead>
<tr>
<th>Maternal characteristic</th>
<th>Women who had never heard of Zika virus, n = 791</th>
<th>Women who had heard of Zika virus during pregnancy</th>
<th>Women who had knowledge of CDC travel advisory, n = 7,204</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Adjusted prevalence ratio (95% CI)†</td>
<td>Adjusted prevalence ratio (95% CI)†</td>
<td>Adjusted prevalence ratio (95% CI)†</td>
</tr>
<tr>
<td>&lt;24</td>
<td>1.77 (1.28–2.44)</td>
<td>1.07 (0.98–1.17)</td>
<td>1.00 (0.97–1.02)</td>
</tr>
<tr>
<td>25–34</td>
<td>1.22 (0.92–1.52)</td>
<td>0.99 (0.93–1.06)</td>
<td>0.99 (0.96–1.01)</td>
</tr>
<tr>
<td>&gt;35</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td>Race/ethnicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White, non-Hispanic</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td>Black, non-Hispanic</td>
<td>1.86 (1.46–2.37)</td>
<td>1.12 (1.04–1.20)</td>
<td>0.97 (0.94–1.00)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>0.69 (0.49–0.99)</td>
<td>1.08 (1.00–1.17)</td>
<td>0.99 (0.97–1.02)</td>
</tr>
<tr>
<td>Other, non-Hispanic</td>
<td>2.41 (1.85–3.13)</td>
<td>0.96 (0.87–1.05)</td>
<td>0.92 (0.89–0.96)</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High school or below</td>
<td>2.22 (1.68–2.92)</td>
<td>0.91 (0.84–0.97)</td>
<td>0.94 (0.92–0.97)</td>
</tr>
<tr>
<td>More than high school</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>1.5 (1.19–1.89)</td>
<td>0.92 (0.85–0.98)</td>
<td>0.98 (0.95–1.00)</td>
</tr>
<tr>
<td>Other</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td>Source of payment for delivery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Private</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td>Medicaid</td>
<td>1.45 (1.3–1.88)</td>
<td>0.88 (0.82–0.94)</td>
<td>0.98 (0.95–1.00)</td>
</tr>
<tr>
<td>None</td>
<td>2.46 (1.62–2.34)</td>
<td>0.77 (0.72–0.96)</td>
<td>0.95 (0.88–1.02)</td>
</tr>
<tr>
<td>Infant birth month</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2016 Mar–Aug</td>
<td>Referent</td>
<td>Referent</td>
<td>Referent</td>
</tr>
<tr>
<td>2016 Sep–2017 Feb</td>
<td>0.81 (0.67–0.98)</td>
<td>1.19 (1.13–1.26)</td>
<td>1.02 (1.00–1.04)</td>
</tr>
</tbody>
</table>


†All prevalence ratio estimates adjusted for maternal age, race/ethnicity, education, marital status, delivery payment source, infant month of birth, and state of residence.

Focusing on the subgroup of women who had heard about ZIKV during their pregnancy, we found that more than half (58.8%) reported talking to a healthcare provider about ZIKV. Nearly two thirds (63.5%) reported that their provider initiated the conversation; the remaining third (36.4%) reported that they initiated the conversation themselves (Table 1). Compared with non-Hispanic white women, non-Hispanic black women were more likely to have talked with a healthcare provider about ZIKV (aPR 1.12, 95% CI 1.04–1.20), as were women who gave birth during September 2016–February 2017 compared with those who gave birth in earlier months (aPR 1.19, 95% CI 1.13–1.26). However, women with a high school education or less (aPR 0.91, 95% CI 0.84–0.97), women who were not married (aPR 0.92, 95% CI 0.85–0.98), and women reporting Medicaid (aPR 0.88, 95% CI 0.82–0.94) or no insurance (aPR 0.77, 95% CI 0.2–0.96) at delivery were less likely to have talked with their healthcare provider about ZIKV (Table 2).

Most (91.9%) women reported knowledge of CDC travel advisories to avoid areas affected by Zika while pregnant (Table 1). Respondents reporting other non-Hispanic race versus non-Hispanic white women were less likely to have knowledge of the travel advisories (aPR 0.92, 95% CI 0.89–0.96), as were those with a high school education or less compared with women with more than a high school education (aPR 0.94, 95% CI 0.92–0.97) (Table 2).

In the adjusted analysis, women ≤24 years old were more likely not to have heard of ZIKV compared with women ≥35 years old (aPR 1.77, 95% CI 1.28–2.44), as were non-Hispanic black women compared with non-Hispanic white women (aPR 1.86, 95% CI 1.46–2.37) and non-Hispanic women of other races compared with non-Hispanic white women (aPR 2.41, 95% CI 1.85–3.13). In contrast, Hispanic women were more likely to have heard of ZIKV. Women with a high school education or less, women whose deliveries were paid for by Medicaid, and those who were uninsured at delivery were less likely to have heard of ZIKV compared with their counterparts with more than a high school education and private health insurance (Table 2).

Conclusions

These findings highlight interactions between pregnant women and their healthcare providers in 17 sites during the height of the 2016 Zika outbreak. Awareness of ZIKV was found to be high, as was awareness of CDC travel advisories to avoid
travel to Zika-affected areas during pregnancy (both >90%). This awareness was likely obtained from multiple sources, given that only half of women who heard of ZIKV reported discussing it with their healthcare provider during pregnancy. Similar results have been reported in other studies (9,10). Even though awareness was high, disparities existed, related to the smaller proportion of Hispanic and non-Hispanic black women who reported initiating discussions with providers about ZIKV. These differences suggest the opportunity to promote patient advocacy so that patients of all backgrounds feel comfortable asking about key topics if they are not raised by the provider, especially in the case of public health threats.

Our assessment is not without limitations. Data represent only women who recently gave birth to live infants in the 17 sites included in this analysis. Women seen in practices that conducted screening for travel history before a woman talked to her provider may not have reported counseling, especially if they were determined to be at low risk during the screening. Information is self-reported by the mother 2–6 months following the birth of her infant and may be subject to recall and social desirability bias.

PRAMS is the largest state- and population-based surveillance system in the United States that samples women who delivered live infants. PRAMS was augmented to collect timely data regarding patient and provider interactions related to ZIKV. Information from this analysis can fill data gaps and address the need to understand interactions between pregnant women and their healthcare providers regarding ZIKV.

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References

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Japanese encephalitis is caused by Japanese encephalitis virus (JEV), a mosquito-borne virus of the family Flaviviridae, genus Flavivirus (1). The JEV genome is composed of a single-stranded, positive-sense RNA of ≈11 kb with a single open reading frame (ORF) encoding a polyprotein. The polyprotein is processed into 3 structural proteins, capsid, membrane, and envelope (E), and 7 nonstructural proteins, NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5 (2).

JEV is distributed in temperate and tropical areas of eastern and Southeast Asia. In 2010, JEV genotype 1 was the predominant virus circulating. However, genotype 5 was also identified in mosquitoes in South Korea (3). Since that time, JEV genotype 5 has been detected in mosquitoes in many areas of South Korea (4). We report isolation of JEV genotype 5 virus from patient specimens and differences in sequences among other JEV strains (genotypes 1–5).

The Study
We isolated JEV (strain K15P38) from samples of a 27-year-old woman who came to a hospital in Kyeonggi-do, South Korea, on November 8, 2015. The patient had mild symptoms, such as fever, headache, apathy, and nausea. The patient recovered. We obtained documentation from the hospital that she had been vaccinated against Japanese encephalitis. Cerebrospinal fluid (CSF) and serum samples were obtained during the acute and convalescent phases.

We detected JEV IgM in serum and CSF samples by using an ELISA (Inbios, https://inbios.com) for convalescent-phase samples, but not acute-phase samples. We isolated virus by inoculating the convalescent-phase CSF sample onto BHK-21 cells. After a cytopathic effect was observed, we confirmed presence of virus by using a quantitative real-time PCR. We performed whole-genome sequence analysis of the virus by using virus genome extracted from 5 passaged culture supernatants and QIAamp Viral RNA Mini Kit (QIAGEN, https://www.qiagen.com).

We performed next-generation sequencing for full-length genes by using the Illumina (https://www.illumina.com) and confirmed gaps from next-generation sequencing by using Sanger sequencing. We assembled nucleotide sequences by using the SeqMan program in DNASTAR software version 5.06 (https://www.dnastar.com). We then conducted molecular phylogenetic analysis of ORF nucleotide sequences with 30 previously reported JEV strains by using MEGA 6.0 software (https://www.megasoftware.net) and the maximum-likelihood method (5) and calculated timescale phylogenies by using BEAST version 2.6.0 software (6). We deposited the polyprotein genome sequence of strain K15P38 in GenBank (accession no. MK541529).

We compared the entire ORF sequences of K15P38 virus with previously reported strains of JEV genotypes 1–5. Phylogenetic analysis showed that K15P38 belonged to JEV genotype 5 by (Figures 1, 2,ogenotypes 1–5). Phylogenetic analysis showed that K15P38 belonged to JEV genotype 5 by (Figures 1, 2,3, and 4).
Figure 1. Phylogenetic tree of Japanese encephalitis virus genotypes 1–5, South Korea. Entire open reading frame is shown. Bootstrap probabilities (values along branches) of each node were calculated by using 1,000 replicates. Branches showing quartet puzzling reliability >70% can be considered well supported. Black circle indicates K15P38 strain from patient samples. Scale bar indicates nucleotide substitutions per site.
Figure 2. Phylogenetic trees of Japanese encephalitis virus (JEV) genotypes 1, 3, and 5, South Korea. A) Entire open reading frame of JEV human isolates. B) Envelope protein genes of JEV genotype 5. Bootstrap probabilities (values along branches) of each node were calculated by using 1,000 replicates. Branches showing quartet puzzling reliability >70% can be considered well supported. Black circles indicate K15P38 strain from patient samples. Scale bars indicate nucleotide substitutions per site.
Japanese Encephalitis Virus Genotype 5, South Korea

Table 2. Comparison of amino acid sequences of envelope protein of Japanese encephalitis viruses of genotype 5, South Korea*

<table>
<thead>
<tr>
<th>Virus</th>
<th>Amino acid position</th>
</tr>
</thead>
<tbody>
<tr>
<td>K15P38 (South Korea 2015)</td>
<td>D E T K I T A P S M E A</td>
</tr>
<tr>
<td>10-1827 (South Korea 2010)</td>
<td>- - - - - - - - - - - -</td>
</tr>
<tr>
<td>K12AS1148 (South Korea 2012)</td>
<td>- - - - - - - - - - - -</td>
</tr>
<tr>
<td>K12AS1151 (South Korea 2012)</td>
<td>- - - - - - - - - - - -</td>
</tr>
<tr>
<td>K12HC95 (South Korea 2012)</td>
<td>- A - - - - - - - - - -</td>
</tr>
<tr>
<td>K12YJ1182 (South Korea 2012)</td>
<td>- L - - - - - - - - - -</td>
</tr>
<tr>
<td>K12YJ1203 (South Korea 2012)</td>
<td>- L - - - - - - - - - -</td>
</tr>
<tr>
<td>K13GB57 (South Korea 2013)</td>
<td>G Q O R S - - - T L D - T</td>
</tr>
<tr>
<td>Muar (Malaysia 1952)</td>
<td>- R - - - - - - - - - -</td>
</tr>
<tr>
<td>XZ0934 (China (Tibet) 2009)</td>
<td>- - - - - - - - - - - -</td>
</tr>
</tbody>
</table>

* Dots indicate 100% amino acid sequence identity.

Panel A; Appendix Table 1, https://wwwnc.cdc.gov/EID/article/26/5/19-0977-App1.pdf. Identities between the entire ORF of K15P38 and Muar genotype 5 virus were 90.4%.

In general, the E gene of JEV plays a major role in the pathogenesis of encephalitis (7). Several amino acids, including 107, 138, and 176 in the E protein, are reported to play major roles in the neurovirulence of JEV. K15P38 virus had conserved amino acids at these sites (8,9). However, the E protein of this virus had 6 different amino acids compared with that of the Muar strain isolated from a human in Malaysia in 1952 (10). Because Muar virus was derived from mouse brain and K15P38 virus was passaged in cell culture, we do not exclude the possibility of sequence variation caused by different culture methods.

Furthermore, the K15P38 strain contained Lys rather than Arg at position 84 of the E protein (Table 2), which was unique in genotype 5 viruses from South Korea strains derived from mosquito and human specimens. The E gene sequences of K15P38 virus showed high identity of ≈98.5% to 99.8% with other genotype 5 strains from South Korea isolated from mosquitoes (Figure 2, panel B). By estimating the root of the time measured on the E gene of JEV genotype 5 viruses, we identified that the XZ0934 strain from Tibet was an ancestor of JEV genotype 5 virus strains from South Korea (Figure 2, panel C). Considering these variations and time estimation of JEV genotype 5, further study is needed to investigate molecular and biologic characteristics of JEV.

Conclusions

JEV genotype 5 was isolated from mosquitoes in China during 2009 and South Korea during 2010. Because the major JEV genotype from mosquitoes in South Korea changed from genotype 1 to genotype 5 during 2010, the number of infected patients increased coincidently, especially adult patients (8,11). Japanese encephalitis is generally more prevalent in southern areas of South Korea, wherein Culex tritaeniorhynchus mosquitoes are more prevalent than in other regions. However, the prevalence of Japanese encephalitis has also increased in northern regions of South Korea, including Seoul, Gyeonggi, and Gangwon since 2010. This finding is consistent with the fact that 5 JEVs with genotype 5 have been reported in more diverse mosquito species, including Cx. orientalis and Cx pipiens, not only in Cx. tritaeniorhynchus (4).

A previous study showed that the prevalence of neutralizing antibodies to JEV were maintained at a level of 98.1% among the general population (12) because of the National Vaccine Program against Japanese encephalitis in South Korea since 1982. The currently used Japanese encephalitis vaccine that contains the JEV genotype 3 strain provides adequate protection against JEV genotype 1 (13).

Even so, the number of adult patients with Japanese encephalitis has increased. It has also been reported that existing JEV genotype 3 vaccines are less effective in protecting against JEV genotype 5 (14), suggesting the need for studies of the protective effect of current Japanese encephalitis vaccine against JEV genotype 5 virus.

Although JEV genotype 5 is highly pathogenic and causes early viremia and central nervous system invasion in animal models, limited information is available on the biological nature of JEV G5. Our results provide potentially useful information regarding JEV genotype 5, including pathogenic characteristics and vaccine efficacy.

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References

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In 2014, a tiered network of facilities to manage patients with Ebola virus disease (EVD) was established in the United States (1). The Centers for Disease Control and Prevention designated 56 hospitals as Ebola treatment centers (ETCs), each equipped with specified capabilities to provide safe high-level isolation care for patients with EVD. This network was enhanced with the later designation of 10 regional Ebola and other special pathogen treatment centers (RE-SPTCs) with enhanced capabilities to care for patients with other highly hazardous communicable diseases (HHCDs). Since that time, efforts have been made to expand existing ETC capabilities beyond EVD in preparation for treating the next HHCD outbreak.

Previous assessments of these 56 ETCs by our team found average costs incurred to train teams, enhance physical infrastructure, and acquire advanced resources totaled nearly $1.2 million/facility (2). Despite these major investments, only 15–18 months after initially establishing their ETCs, by 2016 most hospitals reported challenges in sustaining ETC capabilities, and 3 centers reported they no longer maintained preparedness for EVD care (3).

Now, 3.5 years after our last ETC assessment, these specialized units face intensified threats to their sustainability because federal funding of these centers through the Hospital Preparedness Program (HPP) Ebola Preparedness and Response Activities is set to expire in 2020 (4). We aimed to determine whether additional costs for ETCs have incurred since our assessment in 2015, as well as to assess hospitals’ sustainability plans for maintaining capabilities after federal funding ceases.

The Study
In April 2019, we sent a link to an electronic survey (Indiana University Institutional Review Board exemption #1903160012) by email to representatives from the 56 ETCs. Three additional email attempts were made to nonresponding ETCs through survey closing in June. We collected and analyzed data by using Qualtrics (https://www.qualtrics.com) software and exported them for descriptive statistical analyses.

A total of 37 (66%) ETCs responded. However, the ability to skip questions resulted in differing response rates within the survey. Three hospitals that had previously reported they no longer held ETC designation were still listed as ETCs and were therefore included in survey invitations. However, none responded. All but 1 of the remaining respondents reported they had maintained some degree of ETC capabilities. The 1 decommissioned ETC cited a lack of funding and diminished perceived threat of EVD as factors that led to reversion to a regular unit. The other 36 ETCs sustained their high-level isolation capabilities (Figure).

We compiled details of costs incurred by facilities since their 2014 establishment (Table). Of the 35 facilities that completed the reimbursement section, 29 (83%) reported they had received reimbursement from the federal government and 3 (9%) from the state government for costs in establishing or maintaining their unit. A total of 23 facilities reported total reimbursement amounts by the federal and state governments to date, totaling $26,546,545 (average $1,154,198/
ETC). A total of 21 ETCs reported total costs and reimbursement received as of June 2019; these facilities had expended, on average, $612,664 more than their reimbursements. When we excluded federally funded RESPTCs, this figure increased to $753,015.

Of the 34 ETCs that reported primary funding mechanisms for sustaining unit operations, most cited federal (n = 30, 88%) and institutional (hospital) (n = 28, 82%) funding. A total of 21 (58%) ETCs reported capabilities would be maintained after HPP funds expire in 2020; 3 (8%) reported they would no longer maintain capabilities after funding expires, and 11 (31%) were uncertain if capabilities would be maintained. Of the 21 ETCs that would maintain capabilities, nearly all reported additional funding sources would be internal (n = 17 of 18 hospitals that detailed other funding sources), and 4 explicitly noted the desire for state funding to sustain minimal capabilities and continued staff training. Of the 11 ETCs that were uncertain if capabilities would remain, all noted their commitment to sustain capabilities but voiced ambiguity as to whether their hospital would be able to provide necessary funding within their budget. When queried if ETC capabilities would be maintained once personal protective equipment (PPE) stocks expire, 27 (77%) ETCs responded yes and 8 (23%) were uncertain.

### Table. Costs of establishing and maintaining Ebola treatment centers, United States, 2019*

<table>
<thead>
<tr>
<th>Cost scale</th>
<th>Total costs</th>
<th>Construction/facility modifications</th>
<th>Administration</th>
<th>PPE purchases</th>
<th>Staff training</th>
<th>Laboratory equipment</th>
<th>Other unit purchases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Establishment†</td>
<td>$1,425,640</td>
<td>$1,029,973</td>
<td>NA</td>
<td>$166,825</td>
<td>$290,788</td>
<td>$117,134</td>
<td>$141,158</td>
</tr>
<tr>
<td>Median</td>
<td>$937,500</td>
<td>$561,000</td>
<td>NA</td>
<td>$87,467</td>
<td>$200,000</td>
<td>$75,000</td>
<td>$100,000</td>
</tr>
<tr>
<td>High</td>
<td>$4,650,000</td>
<td>$4,000,000</td>
<td>NA</td>
<td>$900,000</td>
<td>$1,250,000</td>
<td>$500,000</td>
<td>$450,000</td>
</tr>
<tr>
<td>Low</td>
<td>$100,000</td>
<td>$10,000</td>
<td>NA</td>
<td>$3,375</td>
<td>$10,000</td>
<td>$4,600</td>
<td>$4,066</td>
</tr>
<tr>
<td>Total</td>
<td>$45,620,489</td>
<td>$27,809,283</td>
<td>NA</td>
<td>$4,337,454</td>
<td>$7,269,711</td>
<td>$2,940,494</td>
<td>$2,964,324</td>
</tr>
<tr>
<td>Maintenance‡§</td>
<td>$224,664</td>
<td>NA</td>
<td>$103,151</td>
<td>$26,367</td>
<td>$70,562</td>
<td>$40,071</td>
<td>$32,679</td>
</tr>
<tr>
<td>Average</td>
<td>$170,000</td>
<td>NA</td>
<td>$27,500</td>
<td>$17,500</td>
<td>$45,000</td>
<td>$27,500</td>
<td>$18,000</td>
</tr>
<tr>
<td>Median</td>
<td>$600,000</td>
<td>NA</td>
<td>$300,000</td>
<td>$75,000</td>
<td>$250,000</td>
<td>$224,500</td>
<td>$100,000</td>
</tr>
<tr>
<td>High</td>
<td>$10,000</td>
<td>NA</td>
<td>$5,000</td>
<td>$1,500</td>
<td>$1,000</td>
<td>$500</td>
<td>$2,500</td>
</tr>
<tr>
<td>Low</td>
<td>$2,475,626</td>
<td>NA</td>
<td>$685,550</td>
<td>$1,693,500</td>
<td>$561,000</td>
<td>$686,250</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>$49,417,813</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*NA, not applicable; PPE, personal protective equipment.
†As of June 2019, n = 32 units that provided costs to establish their facility.
‡As of June 2019, n = 29 units that provided costs to maintain their facility.
§Survey tool did not differentiate between zeros and nonresponses; therefore, averages for subcategories might be inflated because zeros were considered nonresponses.
¶As of June 2019, n = 28 units that provided total costs to establish and maintain their facility.
Most facilities (n = 26, 76%) used the ETC space as a functional clinical unit when not activated for HHCD care, most commonly as intensive care unit beds (n = 13). Most ETCs have been used for ≥1 person under investigation for EVD since June 2014 (n = 22, 63%).

Conclusions
This assessment of ETC costs and sustainability plans is a follow-up to findings from 2015 and 2016 that surveyed the then newly established ETC network. Reported costs of establishing ETC capabilities increased by >$230,000/ETC from our spring 2015 assessment (2), reflecting the ongoing efforts of ETCs to prepare for EVD cases. To date, since establishment in 2014–2015, an average additional $225,000 has been spent per ETC to maintain HHCD care capabilities. Although total ETC costs have increased since our initial assessments (nearly $1.8 million compared with $1.2 million in 2015), gaps in reimbursement from federal and state funding have also increased (from $650,000 to $750,000 in non-RESPTC ETCs). Since 2016, more ETCs reported using their unit for routine use when not activated (76% vs. 58%), offsetting operational costs.

This study had limitations. ETC responses were self-reported and not validated. The survey tool defaulted nonresponses to zeros; therefore, for reported maintenance costs, averages for cost subcategories might be inflated because zeros were considered nonresponses. Since our previous assessment, 22 facilities reported new primary contacts; although we reached out to multiple persons for each ETC, it is possible personnel from nonresponding facilities have since left their position or hospital. It is also unclear how many nonresponding ETCs no longer maintain their capabilities; authors are aware of several nonresponding hospitals within the network that no longer maintain EVD care capabilities.

Since late 2015, the perceived threat of an HHCD outbreak within the borders of the United States has waned, and the perceived demand for numerous US hospitals to maintain a high level of preparedness for HHCDs has dwindled. In tandem with inadequate funding, more ETCs have elected to forgo high-level isolation capabilities. The establishment of RESPTCs sought to centralize capabilities at a regional level, but many ETCs noted that since 2014, major investments in establishing high-level isolation capabilities could prompt continued internal financial support if federal funding ceases.

However, ETCs reported heavy reliance on federal funding; nearly all reported it as their primary funding stream and leading factor in maintaining capabilities. The 2020 expiration of HPP funds threatens the existence of this network. It is unknown if—and for how long—many ETCs could maintain capabilities solely with internal financial support, or if the United States will revert to the level of HHCD preparedness before 2014. The ongoing EVD outbreak in the Democratic Republic of the Congo and the rise of 2019 novel coronavirus disease in China are reminders that HHCD outbreaks are increasingly regular occurrences. The high proportion of ETCs surveyed that have used their ETC for a person under investigation since 2014 (63%) further underscores the ongoing need of such specialized units across the country. The US healthcare system has made major strides in HHCD domestic preparedness capability since 2014. However, on the basis of study responses, the US health system could again be vulnerable and inadequately prepared for the next HHCD threat if federal HPP funding is not renewed.

About the Author
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References

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Tuberculosis (TB) is a global health emergency (1). The World Health Organization (WHO) End TB Strategy proposes a 90% reduction in TB incidence and 95% reduction in TB deaths by 2035 compared with 2015 (2). To reach this target, effective interventions are needed to interrupt transmission of Mycobacterium tuberculosis. Contact investigations help prevent M. tuberculosis transmission by identifying and treating persons in close contact with persons with TB disease (3). WHO recommends tuberculosis preventive treatment (TPT) for household members of bacteriologically confirmed pulmonary TB patients to prevent progression to active TB disease (4).

Contact investigations are a major tenet of the End TB Strategy but remain ineffective for various reasons (2,5,6). Many TB programs in high-burden areas limit contact investigations to household members (6). Recent studies suggest that such restrictions might miss key exposures in the community (7,8). Targeted, population-based, geographic TB screening is a potential approach to augment contact investigations (9–11) but is resource and time intensive and rarely includes TPT (11,12). We used population-based, molecular epidemiologic data from Botswana to investigate potential use of a neighbor-based approach for contact investigations.

The Study
During August 2012–April 2016, we enrolled participants treated for TB disease at 30 healthcare facilities in Botswana for a prospective molecular epidemiologic study, Kopanyo. In brief, Kopanyo was designed to explore potential clinical, demographic, geographic, social relationships, and M. tuberculosis genotypic characteristics among persons with TB (13,14). We interviewed enrolled patients by using a standardized questionnaire and abstracted clinical data from medical records (13). We collected and processed sputum samples for culture and genotyped isolates with 24-locus mycobacterial interspersed repetitive units–variable-number tandem-repeats by using standard methods (15). We geocoded and validated the primary residence of each enrolled patient (Appendix, https://wwwnc.cdc.gov/EID/article/26/5/19-1568-App1.pdf). We excluded patients without a validated primary residence of each enrolled patient (Appendix, https://wwwnc.cdc.gov/EID/article/26/5/19-1568-App1.pdf). We excluded patients without a validated primary residential geocode and those who resided in locations outside of the study area. The study area included all 11 neighborhoods in Gaborone and 3 villages in the Ghanzi District: Ghanzi, D’Kar, and Kuke.

We defined index patients as the first culture-positive pulmonary TB patient identified and started on treatment in a household. We used residence plots to identify nearest neighbors, which we defined as those who lived immediately next door, and next-nearest neighbors, which we defined as those who lived 2 doors away (Figure). We enumerated all subsequent TB cases identified by bacteriologic confirmation and clinical diagnosis within the index home, nearest-neighbor homes, and next-nearest neighbor homes. We defined
A Neighbor-Based Approach to Identify TB Exposure

future-related patients as culture-positive patients with matching genotypes diagnosed after exposure to an index patient. Concurrent disease was TB diagnosed in a contact within 90 days of the index patient.

We enrolled 4,331 patients but excluded 595 (14%) without a residential geocode and 547 (13%) who resided outside the study area. We analyzed data on the remaining 3,189 patients. Among 1,072 index patients, 143 (13%) had subsequent TB patients in the home (n = 426); 30 (7%) in-home subsequent patients had concurrent disease. Of 1,072 index patients, 73 (7%) had future-related patients (n = 123) in their homes; 5 (3.94%) of those had concurrent TB disease.

When we applied a neighbor-based approach, we noted that 257 (24%) index patients could have subsequent TB patients living next door (n = 749), 41 of which could have concurrent disease. Among next-nearest neighbors of index patients, 390 (36%) could have subsequent TB, 23 of which could have concurrent disease (Table). In addition, 29 (2.7%) index patients could have future-related patients among their nearest neighbors (n = 42), and 5 (0.5%) future-related patients among next-nearest neighbors (n = 10), 3 with concurrent TB disease (Table).

We found that a neighbor-based approach could identify 1,565 additional subsequent TB patients, including 175 future-related patients, and 102 patients with concurrent TB disease. The number of persons living with a bacteriologically positive patient varied by geography; however, ≈23,630 contacts potentially could benefit from TPT. Of note, 9% (97/1,072) of index patients interviewed stated they lived alone, but 91 (94%) had subsequent patients identified in the home, and 84 (87%) had subsequent future-related patients living in the home.

Conclusions

We explored the use of a nearest-neighbor approach to expand TB contact investigations. This approach does not rely on name-based contact identification, which has been shown to be ineffective (6,16–18). In addition, the neighbor-based approach would not require mobile screening units or mass screening campaigns in the community. By simply expanding the number of homes visited to nearest and next-nearest neighbors, the Botswana National TB Program could increase the number of TB case diagnoses by 146% and potentially interrupt 175 secondary patient transmission events.

Preventing future TB disease through TPT could also hasten TB elimination in at-risk neighborhoods and reduce deaths in the community (11,12). Cegielski et al. effectively used TPT to eliminate TB from 2 at-risk neighborhoods in Texas, USA (11). The focus on nearest and next-nearest neighbors gives programs a tangible and practical approach to locating persons at risk for TB exposure and progression to TB disease.

Figure. Illustration of possible nearest neighbors and next-nearest neighbors for tuberculosis (TB) screening and possible TB preventive treatment. Black box represents the home of a TB index patient; dark-gray boxes represent the nearest-neighbor homes; light-gray boxes represent the next-nearest neighbor homes. This figure does not reflect the true number of neighbor homes, and index patients might have >4 next-door neighbors, depending on the geographic orientation of residential plots.
The neighbor-based approach differs from a neighborhood screening, which places an additional burden on TB programs by unnecessarily screening many persons at lower risk. For example, 59,100 persons reside in neighborhood C in Gaborone (data not shown). Under the neighbor-based approach, only 5,470 (9%) persons, including in-home and nearest neighbor residents, would be targeted for testing.

Previous reports suggest that contact investigations fail to identify key relationships, even within households (16,17). Potential stigma and lack of trust in government officials also play a role in contact investigations (16–18). In our cohort, many (n = 97) index patients said they lived alone, but 94% of them had subsequent cases identified in the home. In addition, 48% of future-related patients were linked to index patients who claimed no household contacts during name-based contact solicitation interviews conducted at the enrollment clinic. Household membership composition could have changed over time, and some connections might not have existed at the time of the interview. However, our study reinforces the necessity of home visits at times convenient to the index patient and when most household members are in the home, which might warrant home visits outside of business hours and flexibility in staff workplans.

Our analysis emphasizes the opportunity to prevent future TB patients and future-related TB patients by providing TPT. Household contacts, especially young children and persons living with HIV, are eligible for TPT by national policy, but TPT has not been practiced routinely in Botswana. As the Botswana Ministry of Health scales up access to TPT throughout the country, the neighbor-based approach could improve identification of most likely contacts and help target interventions where they are most needed.

Our study has limitations. Living in proximity to an index patient is not the only opportunity for transmission and might not always translate into time spent together. In addition, our analysis of future-related patients included only patients with culture-positive disease and genotyping results; excluding them did not affect the main analysis enumerating subsequent patients but might have underestimated the number of future-related patients. Also, our estimates for TPT represent the maximum number of persons who could benefit because we used the average number of persons per household and assumed all household members would be eligible for TPT without a reliable and available test for infection.

A neighbor-based approach should not supplant household investigations, and community-based interventions should not divert essential resources from those already devoted to finding and treating TB patients. Wide-scale implementation of this approach would require adequate resources to ensure that all patients complete the full cascade of treatment. To reach the ambitious global goal of TB elimination, we need simple, easy to implement, location-based approaches. Screening index patient households and nearest neighbors could help identify additional TB patients and persons who could benefit from TPT.

Table. Number of index patients and possible additional subsequent contacts and future-related patients identified by using a nearest-neighbor approach to tuberculosis screening, Botswana*

<table>
<thead>
<tr>
<th>Geographic area</th>
<th>No. index patients†</th>
<th>No. household members (FR)‡</th>
<th>No. nearest-neighbors (FR)‡</th>
<th>No. next-nearest neighbors (FR)‡</th>
<th>Total subsequent patients (FR)‡</th>
<th>No. screened to identify 1 TB patient (95% CI)§</th>
<th>Household contacts that could benefit from TPT¶</th>
<th>Neighbor contacts that could benefit from TPT¶</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gaborone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>123</td>
<td>57 (16)</td>
<td>93 (0)</td>
<td>47 (2)</td>
<td>197 (18)</td>
<td>21 (13–32)</td>
<td>861</td>
<td>3,472</td>
</tr>
<tr>
<td>B</td>
<td>58</td>
<td>19 (4)</td>
<td>41 (0)</td>
<td>21 (0)</td>
<td>81 (4)</td>
<td>18 (11–28)</td>
<td>307</td>
<td>1,230</td>
</tr>
<tr>
<td>C</td>
<td>210</td>
<td>83 (22)</td>
<td>146 (8)</td>
<td>84 (1)</td>
<td>313 (31)</td>
<td>16 (9–26)</td>
<td>1,092</td>
<td>4,368</td>
</tr>
<tr>
<td>D</td>
<td>195</td>
<td>58 (10)</td>
<td>110 (0)</td>
<td>56 (2)</td>
<td>224 (12)</td>
<td>19 (11–30)</td>
<td>878</td>
<td>3,510</td>
</tr>
<tr>
<td>E</td>
<td>79</td>
<td>28 (6)</td>
<td>46 (0)</td>
<td>30 (0)</td>
<td>104 (6)</td>
<td>11 (5–20)</td>
<td>253</td>
<td>1,011</td>
</tr>
<tr>
<td>F</td>
<td>129</td>
<td>53 (2)</td>
<td>84 (2)</td>
<td>51 (2)</td>
<td>188 (6)</td>
<td>15 (8–25)</td>
<td>593</td>
<td>2,374</td>
</tr>
<tr>
<td>G</td>
<td>51</td>
<td>14 (0)</td>
<td>29 (0)</td>
<td>18 (0)</td>
<td>61 (0)</td>
<td>9 (4–17)</td>
<td>128</td>
<td>510</td>
</tr>
<tr>
<td>H</td>
<td>20</td>
<td>5 (0)</td>
<td>12 (0)</td>
<td>6 (0)</td>
<td>23 (0)</td>
<td>7 (3–14)</td>
<td>38</td>
<td>152</td>
</tr>
<tr>
<td>I</td>
<td>6</td>
<td>2 (0)</td>
<td>9 (0)</td>
<td>4 (0)</td>
<td>15 (0)</td>
<td>2 (0–7)</td>
<td>10</td>
<td>41</td>
</tr>
<tr>
<td>J</td>
<td>6</td>
<td>2 (0)</td>
<td>2 (0)</td>
<td>1 (0)</td>
<td>5 (0)</td>
<td>22 (14–33)</td>
<td>23</td>
<td>94</td>
</tr>
<tr>
<td>K</td>
<td>11</td>
<td>6 (0)</td>
<td>11 (0)</td>
<td>6 (0)</td>
<td>23 (0)</td>
<td>7 (3–14)</td>
<td>35</td>
<td>141</td>
</tr>
<tr>
<td>Ghanzi District</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ghanzi</td>
<td>141</td>
<td>83 (57)</td>
<td>143 (24)</td>
<td>57 (3)</td>
<td>283 (84)</td>
<td>6 (2–16)</td>
<td>398</td>
<td>1,590</td>
</tr>
<tr>
<td>D’kar</td>
<td>35</td>
<td>9 (2)</td>
<td>14 (8)</td>
<td>7 (0)</td>
<td>30 (10)</td>
<td>11 (5–20)</td>
<td>86</td>
<td>280</td>
</tr>
<tr>
<td>Kuke</td>
<td>8</td>
<td>7 (4)</td>
<td>9 (0)</td>
<td>2 (0)</td>
<td>18 (4)</td>
<td>8 (3–15)</td>
<td>28</td>
<td>128</td>
</tr>
<tr>
<td>Total</td>
<td>1,072</td>
<td>426 (123)</td>
<td>749 (42)</td>
<td>390 (10)</td>
<td>1,565 (175)</td>
<td>16 (9–26)</td>
<td>4,730</td>
<td>18,901</td>
</tr>
</tbody>
</table>

*FR, future related; TB, tuberculosis; TPT, tuberculosis preventive treatment.
†No. index patients is equivalent to the number of standard contact investigations.
‡Future related, i.e., all culture-positive patients with matching M. tuberculosis genotype as an index patient.
§Limits of 95% CI assume a Poisson distribution.
¶Number exposed to bacteriologically confirmed pulmonary TB who do not have TB disease.
Members of the Kopanyo Study Group: Joyce Basotli, Ebi Bile, Cynthia Caiphus, Eleanor Click, Rosanna Boyd, Mbatshi Dima, Othusitse Fane, Alyssa Finlay, Sambayawo Gwebe-Nyirenda, Thandi Katlholo, Pilara Khumongwana, Chawangwa Modongo, Patrick Moonan, John Oeltmann, Matsiri Ogopotse, Kitso Ramogale, Christopher Serumola, James Shepherd, Tsaoe Tamuhla, James Tobias, Goitseone Thamae, Onani Zimba, and Nicola Zetola.

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References


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Nontuberculous mycobacteria (NTM) are comprised of >150 species (1) and are increasingly recognized as opportunistic pathogens (2). In addition, frequency of disease-causing NTM isolation has been rising in many settings (3, 4). The exact epidemiology of NTM pulmonary disease, the most common manifestation in adults (3), has been difficult to determine because reporting is not mandatory in most countries and identification of true disease is often difficult (5).

The distribution of NTM species isolated from human clinical samples varies greatly by region (6). In South America, data on NTM isolation frequencies are available for São Paulo and Rio de Janeiro, Brazil (7–9) and Buenos Aires, Argentina (6). Because clinical and laboratory observations suggested an emergence of NTM isolates, we conducted a retrospective study of isolation frequency of NTM in Uruguay.

The Study
We conducted a retrospective study of data on all NTM isolates identified at the Comisión Honoraria de Lucha Anti-Tuberculosis (Montevideo, Uruguay), the national tuberculosis reference laboratory, during January 2006–December 2018. The laboratory receives samples from all suspected tuberculosis (TB) cases across the country and performs universal acid-fast bacillus smear testing and solid culture in Löwenstein-Jensen and Ogawa egg-based media as a part of routine diagnostic testing. We abstracted basic patient demographic information and NTM identification results from the laboratory databases.

Phenotypic characterization of isolated NTM was performed by using biochemical methods, and complementary genotyping identification was introduced in 2012 by using GenoType Mycobacterium CM (Hain Lifescience GmbH, https://www.hain-life-science.de). In 2016, the laboratory included GenoType Mycobacterium AS (Hain Lifescience GmbH) in its pipeline and retrospectively identified previously unidentified isolates.

For comparison, we also collected data on the number of culture-positive TB cases during the study period from the laboratory databases. We used official population information from the National Institute of Statistics Uruguay (Instituto Nacional de Estadística, http://www.ine.gub.uy) to calculate NTM incidence (Appendix 1, https://wwwnc.cdc.gov/EID/article/26/5/19-1631-App1.xlsx).

During 2006–2018, a total of 255 NTM isolates were collected from pulmonary and extrapulmonary samples from 204 patients, 143 male and 61 female, in Uruguay (Table 1). Most (147/255; 57.6%) isolates identified were members of the Mycobacterium avium complex (MAC), which includes M. intracellulare and M. avium; 21 (8.2%) were M. kansasi; 15 were M. gordonae (5.9%); and 12 were M. peregrinum (4.7%) (Table 2). We observed an increase in NTM isolation frequency and an increase in TB cases during 2011–2018 (Figure 1, panel A). We also calculated an age-adjusted isolation frequency to determine population aging effect (Figure 1, panel B).

Among 204 cases, 23 patients had >1 NTM-positive isolate 6 months apart, and 13 had positive cultures for >2 years. Patients >50 years of age (mean...
Table 1. Specimen sources for isolation of nontuberculous mycobacteria. Uruguay*  

<table>
<thead>
<tr>
<th>Specimen source</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pulmonary</strong></td>
<td></td>
</tr>
<tr>
<td>Sputum</td>
<td>170 (66.6)</td>
</tr>
<tr>
<td>Bronchoalveolar lavage</td>
<td>18 (7.0)</td>
</tr>
<tr>
<td>Bronchoalveolar secretions†</td>
<td>8 (3.1)</td>
</tr>
<tr>
<td>Lung biopsy</td>
<td>6 (2.3)</td>
</tr>
<tr>
<td>Pleural fluid</td>
<td>3 (1.2)</td>
</tr>
<tr>
<td>Ear, nose, throat aspirate</td>
<td>2 (0.8)</td>
</tr>
<tr>
<td>Puncture fluid</td>
<td>3 (1.2)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>210 (82.3)</td>
</tr>
<tr>
<td><strong>Extrapulmonary</strong></td>
<td></td>
</tr>
<tr>
<td>Blood culture</td>
<td>15 (5.9)</td>
</tr>
<tr>
<td>Myelocyte culture</td>
<td>9 (3.5)</td>
</tr>
<tr>
<td>Skin or soft tissue abscess</td>
<td>4 (1.6)</td>
</tr>
<tr>
<td>Urine</td>
<td>1 (0.4)</td>
</tr>
<tr>
<td>Feces</td>
<td>2 (0.8)</td>
</tr>
<tr>
<td>Gastric lavage</td>
<td>1 (0.4)</td>
</tr>
<tr>
<td>Ganglion biopsy</td>
<td>3 (1.2)</td>
</tr>
<tr>
<td>Ascites fluid</td>
<td>3 (1.2)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>38 (14.9)</td>
</tr>
<tr>
<td><strong>Multiple isolation sources‡</strong></td>
<td>3 (1.2)</td>
</tr>
<tr>
<td><strong>Missing data</strong></td>
<td>4 (1.6)</td>
</tr>
</tbody>
</table>

*The data include isolates from the same patient over time.
†Includes tracheal aspirations.
‡Includes pulmonary and nonpulmonary samples of the same patient.

52 years of age) were more likely to have prolonged culture-positivity, a statistically significant difference from patients with a single positive culture (mean 42.6 years of age; p = 0.0099). Most (7/13; 53.8%) prolonged infections were caused by M. intracellulare, and 4 prolonged cases remained NTM-positive for ≤6 years, including 2 infections with M. intracellulare and 1 each with M. kansasii and M. heckeshornense. Most (10/13; 76.9%) prolonged cases were detected in pulmonary isolates.

Only 4 (1.9%) cases showed disseminated infections in isolates obtained from hemoculture, bone marrow culture, ganglion biopsy, feces, urine, or sputum of the same patient. Among the 4 disseminated infections, M. avium was isolated from 2 cases, M. genavense from 1 case, and M. intracellulare from 1 case.

The incidence of NTM in Uruguay increased from 0.33 cases/100,000 inhabitants in 2006 to 1.57 cases/100,000 inhabitants to 2018 (Figure 1, panel A). In 2018, the incidence of NTM was 2.73/100,000 inhabitants in the north, which has only 16.72% of the total population of the country. In the south, where 83.28% of the population lives, the incidence was much lower, 1.34 cases/100,000 inhabitants. Culture-positive TB cases showed a reverse tendency with statistically significant differences. The incidence rate for TB was higher in the south, 30.01 cases/100,000 inhabitants, and lower in the north, 21.50 cases/100,000 inhabitants (Figure 2).

During the period studied, we observed a 4-fold increase in the NTM isolation rate (Appendix 1). Implementation of new detection techniques during this timeframe could account for part of the increase (10). However, we noted a >2-fold increase (2.49) in number of isolates recorded during 2017–2018, when no laboratory protocol changes were introduced, suggesting the NTM incidence is rising in Uruguay (Appendix 1).

Rivero-Lezcano et al. (10) suggested changes in the pathogen and an aging population could explain the rising incidence of NTM isolation and its clinical manifestation.

Table 2. Nontuberculous mycobacteria species isolated in Uruguay, 2006–2018*  

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td><strong>Mycobacterium tuberculosis</strong></td>
<td>12</td>
<td>7</td>
<td>4</td>
<td>5</td>
<td>15</td>
<td>12</td>
<td>14</td>
<td>19</td>
<td>26</td>
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<td>27</td>
<td>28</td>
<td>53</td>
<td>255</td>
<td>100.0</td>
</tr>
<tr>
<td><strong>M. avium</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>M. kansasii</strong></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>0</td>
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<td>0</td>
<td>1</td>
<td>0</td>
<td>4</td>
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<tr>
<td><strong>M. gordonae</strong></td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>4</td>
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<td>0</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>21</td>
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<tr>
<td><strong>M. peregrinum</strong></td>
<td>0</td>
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<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
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<td>1</td>
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<td>3</td>
<td>2</td>
<td>2</td>
<td>12</td>
<td>4.7</td>
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<tr>
<td><strong>M. chelonae</strong></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>8</td>
<td>3.1</td>
<td></td>
</tr>
<tr>
<td><strong>M. fortuitum</strong></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>8</td>
<td>3.1</td>
<td></td>
</tr>
<tr>
<td><strong>NTM, no species</strong></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>5</td>
<td>2.0</td>
<td></td>
</tr>
</tbody>
</table>

*MTBC, Mycobacterium tuberculosis complex; NTM, nontuberculous mycobacterium.
†Mixed infections
‡Other species included 1 isolate each of M. arupense, M. asiaticum, M. koreense, M. shimodei, M. interjectum, M. marinum and M. malmoense, and mixed infections of M. avium–M. intracellulare, M. gordonae–M. chelonae, M. intracellulare, M. fortuitum, M. intracellulare-MTBC.
From 2017 to 2018, we observed a higher increase in NTM incidence (2.94 cases/100,000 persons) among persons >60 years of age compared with persons 15–60 years of age (2.39 cases/100,000 persons) (Figure 1, panel B). However, we noted that the age composition of the population remained stable over time (Appendix 1), suggesting other factors are driving increases in NTM isolation rates.

MAC is reported to be the most frequently isolated NTM species in all continents (3), but we observed a different species distribution than previously reported for South America. We saw a higher prevalence of M. intracellulare (33.7% of isolates) than M. avium (23.9% of isolates) (Table 2). We found species not previously reported in Latin America, including M. xenopi and M. arupense, and M. malmoense, which typically is found in northern Europe (11). We also found M. parakoreense, for which only 2 cases are reported in the literature, 1 case in 2013 isolated from a single clinical sample (12) and another in Africa (13). In addition, we noted 2 cases of M. heckeshornense, a species previously reported by our group (14).

We noted prolonged positivity in 6% (13/204) of NTM cases. MAC was responsible for most (69%) prolonged culture positives and M. kansasii was responsible for 15% of prolonged positivity.

We believe the differences observed in NTM isolation rates between regions of Uruguay could be explained by climate factors. The higher temperatures and increased rainfall in the north could favor the incidence ambient pathogens (Appendix 2, https://wwwnc.cdc.gov/EID/article/26/5/19-1631-App2.pdf). We also cannot disregard differences associated with other factors, such as the age of the population.

Our study has several limitations. First, the public health system of Uruguay only registers M. tuberculosis; clinical data of NTM patients are difficult to obtain. In addition, the GenoType AS assay used to identify NTM does not detect all NTM species; including genomic sequencing-based identification methods would improve detection in the future. Identification of NTM species is crucial for determining appropriate clinical and antimicrobial treatment for pulmonary NTM, which are dependent on the characteristics of the species (8).
Conclusions
Our findings indicate that rate of NTM have risen nearly 5-fold (4.79) in Uruguay, from 0.33 cases/100,000 inhabitants in 2006 to 1.57 cases/100,000 inhabitants in 2018. The species distribution largely aligns with existing data from elsewhere in South America, which demonstrates a predominance of MAC. Prolonged culture positivity and various isolation sites sources suggest that NTM disease is prevalent in Uruguay and warrants further studies to optimize diagnosis and treatment.

Acknowledgments
We thank Carlos Rivas for his support and efforts to preserve nontuberculous mycobacteria data at the Comisión Honoraria de Lucha Anti-Tuberculosis. G.G. and C.R. are supported by the Sistema Nacional de Investigadores, Uruguay.

About the Author
Dr. Greif is a molecular biologist at the Institut Pasteur Montevideo, Montevideo, Uruguay. His research interests include developing molecular methods for diagnosing mycobacteria.

References:
Plague is a globally distributed, zoonotic disease caused by the bacterium Yersinia pestis. In the late 1890s, rat-infested steamships introduced the disease into the continental United States. The first documented autochthonous human infection occurred in the Chinatown section of San Francisco, California, in March of 1900. Cases were soon reported in other port cities, including New Orleans, Galveston, Seattle, and Los Angeles. Along the Pacific Coast, infection spread from urban rats to native rodent species, and by the 1950s, Y. pestis had spread eastward to reach western portions of the Dakotas, Nebraska, Kansas, Oklahoma, and Texas. This distribution has remained static for more than 60 years, presumably the result of climatic and ecologic factors that limit further spread. Although poorly defined, these factors may be related to the ecology of vector species rather than that of rodent hosts.
Crimean-Congo Hemorrhagic Fever Virus Endemicity in United Arab Emirates, 2019

Jeremy V. Camp, Dafalla O. Kannan, Babiker Mohammed Osman, Moayyed Sher Shah, Brigitte Howarth, Tamer Khafaga, Pia Weidinger, Noushad Karuvantevida, Jolina Kolodziejek, Hessa Mazrooei, Nadine Wolf, Tom Loney, Norbert Nowotny

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We conducted a cross-sectional survey of Crimean-Congo hemorrhagic fever virus (CCHFV) in dromedary camels and attached ticks at 3 locations in the United Arab Emirates. Results revealed a high prevalence of CCHFV-reactive antibodies in camels and viral RNA in ticks and camel serum, suggesting the virus is endemic in this country.

C

rimean-Congo hemorrhagic fever virus (CCHFV; order Bunyavirales, family Nairoviridae, genus Orthornavirus) is a geographically widespread species of tickborne virus. Enzootic transmission cycles involve livestock (cattle, sheep, goats) and tick species of the genus Hyalomma (Acari: Ixodidae) (I). Spillover into humans typically occurs through tick bites; however, some severe (and even fatal) CCHFV infections have occurred as a result of exposure to blood or tissue from infected animals. The virus is genetically diverse, and evidence indicates that frequent reassortment of viral gene segments occurs, potentially as a result of animal contact into this region. Therefore, we conducted a cross-sectional survey of ticks and dromedary camels in the UAE to determine exposure status and detect active CCHFV infections.

We collected whole blood samples from camels at 3 sites within the UAE that differed by frequency of camel use: a family farm, a desert conservation reserve with multiple tour operators, and a large livestock market (Appendix, https://wwwnc.cdc.gov/EID/article/26/5/19-1414-App1.pdf). We found CCHFV antibodies in the serum samples of 67% (84/125) of camels. CCHFV antibody prevalence was highest in older camels (96% in camels >10 years of age), and no difference in antibody prevalence was detected between sexes (68% [51/75] male, 71% [29/41] female) (Appendix Table 1). The prevalence of reactive antibodies differed between sampling locations, potentially because of differences in animal ages at the respective sites.

We removed 314 adult ticks and 33 tick nymphs (0–5 ticks/camel) from camels and identified the species under a stereomicroscope. Most (99%, 311/314) adults were Hyalomma dromedarii ticks, and 3 were H. scupense ticks. Two pools of adult H. dromedarii ticks (1 containing 3 males and the other containing 1 male) from 2 separate camels (both 6-year-old females, one of which was antibody positive) and serum samples from 2 camels (a 3-year-old female and 2-year-old male, both antibody negative) were positive for CCHFV nucleocapsid acid (Appendix Table 2). These 4 camels were all from the livestock market but originated from different regions of the UAE. The 2 camels with CCHFV RNA–positive serum were only briefly at the livestock market (for 1 and 2 days), and the 2 with CCHFV RNA–positive ticks were housed at the market for 7 and 41 days.

We performed 2 conventional reverse transcription PCRs on the RNA-positive serum samples and on each tick from the 2 RNA-positive pools, 1 amplifying a 492-bp portion of the viral small (S) segment and 1 amplifying a 672-bp portion of the viral medium (M) segment (Appendix). We then subjected these PCR products to Sanger sequencing (GenBank accession nos. MN516481–8; Appendix Table 3). The S segment sequences from 3 ticks (from 2 camels) and 2 serum samples were all identical to each other, except for a single synonymous substitution in the sequence from 1 serum sample; these sequences were genetically similar to sequences of isolates from West and South Africa (group III; Figure, panel B). We obtained the M segment sequences from only 3 ticks from 2 camels. These sequences were 85% identical to available sequences in GenBank, and the isolate with the closest identity (AP92, GenBank accession no. DQ211625) was from Greece (Figure panel B). Thus, the 2019 UAE
Figure. Molecular phylogeny of Crimean-Congo hemorrhagic fever viruses from dromedary camel serum samples and ticks (green circles, thick branches), United Arab Emirates, 2019. A maximum-likelihood analysis of a 492-nt sequence of the viral small (S) segment (A) and 672-nt sequence of the viral medium (M) segment (B) were performed. Viruses are labeled by GenBank accession number, country of origin, isolate name, and year of identification and are colored according to S segment lineages following the group nomenclature (2): group I, West Africa 1; group II, Democratic Republic of the Congo; group III, South Africa and West Africa 2; group IV, Asia and the Middle East; group V, Europe and Turkey; group VI, Greece; and group VII (M segment only). Numbers beside branches are bootstrap values from 500 bootstrap replicates; only values >60% are shown. Scale bars indicate number of substitutions per site.
isolates did not fall within previously defined phylogenetic groups (2).

Our data indicate that exposure to CCHFV is common among camels in the UAE, and transmission to camels might be occurring via native infected H. dromedarii ticks. A previous survey of UAE livestock that occurred shortly after the 1994–1995 outbreak ruled out camels and camel ticks as CCHFV reservoirs (7). Our data might indicate increased transmission activity in the region, potentially explaining the human case in Sharjah, UAE, in August 2019 associated with handling infected meat (5). The largest outbreak of CCHFV infection in the UAE (1994–1995) was associated with a high case-fatality ratio (73%) and was limited to abattoir workers (8,9); however, hospital outbreaks have also previously occurred in the UAE (6).

All previously characterized CCHFV isolates from the Arabian Peninsula and the Middle East (including viruses from the UAE and Oman) were genetically similar to each other, clustering together according to the S segment (group IV, Figure panel A). The M segments of the isolates from UAE and Oman were similar to those of viruses from Asia, the Middle East, West Africa, and South Africa (Figure panel B) (2,3,7). Overall, the data suggest that CCHFV is endemic in the UAE, where enzootic transmission cycles involve camels and camel ticks.

Acknowledgments
The authors thank Matter Mohammed Saif Alnuaimi (General Manager of Al Ain City Municipality) and his team for supporting the study; Hashim Ahmed Saeed and Md. Helal Ahmed for assistance with sampling at the livestock market; Greg Simkins and the Dubai Desert Conservation Reserve staff for access and support during our sampling of the reserve; staff of the camel tour providers Al Maha, Arabian Adventures, Desert Star, Alpha Tours, and Travco Tours; and the Mazrooei family for their support and generosity during the sampling of their farm. We are grateful for the assistance of Athiq Ahmed Wahab and Abubakkar Babuhan in facilitating the study.

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References

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Zika Inquiries Made to the CDC-INFO System, December 2015–September 2017

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Author affiliations: Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA (T.K. Sell, C. Watson, D. Meyer, M.R. Snyder, S.J. Ravi, E.E. McGinty); Centers for Disease Control and Prevention, Atlanta, Georgia, USA (L.E. Pechta, D.A. Rose, M.N. Podgornik, K.M. Lubell)

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We examined Zika-related inquiries to CDC-INFO, the national contact center for the Centers for Disease Control and Prevention, to identify potential communication gaps. The most frequently asked questions related to travel or geographic location of Zika (42% of all inquiries), information about laboratory testing (13%), or acquiring a Zika test (11%).

A rapid increase in Zika virus disease transmission rates throughout Latin America in 2015, followed by transmission in some US states and outbreaks in several US territories, sparked widespread attention (1–3). We systematically examined Zika-related inquiries to the CDC-INFO system, the national contact center for the Centers for Disease Control and Prevention to determine public concerns and questions about Zika and potential communication gaps. We analyzed inquirer type, inquiry topic, and number of inquiries. In this article, “question” refers to the content of each call/email and “inquiry” to individual calls/emails, regardless of content. Inquiries may include >1 question.

The CDC-INFO Zika dataset contained 32,668 English-language inquiries (calls/emails) about Zika made from December 1, 2015 (when inquiries about Zika began to be tracked), through September 29, 2017 (when CDC’s emergency activation for Zika response ended). We analyzed the number of inquiries over time using all database records and information on inquirers and topics using a 10% simple random sample (n = 3,268). After an initial pilot process, 2 study authors coded notes made by operators for information on the types of inquirers and types of questions (4) (Appendix, https://wwwnc.cdc.gov/EID/article/26/5/18-1694-App1.pdf).

We grouped inquirers into 3 different categories; however, most did not specify a category affiliation. The first category, the pregnancy group, made up 19% of all inquirers and was composed of pregnant women (10%), women planning to become pregnant and their partners (6%), and partners of pregnant women (3%). The second category, clinicians, made up 14% of all inquirers. (CDC also initiated a separate hotline for clinicians during the Zika response; information from those calls was not included in this data analysis.) The third category, all other inquirers (67%), included family members and parents (8%); public health practitioners, students and educators, politicians and political staff, media, and salespeople (3%); and inquirers who did not specify identity (56%).

The most frequent questions (present in 42% of inquiries) were about travel or geographic location (geolocation) of Zika outbreaks (Table). Approximately 13% of all inquiries included questions seeking factual background information about laboratory testing, including how to obtain results, how to administer tests, how to interpret results, and criteria for testing. Questions about getting tested for Zika were present in 11% of inquiries. Questions related to transmission factors (e.g., incubation, persistence, immunity, semen, mosquitoes) were present in 9% of inquiries. Only 4% of inquiries included questions about health effects and related issues (e.g., potential harm to fetus, self, children) (Table).

Some types of questions were asked more frequently than others by certain groups. For example, the pregnancy group most frequently asked questions about travel or geolocation of the disease (65% of all inquiries from this group), whereas clinicians most frequently sought information about tests (46%) (Appendix Table 1).

Analysis of the number of inquiries over time showed 2 distinct peaks. The first occurred early in the response, with ≈4,000 biweekly inquiries at the peak in late January/early February 2016, when news media coverage of the outbreak increased. The second occurred in late July/early August 2016, after local US transmission was confirmed, with nearly 2,000 biweekly inquiries (Appendix Figure 1). The number of inquiries by date for the 4 most frequently asked questions (about travel or geolocation of Zika, seeking information about tests, seeking to be tested for Zika, and transmission) generally reflected the same overall pattern as all inquiries. One exception was questions about travel/geolocation of the disease, which showed a third, smaller rise in volume in late 2016/early 2017 (Appendix Figure 2). The frequency of
questions about transmission, signs/symptoms, health effects, long term health effects, and insect management was significantly greater early in the outbreak, before local transmission was confirmed in the United States (p<0.05 by χ² test). Questions about waiting to get pregnant and geolocation were made significantly more often after local transmission occurred (p<0.05 by χ² test).

Outreach to CDC-INFO might indicate that Zika messages reached intended target populations. Of those who volunteered demographic information, 44% were in the pregnancy group and so might have been made aware of heightened risks and sought more specific information about ways to reduce them. Results show that information needs were most intense at the time the threat emerged, although events during the outbreak, such as news of local transmission cases in the United States or new transmission routes, might also have increased public interest.

Inquiries made to CDC-INFO about Zika might represent potential information gaps from other sources. Inquirers most frequently asked about travel/geolocation of disease and testing, which was already included in CDC messaging and disseminated through various channels. Our findings could indicate that the information previously provided was perceived to be insufficient or difficult to locate or understand or that it was hard to keep up with changes in messaging during the response.

CDC-INFO records are a source of data that has been underanalyzed but that provides critical information about inquiries from the public about active efforts to obtain information from CDC. Our findings may help with future messaging efforts around infectious disease outbreaks by identifying topics of information that should be emphasized to improve public understanding.

Acknowledgment
The authors thank Rachel Ciccarone and Hoang-Kheim Ha for providing us the CDC-INFO Zika dataset and their technical assistance for this project.

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About the Author
Dr. Sell is a senior scholar at the Johns Hopkins Center for Health Security and an assistant professor in the Department of Environmental Health and Engineering at the Johns Hopkins Bloomberg School of Public Health. Her work focuses on improving public health policy and practice in order to reduce the health impacts of disasters and terrorism.

Table. Percentage of inquiries with specific question topics to CDC-INFO, December 1, 2015–September 29, 2017*

<table>
<thead>
<tr>
<th>Question topic</th>
<th>% Inquiries with question topic, n = 3268</th>
</tr>
</thead>
<tbody>
<tr>
<td>Information gathering</td>
<td></td>
</tr>
<tr>
<td>Transmission: includes persistence, presence in semen, mosquitoes, and immunity</td>
<td>9</td>
</tr>
<tr>
<td>Incubation period</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Signs and symptoms</td>
<td>2</td>
</tr>
<tr>
<td>Outbreak response processes</td>
<td>3</td>
</tr>
<tr>
<td>Seeking information about diagnostic tests</td>
<td>13</td>
</tr>
<tr>
<td>Seeking information about treatments, countermeasures, vaccines</td>
<td>1</td>
</tr>
<tr>
<td>Clinician seeking clinical recommendation/assistance for a patient with Zika</td>
<td>1</td>
</tr>
<tr>
<td>Information about risks</td>
<td></td>
</tr>
<tr>
<td>Health effects/issues: includes harm to self, fetus, pregnant woman, or child</td>
<td>4</td>
</tr>
<tr>
<td>Health effects: specifically long-term reproductive effects</td>
<td>4</td>
</tr>
<tr>
<td>Exposure: mosquito-related or sexual exposure</td>
<td>2</td>
</tr>
<tr>
<td>Infection: asking if inquirer could have Zika</td>
<td>1</td>
</tr>
<tr>
<td>Safety of protective actions: includes spraying or repellant</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Actions</td>
<td></td>
</tr>
<tr>
<td>Protective actions</td>
<td></td>
</tr>
<tr>
<td>What activities should be done to protect from getting Zika</td>
<td>3</td>
</tr>
<tr>
<td>Waiting to get pregnant</td>
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</tr>
<tr>
<td>Safe sex practices</td>
<td>1</td>
</tr>
<tr>
<td>Insect repellent/preventioning bug bites/mosquito control</td>
<td>3</td>
</tr>
<tr>
<td>What action to take following possible exposure</td>
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<tr>
<td>Acquiring a Zika test</td>
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</tr>
<tr>
<td>Travel and geolocation</td>
<td>42</td>
</tr>
<tr>
<td>Actions for infected persons</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Other</td>
<td></td>
</tr>
<tr>
<td>Seeking access to materials/tools</td>
<td>4</td>
</tr>
</tbody>
</table>
| *When inquirers asked >1 type of question, each type was counted separately. As a result, total percentage adds up to >100%. CDC-INFO, the national contact center for the Centers for Disease Control and Prevention.
Serologic Detection of Middle East Respiratory Syndrome Coronavirus Functional Antibodies


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We developed and validated 2 species-independent protein-based assays to detect Middle East respiratory syndrome coronavirus functional antibodies that can block virus receptor-binding or sialic acid-attachment. Antibody levels measured in both assays correlated strongly with virus-neutralizing antibody titers, proving their use for serologic confirmatory diagnosis of Middle East respiratory syndrome.

The zoonotic introductions and ongoing outbreaks of Middle East respiratory syndrome (MERS) coronavirus (MERS-CoV) pose a global threat (1,2) necessitating continuous serosurveillance to monitor virus spread alongside the development of vaccine and antibodies as countermeasures. Both approaches require validated assays to evaluate specific antibody responses. Although MERS-CoV serologic assays have been developed (2–6), those detecting functional antibodies cannot be applied in all laboratories and can require Biosafety Level 3 (BSL-3) containment. Recombinant protein-based immunoassays are easier to operate and standardize and do not require BSL-3 containment. However, MERS-CoV protein-based assays developed thus far can only detect antibody binding and give no information on antibody functionality. The MERS-CoV spike protein N terminal subunit (S1) contains 2 functional domains: the N-terminal domain (S1A), which binds sialic acid, the viral attachment factor; and the receptor-binding domain (RBD) (S1B), which binds dipeptidyl peptidase 4, the virus receptor (7,8). Antibodies against those 2 domains can block MERS-CoV infection (9). Based on this fundamental knowledge, we developed 2 recombinant protein-based functional assays.

First, we developed an S1-based competitive ELISA, a receptor-binding inhibition assay (RBI), to test for antibodies that block the interaction with dipeptidyl peptidase 4, the viral receptor (Appendix Figure 1, https://wwwnc.cdc.gov/EID/article/26/5/19-0921-App1.pdf). We validated the specificity of the assay for human diagnostics using serum samples from healthy blood donors, PCR-confirmed non-coronavirus-infected patients and non–MERS-CoV–infected patients (cohorts H1–H3) (Appendix Table 1). At a 1/20 dilution, none of the samples from non-MERS-CoV-infected humans showed a ≥50% reduction in signal (RBl50) (Figure, panel A), indicating a high specificity of the assay. MERS-CoV–specific RBI antibodies were detected in all the 90% plaque reduction neutralization assay (PRNT)50–positive serum samples of the PCR-confirmed MERS-CoV patients tested (Appendix Table 2, Figure 2). The percentage reduction in signal strongly correlated with neutralizing antibody titers (Figure, panel B). The RBI50 assay showed similar sensitivity to the PRNT50 assay.

Because the RBI assay is species-independent, we validated its ability to detect RBI antibodies in dromedaries. At a 1/20 dilution, none of the naive dromedary serum samples (10) reacted in the assay, whereas all samples from MERS-CoV–infected dromedaries (2) resulted in a >90% reduction in signal (Appendix

References

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Table 1, Figure 3, panel A). We detected RBI antibodies in the samples of vaccinated and experimentally infected dromedaries (Appendix Figure 3, panel B). Overall, the RBI was highly specific and showed comparable sensitivity to PRNT for detection of MERS-CoV-specific RBI (neutralizing) antibodies after infection and vaccination (Appendix Figure 3, panel C).

Apart from the RBD, the MERS-CoV S1 contains an α2,3 sialic acid–binding S1 domain (7). When this domain was multivalently presented on self-assembling lumazine synthase (LS) nanoparticles (S1 A-Np), it was able to hemagglutinate human erythrocytes. To generate S1 A-Np, we genetically fused the S1 A domain to LS and expressed the particles in HEK-293S cells (Appendix Figure 4, panel 7).

**Figure.** MERS-CoV–specific RBI and HI assays for MERS-CoV human diagnostics. A) Validation of the specificity of the RBI assay for the detection of MERS-CoV–specific antibodies in humans. Red dots indicate severe illness. Green dots indicate mild illness. B) Correlation between neutralizing and RBI antibody responses after MERS-CoV infection. C) Hemagglutination of turkey erythrocytes using S1 A–nanoparticles. S1 A, S1 B, or empty self-assembling lumazine synthase nanoparticles were serially diluted and tested for the ability to agglutinate turkey RBCs. D) Specificity of the HI assay for the detection of MERS-CoV S1 A–directed antibodies. Rabbit anti-S1, anti S1 B, or anti-S1 serum samples were serially diluted and tested for the ability to block S1 A–nanoparticles–induced hemagglutination of turkey RBCs. E) Validation of HI assay for the detection of MERS-CoV–specific antibodies in humans. F) Scatter plot correlating PRNT 90 neutralization titers and HI titers after MERS-CoV infection. CoV, human coronavirus; HI, hemagglutination inhibition; MERS-CoV, Middle East respiratory syndrome coronavirus; PRNT 90, 90% reduction in plaque reduction neutralization test; RBI, receptor-binding inhibition.
By using S1A-Np, we developed a hemagglutination inhibition (HI) assay to detect antibodies capable of blocking virus interaction with sialic acids (Appendix Figure 4, panel B). To set up the assay using turkey RBCs, we tested the ability of S1A-Np to agglutinate turkey erythrocytes by using empty (LS)-Np and S1B-Np as negative controls. Although neither the lumazine synthase-Np nor the S1B-Np showed any hemagglutination at any temperature tested, the S1A-Np induced hemagglutination at 4°C; we also noted hemagglutination when using dromedary erythrocytes (Figure, panel C; Appendix Figure 4, panel C). Although antibodies against the S1 and S1A domain inhibited hemagglutination showing high HI titers, S1B antibodies were negative for HI (Figure, panel D).

Next, we used the same cohort of serum samples for validating the RBI assay. None of the samples from healthy blood donors, PCR-confirmed non–coronavirus-infected and non–MERS-CoV-infected patients (cohorts H1–H3) showed any HI at the 1/20 dilution (Figure, panel E). HI antibodies were detected in the samples of all severely infected MERS-CoV patients and that of 1 mildly infected MERS-CoV patient (Figure, panel E; Appendix Figure 5); only 2 of the mildly infected MERS-CoV patients were PRNT90-positive (Appendix Table 2). Serum HI titers correlated strongly with neutralizing antibody titers detected by a whole virus neutralization assay (PRNT90); nonetheless, the PRNT90 assay was more sensitive (Figure, panel F). Similarly, only serum samples from MERS-CoV–infected dromedaries were HI-positive (10/13), whereas none of the naive dromedary camel serum samples showed any HI (Appendix Figure 6, panel A). HI antibodies were detected in serum samples of vaccinated dromedaries after booster immunization (Appendix Figure 6, panel B). Overall, although less sensitive, the antibody titers detected by the HI assay correlated strongly with the neutralizing antibody titers detected by PRNT90 assay (Appendix Figure 6, panel C).

The RBI and HI assays we developed are easy to operate and standardize and can detect functional antibodies against 2 MERS-CoV S1 domains responsible for virus entry (RBD) and attachment (S1A). Both assays are protein-based and can be carried out in a 96-well plate format, therefore providing BSL-1 high-throughput platforms. The assays can be used as confirmatory assays for human and dromedary MERS-CoV diagnostics and for antibody and vaccine evaluation.

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Novel Ehrlichia Strain Infecting Cattle Tick
Amblyomma neumanni, Argentina, 2018
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In 2018, we detected a novel Ehrlichia strain infecting Amblyomma neumanni ticks in Argentina. The novel strain is phylogenetically related to the ruminant pathogen E. ruminantium and represents a potential risk for veterinary and public health because A. neumanni ticks parasitize domestic and wild ruminants and bite humans.

etymologia

Coronavirus [kə-ro'nə-viˈrəs]
Ronnie Henry

The first coronavirus, avian infectious bronchitis virus, was discovered in 1937 by Fred Beaudette and Charles Hudson. In 1967, June Almeida and David Tyrrell performed electron microscopy on specimens from cultures of viruses known to cause colds in humans and identified particles that resembled avian infectious bronchitis virus. Almeida coined the term “coronavirus,” from the Latin corona (“crown”), because the glycoprotein spikes of these viruses created an image similar to a solar corona.

Strains that infect humans generally cause mild symptoms. However, more recently, animal coronaviruses have caused outbreaks of severe respiratory disease in humans, including severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), and coronavirus disease (COVID-19).

Sources

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**Ehrlichia** spp. are intracellular gram-negative bacteria relevant to human and animal health; they infect monocytes, neutrophils, or endothelial cells, depending on the species involved (1). The genus *Ehrlichia* (Rickettsiales: Anaplasmataceae) comprises 6 formally recognized tick-transmitted species: *E. canis*, *E. muris*, *E. chaffeensis*, *E. ewingii*, *E. minasensis*, and *E. ruminantium* (2,3). Recently, other *Ehrlichia* species have been reported and different strains of putative novel *Ehrlichia* species have been molecularly detected, but their taxonomic positions are still not clearly defined (4–6). Current knowledge about this group of pathogens suggests that a large number of *Ehrlichia* species might be not yet described.

*Amblyomma neumannii* ticks are relevant to human and veterinary medicine because in all stages they commonly parasitize wild and domestic ruminants and other large mammals, including humans (7). Moreover, *A. neumannii* ticks can reportedly be infected with *Rickettsia bellii* and *Rickettsia amblyomnensis* and are potential vectors of the cattle pathogen *Anaplasma marginale* (7). To determine the presence of tickborne bacteria of the genus *Ehrlichia* in questing *A. neumannii* ticks in northwestern Argentina, we performed phylogenetic analyses on Anaplasmataceae-positive tick DNA samples. All procedures were approved by the Ethics and Biosafety Committee of the Facultad de Ciencias Veterinarias, Universidad Nacional del Litoral, Espeanza, Argentina.

During May 2018 (late autumn), we collected free-living ticks by dragging and by using dry ice-baited traps in Dean Funes (30°22’S, 64°21’W) and San José de la Dormida (30°21’S, 63°58’W), Córdoba Province, Argentina. Both sites are located in the Chaco Seco ecoregion. We identified all ticks by using standard taxonomic keys (7) and individually processed them for DNA extraction by using a boiling method (8). We screened DNA extracts for Anaplasmataceae by real-time PCR targeting the 16S rRNA gene, as previously described (9). We further tested samples positive for Anaplasmataceae by amplification of *Ehrlichia* genes *dsb* and *groEL*, as described elsewhere (5). We sequenced all amplicons and performed phylogenetic analyses with the maximum-likelihood method.

We collected 229 ticks from Dean Funes (70 adults, 159 nymphs) and 62 from San José de la Dormida (24 adults, 38 nymphs) and identified all ticks as *A. neumannii*. Only 1 adult tick from San José de la Dormida was positive for Anaplasmataceae by PCR. Further analysis of that sample resulted in 2 sequences of 355 bp (*dsb*) and 784 bp (*groEL*). Phylogenetic analysis of the *dsb* sequence (GenBank accession no. MN176580) showed that the *A. neumannii* tick was infected with a species of *Ehrlichia*, which we named *Ehrlichia* sp. strain La Dormida, closely related to *E. ruminantium* (82.0%, GenBank accession no. CR925677) (Figure, panel A). Other ehrlichiae from South America included in the analysis, such as *Ehrlichia* sp. strain Córdoba from Argentina (78.0%, GenBank accession no. KY413807) and *Ehrlichia* sp. strain Natal from Brazil (79.3%, GenBank accession no. KY207546), were placed in a clade sister to the group formed by *Ehrlichia* sp. strain La Dormida and *E. ruminantium*. Furthermore, the phylogenetic analysis performed by using the *groEL* sequence (GenBank accession no. MN176581) confirmed these results (Figure, panel B).

Several recent studies conducted in South America reported finding novel ehrlichial agents infecting jaguars, horses, crab-eating foxes, opossums, sloths, and peccaries (6). Unfortunately, only short *dsb* sequences are available for those ehrlichiae from South America. Phylogenetic analysis including these sequences (210 positions included in the final dataset) placed them all together in a clade sister to the group formed by *Ehrlichia* sp. strain La Dormida and *E. ruminantium* (Appendix Figure, https://wwwnc.cdc.gov/EID/article/26/5/19-0940-App1.pdf).

*Ehrlichia* sp. strain La Dormida, associated with *A. neumannii* ticks, circulates in rural areas of northwestern Argentina. In our phylogenetic analyses, *Ehrlichia* sp. strain La Dormida genotype was unique and well separated from all other available ehrlichial sequences, suggesting that it could represent a distinct species yet to be properly characterized. In addition, these analyses positioned *Ehrlichia* sp. strain La Dormida in a separate group together with *E. ruminantium*. The species *E. ruminantium* is native to the Africa continent, where it is the etiologic agent of heartwater, a tickborne disease of major economic relevance with regard to domestic ruminants throughout sub-Saharan Africa (10). Besides *E. ruminantium*, the only other species of *Ehrlichia* known to naturally infect and cause clinical manifestations of ehrlichiosis in cattle is *E. minasensis* (3). However, *E. minasensis* is phylogenetically distant from *E. ruminantium* and closely related to *E. canis* genotypes (3).

We report *A. neumannii* ticks as a potential vector of *Ehrlichia* sp. strain La Dormida. Because it is assumed that bacteria of the genus *Ehrlichia* are not transmitted transovarially in ticks (1), infection with *Ehrlichia* must be acquired during feeding of immature ticks, which then pass the infection to adults by transstadial transmission. Regarding *E. ruminantium*, wild African ruminants are reservoirs...
Figure. Maximum-likelihood trees constructed from dsb and groESL sequences of Ehrlichia sp. infecting Amblyomma neumanni ticks in Argentina compared with reference strains. A) Tree constructed by using dsb Ehrlichia sequences of approximately the same length as the sequence identified in this study (341 positions included in the final dataset). B) Tree constructed by using groESL Ehrlichia sequences of approximately the same length as the sequence identified in this study (767 positions included in the final dataset). Phylogenetic trees were constructed by using MEGA 7.0 (https://www.megasoftware.net), and best-fitting substitution models were determined with the Akaike Information Criterion, using the maximum-likelihood model test. Numbers represent bootstrap support generated from 1,000 replications. GenBank accession numbers are shown in parentheses. Boldface indicates the strain identified in this study. Scale bars indicate nucleotide substitutions/site.
of the bacteria (10). The novel *Ehrlichia* sp. strain La Dormida is phylogenetically related to the ruminant pathogen *E. ruminantium* and represents a potential risk for veterinary and public health because *A. neumanni* ticks parasitize domestic and wild ruminants and bite humans.

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**Multidrug-Resistant *Salmonella* Serotype Anatum in Travelers and Seafood from Asia, United States**

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A multidrug-resistant *Salmonella enterica* serotype Anatum strain reported in Taiwan was isolated in the United States from patients and from seafood imported from Asia. Isolates harbored 11 resistance determinants, including quinolone and inducible cephalosporin resistance genes. Most patients had traveled to Asia. These findings underscore the need for global One Health resistance surveillance.

A sharp increase in *Salmonella enterica* serotype Anatum infections reported in Taiwan during 2016–2017 was associated with emergence of...
multidrug-resistant (MDR) strains harboring 11 resistance genes: *aadA2*, *bla*DHA-1*, *dfrA23*, *floR*, *lnu*(F), *qnrB4*, *strA*, *strB*, *sul1*, *sul2*, and *tet*(A) (1). Isolates had intermediate susceptibility to ciprofloxacin and resistance to many antimicrobial agents, including third-generation cephalosporins. We report human cases and related isolates in the United States.

We found 43 isolates genetically related to MDR *Salmonella* Anatum from Taiwan in the National Center for Biotechnology Information Pathogen Detection Isolates Browser (http://www.ncbi.nlm.nih.gov/pathogens). We analyzed genome assemblies for resistance determinants and plasmids by using databases adapted from ResFinder and PlasmidFinder (Center for Genomic Epidemiology, https://cge.cbs.dtu.dk). To assess strain relatedness, we constructed a core genome multilocus sequence typing (cgMLST) phylogenetic tree and pairwise matrix of allele differences by using BioNumerics version 7.6 (Applied Maths, http://www.applied-maths.com). We contacted US health departments to obtain patient information and isolates for susceptibility testing by broth microdilution (Appendix Table 1, http://wwwnc.cdc.gov/EID/article/26/5/19-0992-App1.pdf).

**Figure.** Core genome multilocus sequence typing (cgMLST) phylogenetic tree of 40 *Salmonella enterica* serotype Anatum isolates, 2015–2019. The tree was constructed by using BioNumerics version 7.6 (Applied Maths, http://www.applied-maths.com). Isolate sources, collection years, and National Center for Biotechnology Information strain or isolate numbers are shown. For isolates from the United States, international travel destinations of patients and sources of imported foods are provided. Dark gray boxes indicate resistance determinants detected: 1) *aadA2*; 2) *aph(3″)-Ib* (*strA*); 3) *aph(6)-Ia* (*strB*); 4) *bla*TEM-1B; 5) *dfrA23*; 6) *floR*; 7) *lnu*(F); 8) *qnrB4*; 9) *sul1*; 10) *sul2*; 11) *tet*(A); 12) *aadA1*; 13) *bla*TEM-1B; 14) *dfrA1*; 15) *dfrA12*; 16) *mcr-1*; 17) *mph*(A); 18) *qnrA6*; 19) *sul3*. Scale bar indicates percentage similarity.

JAP, Japan; MX, Mexico; NA, not available; PHL, Philippines; TWN, Taiwan; UK, United Kingdom; USA, United States.
We created a cgMLST phylogenetic tree showing resistance determinants detected for 40 isolates with >99.5% similarity and 0–20 allele differences (Figure; Appendix Figure). We excluded 3 more distantly related isolates. A total of 25 isolates were from Taiwan (16 from humans, 3 each from chickens and pigs, 2 from geese, and 1 from a duck); 12 were from the United States (7 from humans, 4 from tilapia imported from Taiwan, and 1 from shrimp imported from the Philippines). We detected IncC plasmids in all isolates, except PNUAS038936; 15 had additional plasmids (Appendix Table 2). Most (38/40) had the previously reported 11 resistance genes (I). Two isolates from the Philippines had additional resistance genes, including mph(A), qnrA6, and oqxAB; 3 isolates from tilapia in the United States and 1 human isolate from Taiwan had mcr-1.1. We found no quinolone resistance–determining region mutations.

The 7 patients from the United States were 19–71 (median 48) years of age; 3 were women and 4 men. Among 5 patients with data on race, 3 were Asian and 2 white. All patients reported illness, including diarrhea (7/7), abdominal pain (4/7), nausea (2/7), and fever (1/7). None were hospitalized or died. Four became ill ≤3 days after returning from travel to the Philippines; 1 visited Japan before the Philippines. Two additional patients reported travel before illness onset; 1 traveled to the Philippines and the other to Taiwan and Mexico, but travel and illness onset dates were unavailable.

One patient had never travelled internationally. Her isolate was indistinguishable from 1 from a patient who traveled to Asia and differed by only 2 alleles from an isolate from shrimp imported from the Philippines. Before illness onset, she ate at several restaurants and had shrimp at an Asian restaurant and sushi bar.

In patient isolates from the United States, bla\_DHA-1 appeared to be carried in a complex integron, with the regulatory ampR gene positioned upstream and qnrB4 downstream. Six isolates had IncC plasmids similar to pR16.0676_90k (GenBank accession no. CP029802) (I), which likely carried all 11 resistance genes, but long-reading sequence is required for confirmation. Isolate PNUAS038936 lacked the IncC plasmid replicon but appeared to have an IS26-mediated integration of the entire resistance region from the plasmid (≈60 kb) into the chromosome.

We performed antimicrobial susceptibility testing on 6 patient isolates, including PNUAS038936. All had intermediate susceptibility to ciprofloxacin (MIC 0.25 µg/mL) and were resistant to amoxicillin/clavulanic acid, ampicillin, cefoxitin, chloramphenicol, streptomycin, sulfisoxazole, tetracycline, and trimethoprim/sulfamethoxazole. One isolate had intermediate susceptibility to ceftriaxone (MIC 2 µg/mL) and 5 were ceftriaxone susceptible; 1 had a MIC of ≤0.25 µg/mL, 3 MICs of 0.5 µg/mL, and 1 a MIC of 1 µg/mL.

The emergence and spread of *Salmonella* carrying *bla\_DHA-1* has both clinical and public health implications. Unlike most plasmid-mediated AmpC β-lactamase genes, *bla\_DHA-1* is inducible (2,3), which can complicate detection and treatment. Isolates can appear susceptible to third-generation cephalosporins in vitro, but treatment may fail if AmpC induction occurs (3,4). The co-occurrence of *qnrB4*; the plasmid-mediated quinolone resistance gene *qnrB4*; and in the isolates from the Philippines, *mph(A)*, a macrolide-resistance gene, is worrisome because third-generation cephalosporins (e.g., ceftriaxone), fluoroquinolones (e.g., ciprofloxacin), and the macrolide azithromycin are recommended for *Salmonella* infections requiring treatment (5,6). In addition, the presence of *mcr-1.1*, which confers resistance to colistin, a drug of last resort for treating MDR gram-negative bacterial infections, is concerning.

Our findings underscore the need for global, One Health surveillance. Most infections likely were acquired during travel in Asia. International travel, particularly to Asia, has been associated with acquisition of *Salmonella* with clinically important resistance (7,8). Resistance also can be disseminated via food and animals. Imported food likely was the source of infection for 1 patient without international travel. Among imported foods tested by the US Food and Drug Administration, seafood from Asia is a frequently reported source of antimicrobial-resistant *Salmonella* (9,10). Given the extent of international travel and trade, data sharing among human health, animal health, and food production sectors and across geographic borders is essential to detect MDR strains and inform strategies and interventions to prevent spread.

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The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

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Fatal Rodentborne Leptospirosis in Prison Inmates, South Africa, 2015

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Leptospirosis is a neglected zoonotic disease. In 2015, leptospirosis was diagnosed in 2 prison inmates in South Africa. Using real-time PCR and DNA sequencing, we identified \textit{Leptospira interrogans} serogroup Icterohaemorrhagiae in rodents and water samples within the prison. Leptospirosis might be frequently underdiagnosed in South Africa.

Although leptospirosis, a bacterial zoonosis, is responsible for ≈1 million cases per year worldwide, estimates of its incidence in Africa are limited by a lack of quality-assured studies (1). Humans become infected through mucosal membranes or skin breaks by direct contact with reservoir animals or exposure to urine-contaminated soil or water. We describe an outbreak of leptospirosis in prison inmates in Cape Town, South Africa, and identification of probable animal sources and environmental routes of infection.

In September 2015, the South Africa Department of Correctional Services requested the National Institute for Communicable Diseases to assist with investigation and management of leptospirosis infections in 2 inmates at a maximum-security prison in Cape Town. The National Health Laboratory Service Animal Ethics Committee clearance 131/11 granted approval for rodent trapping and testing; ethical clearance certificate no. M160667 from the Human Research Ethics Committee (Medical) of the University of the Witwatersrand covered the outbreak investigation.

Case-patient 1, a 52-year-old man, was admitted to a hospital in Cape Town. He had jaundice, overwhelming sepsis, disseminated intravascular
coagulation, and multiorgan failure after ≈1 week of worsening illness that included conjunctivitis, myalgia, and fever. At admission, his leukocyte count was 18.64 × 10⁹ cells/L (reference 4–10 × 10⁹ cells/L), platelets 65 × 10⁹/L (reference 137–373 × 10⁹/L), total bilirubin 30.23 mg/dL (reference 0–1.23 mg/dL) (91% conjugated), alanine transaminase 95 U/L (reference 5–40 U/L), and creatinine 10 mg/dL (reference 0.72–1.18 mg/dL). He died the following day.

Case-patient 2, a 49-year-old man occupying the same prison cell as case-patient 1, was hospitalized 10 days later. He had jaundice, abdominal pain, anorexia, weakness, and body aches. The initial leukocyte count was 21.71 × 10⁹ cells/L, platelets 100 × 10⁹/L, total bilirubin 17.89 mg/dL (88% conjugated), alanine transaminase 66 U/L, and creatinine 4.66 mg/dL. The patient received treatment and was discharged after 9 days of hospital stay.

A physician diagnosed leptospirosis on the basis of clinical signs and clinical pathology consistent with severe leptospirosis (leukocytosis, thrombocytopenia, hyperbilirubinemia, mild transaminasemia, acute renal failure). For both patients, blood cultures and hepatitis virus tests were negative. However, IgM to Leptospira spp. was detected by the Panbio Leptospira IgM ELISA (Standard Diagnostics, https://www.alere.com), confirming the clinical diagnosis. The reference serologic method (microagglutination test) is not available in South Africa, and neither blood nor urine samples were available for subsequent analysis by the reference laboratory.

An initial assessment of the prison by the National Institute for Communicable Diseases outbreak investigation team, together with provincial and national Department of Health officials, identified conditions favorable for Leptospira transmission (rodent infestation, standing water, and food waste accumulation). Within 3 weeks after hospital admission of case-patient 2, 12 Rattus norvegicus rats, identified by morphologic characteristics, were live-trapped around the prison section housing the patients and humanely euthanized, and their kidneys were harvested. Five stagnant drain water samples were collected in the same area.

We extracted DNA from rodent kidney samples using the QIAamp DNA Mini kit on a QIAcube system (QIAGEN, https://www.qiagen.com) and from water samples using ZR Soil Microbe DNA MicroPrep kit (Zymo Research, https://www.zymoresearch.com). We detected Leptospira DNA with an Applied Biosystems 7500 qPCR instrument (Applied Biosystems, https://www.thermofisher.com) using published primers targeting ≈300 bp of the lfb1 gene (2) and sequenced the amplicons as previously described (3). Leptospira lfb1 sequences are available in GenBank under accession nos. MH795484–MH795490 (rodents) and MH795482–MH795483 (water).

The prevalence of Leptospira infection in rodents was 66.7% (8/12), and we detected Leptospira DNA in 40% (2/5) of water samples. Sequencing of the lfb1 amplicons from both rodent and water samples identified a single genotype. Phylogenetic analysis using

![Figure](https://www.cdc.gov/eid/)

**Figure.** Neighbor-joining phylogenetic tree based of 256 bp of Leptospira lfb1 gene of isolates from South Africa and reference sequences. Leptospira sequences collected during this study are labeled according to sample source (rodent, water) and the number of sequences indicated. Reference sequences include lfb1 sequences from isolates obtained from human cases of leptospirosis in New Caledonia (3). GenBank accession numbers are provided in parentheses. Scale bar indicates nucleotide substitutions per site.
MEGA7 (4) revealed 100% identity to strains isolated from humans in New Caledonia and typed as *L. interrogans* serogroup Icterohaemorrhagiae (Figure) (5), a serogroup identified in historical cases from Cape Town (6,7).

*L. interrogans* serogroup Icterohaemorrhagiae, traditionally associated with rats, is frequently implicated in fatal leptospirosis and survives for prolonged periods in fresh water (8). Therefore, rodent infestations, the high prevalence of infected rodents, and increased environmental contamination are likely to have contributed to this prison outbreak of human leptospirosis. To reduce inmate exposure, the affected section of the prison was evacuated before intensive clean-up and rodent control activities were undertaken. Moreover, early referral of inmates with nonspecific febrile illness for preemptive treatment of leptospirosis was implemented. Thirty serum samples from symptomatic inmates were referred for *Leptospira* spp. IgM ELISA and real-time PCR; all tested negative, possibly because of the intermittent nature of leptosiraemia and the lack of detectable antibodies during early stages of the disease (9). No additional cases of leptospirosis were diagnosed.

We found evidence that rodent infestation and contaminated environments within confined settings, such as prisons, are risk factors for leptospirosis in humans, supporting previous findings from an Ecuador prison (10). Moreover, the finding that highly pathogenic *Leptospira* spp (6,7) continue to circulate in rodents suggests that human leptospirosis may be an underreported public health problem in South Africa, particularly among persons living in informal settlements, where rodent infestations are common and environmental conditions favor disease transmission.

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**References**


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Diplorickettsia Bacteria in an Ixodes scapularis Tick, Vermont, USA

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The blacklegged tick, Ixodes scapularis, is a generalist arthropod ectoparasite that serves as a vector for an array of common human pathogens; novel disease-causing microbes have been discovered consistently in the tick for the past several decades. Tickborne bacterial infections causing illnesses such as anaplasmosis, Borrelia miyamotoi disease, and ehrlichiosis have all emerged in the United States in recent years (1–3), and it has been estimated that as many as half of all tickborne illnesses are caused by unknown pathogens (4).

We used 16S rRNA sequencing to survey for bacterial pathogens in I. scapularis ticks in western Vermont, USA. We collected ticks by drag sampling along 100 m transects using a 1 m² square of white denim. We collected ticks from 6 deciduous forest sites in Addison and Chittenden counties, Vermont (Appendix Table 1, https://wwwnc.cdc.gov/EID/article/26/5/19-1135-App1.pdf), during May–July 2015. We extracted DNA from ticks using a phenol-chloroform extraction (5). In total, we extracted DNA from 97 ticks, 20 of which we selected based on DNA quality and quantity for 16S rDNA sequencing at Hudson Alpha Genomic Services Laboratory (Huntsville, AL, USA). We PCR amplified the V3 and V4 regions of the 16S rRNA gene from these ticks and 1 blank using 341F and 875R primers (6) and sequenced them on a MiSeq platform (Illumina, https://www.illumina.com), yielding a total of 15,302,568 reads. We used the DADA2 R package to identify amplicon sequence variants (ASVs) and assign taxonomy (7) (Appendix Figure 1). We used default settings in the DADA2 pipeline; however, we estimated error rates using the first 10 billion base pairs.

In a single adult male tick, an ASV assigned to the genus Diplorickettsia comprised 82% of the microbiome sequencing reads. The genus Diplorickettsia was originally defined by the species D. massiliensis, discovered in I. ricinus ticks in Europe (8). The ASV we identified shared 425 of 427 nt in the sequenced V3–V4 region of the 16S rRNA gene with the reference 1036

Figure. Neighbor-joining phylogenetic tree of a MAFFT alignment (https://mafft.cbrc.jp/alignment/server) of the V3–V4 region of the Diplorickettsia 16S rRNA gene, including the novel amplicon sequence variant identified in Vermont, USA (bold). A total of 427 bases were aligned and 363 conserved sites were used for neighbor-joining phylogeny, with 100 bootstrap iterations. The 341F and 875R primers were used to amplify these regions (6). Default alignment parameters were used for alignment and generation of phylogenetic tree. Numbers at nodes indicate bootstrap values after 1,000 bootstrapping iterations. GenBank accession numbers are indicated. Scale bar represents average number of substitutions per site.
sequence of *D. massiliensis* strain 20B (GenBank accession no. NR_117407.1) and was more closely related to this strain than any other previously sequenced *Diplorickettsia* in the National Center for Biotechnology Information (NCBI) nucleotide database (Figure). This tick was collected at the Sunny Hollow Colchester site (coordinates 44.518353°, -73.17112°) (Appendix Table 1).

*D. massiliensis* has been identified as a possible human pathogen; of patients in a hospital in France, 3 were found seropositive, and 1 found positive by quantitative PCR for the *D. massiliensis rpoB* gene (9). Our findings represent evidence of a *Diplorickettsia* bacteria in ticks in North America.

To confirm the presence of *Diplorickettsia* in the positive tick, we designed PCR primers to regions of the *D. massiliensis parC* and *ftsY* genes (Appendix Table 2) using primer-BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast). We aligned primers against the NCBI nr database to ensure specific binding to *Diplorickettsia*. We also used DNA from a *Diplorickettsia*-negative tick (as determined by 16S sequencing) as PCR template to serve as a negative control. Successful amplification of regions of both genes confirmed the presence of *Diplorickettsia* DNA in the positive tick (Appendix Figure 2).

We Sanger sequenced amplicons from these PCR tests. We combined forward and reverse reads and trimmed them using the PEAR utility (https://cme.h-its.org/exelixis/web/software/pear). Each sequence showed high identity with previously sequenced *D. massiliensis* reference sequences via gapped alignment (247/252 bp *parC*, 298/310 bp *ftsY*). These results further suggest a close relationship between *Diplorickettsia* species we identified and *D. massiliensis*, but a lack of reference sequences for these genes from other species of *Diplorickettsia* makes it impossible to definitively assign this uncultured specimen to a particular species.

The high sequence similarity between the *Diplorickettsia* we identified and the previously identified pathogenic variety suggests the need for further study of the pathogenicity of this variant. Many genera of tickborne bacteria contain both pathogenic and non-pathogenic strains, and genetic similarity alone cannot confirm pathogenicity. Future work is needed to isolate this strain of *Diplorickettsia* and determine its ability to infect mammalian hosts and its transmissibility via tick bite. Experiments to test its ability to induce febrile illness in mammals would also help determine if *Diplorickettsia* spp. could cause a clinically significant infection in humans. Furthermore, serologic studies of patients with suspected tickborne diseases in the area surrounding the collection site are necessary to determine if this bacterium has infected persons in Vermont.

In addition, our findings suggest the need for further study of the prevalence of *Diplorickettsia* in North America ticks. We have developed PCR primers (Appendix Table 2) to facilitate future study of this bacterium and have demonstrated via sequencing that these primers accurately amplify their target *Diplorickettsia* genes. We have deposited the partial *Diplorickettsia* 16S rRNA, *ftsY*, and *parC* sequences in this study into the NCBI GenBank database (accession nos. MN192053, MN640996, MN640997). The raw sequencing reads are available through the NCBI sequence read archive (accession no. PRJNA557440).

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### References


Case of Babesia crassa–Like Infection, Slovenia, 2014

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We report a case of Babesia crassa–like infection in an asplenic patient in Slovenia in 2014. We diagnosed the infection using microscopy, 18S rRNA sequencing, and serology and monitored parasitemia using digital PCR. With its increasing occurrence, babesiosis should be included in differential diagnoses for immunocompromised patients displaying fever.

Babesia infections occur worldwide and cause disease mainly in animals, but disease occurs occasionally in humans. Infections in humans are mostly attributable to B. microti, B. duncani, and Babesia sp. MOI in North America; B. divergens, B. venatorum, and B. microti in Europe; and B. venatorum, B. crassa–like parasite, B. microti, Babesia sp. XXB/HangZhou, and Babesia sp. KO-1 in Asia (1,2). Transmission occurs predominantly through tick bites, but humans have acquired infections via contaminated blood products and through the transplacental and perinatal routes (1). Most patients with Babesia infections in Europe were reported to be asplenic or immunocompromised. Typical clinical signs and symptoms include fever (up to 40°C), parasitemia (20%–80%), severe anemia, muscle weakness, fatigue, and late-onset jaundice with dark urine, and sometimes complications develop. Long-term clinical follow-up that includes blood smear examination and PCR analysis is necessary because relapse and persistence of parasitemia can occur in spite of treatment. The application of novel molecular methods has revealed that the host range of many Babesia species is less restricted than previously thought. New species or animal pathogens are increasingly being discovered as causing Babesia infections in humans. We report a B. crassa–like infection in a patient in Slovenia in 2014.

In 2014, a 55-year-old woman, living on the outskirts of Murska Sobota, Slovenia, sought medical treatment for a 6-day history of intermittent fever up to 39°C, myalgia, headache, poor appetite comitant with weight loss, fatigue, sweating, and dark urine. She previously had a splenectomy and partial pancreatectomy 5 years previous because of cystic adenoma and adrenal incidentaloma without hormonal activity. She reported no history of travel, tick bite, animal contact, or blood transfusions.

Her blood pressure was 115/70 mm Hg, heart rate 83 beats/min, and body temperature 36.6°C, and a physical examination indicated no significant clinical findings. The first basic blood analysis revealed thrombocytopenia (platelets 85 × 10^9/L). A differential blood analysis indicated that the concentration of large unstained cells was elevated (0.41 × 10^10/L, reference range 0–0.4 × 10^10/L). Biochemical laboratory testing showed mild fluctuations in liver functioning: aspartate aminotransferase 1.22 (reference range 0.17–0.51 µkat/L, alanine aminotransferase 1.13 (reference range 0.17–0.68) µkat/L, γ-glutamyltransferase 1.08 (reference range 0.03–0.51 µkat/L) µkat/L, and alkaline phosphatase 1.88 (reference range 0.5–2.0) µkat/L. C-reactive protein was 51 mg/L (150 [reference range 0.76–28.5] nmol/L), and mild erythrocytosis was present. Giemsa-stained blood smears showed unusual inclusions in erythrocytes, Howell-Jolly bodies, mild anisocytosis, some atypical lymphocytes, and some large thrombocytes. We observed many ring forms and some paired atypical shapes of Babesia spp. in blood smears (Figure), and parasitemia was 1% (Appendix Table,
We confirmed diagnosis by conventional PCR and sequencing of the 18S rRNA gene (3). A phylogenetic analysis indicated the pathogen was the *B. crassa*–like parasite (Appendix Figure).

We gave the patient an oral treatment of clindamycin (600 mg 3×/d) and quinine (600 mg 3×/d). Three days later, the patient was normothermic, and after a total of 6 days, she was discharged from the hospital with platelet levels within the reference range (150–350 × 10⁹/L). She continued the dual therapy for 14 days. To follow up on the patient’s response to treatment, we measured parasitemia levels by blood smear microscopy, PCR (3), and digital PCR (Appendix).

We later confirmed the infection by serology using an indirect immunofluorescence assay specific to another member of the large *Babesia* group, *B. divergens* (MegaFLUO BABESIA divergens; Megacor, https://www.megacor.at). Antibodies were cross-reactive, and results demonstrated a 4-fold increase in IgG titer (Appendix Table).

Reports of babesiosis in humans are increasing with the increase in number of immunocompromised persons; a species previously known only as an animal pathogen is posing a greater threat to those with weakened immune systems. *B. crassa* has been detected in sheep in Iran (4), goats and ticks in Turkey (5,6), and ticks in Hungary (7), and a case series of infections with *B. crassa*–like parasite in humans, sheep, and ticks was reported in northeastern China (8).

We report an infection of *B. crassa*–like parasite in an asplenic person in Europe that was confirmed by blood smear examination, PCR, sequencing, and serology (with assay specific to distant relative *B. divergens*). The patient recovered after treatment with the standard dual antimicrobial regimen. In addition to blood smear, we used a unique digital PCR assay to follow the decrease in concentration of babesial DNA in the patient’s blood until complete recovery. Note that DNA levels in blood do not necessarily correlate with levels of live pathogen (i.e., active infection).

With the development of new and more sensitive diagnostic techniques, parasites like *Babesia* spp., primarily recognized as animal pathogens, are becoming increasingly reported as human pathogens too, even in areas where the parasite has not been reported previously. Babesiosis should be included in the differential diagnoses for immunocompromised patients displaying fever worldwide.

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### References


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**Figure.** Piriform (A) and ring shapes (B) in blood smear of sample taken from patient with *Babesia crassa*–like infection, Slovenia, 2014. Smear was Wright-Giemsa stained. Scale bars indicate 50 µm.
Hepatitis A Hospitalization Costs, United States, 2017

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The United States is in the midst of unprecedented person-to-person hepatitis A outbreaks. By using Healthcare Cost and Utilization Project data, we estimated the average costs per hepatitis A–related hospitalization in 2017. These estimates can guide investment in outbreak prevention efforts to stop the spread of this vaccine-preventable disease.

The introduction of hepatitis A vaccine has dramatically changed the epidemiology of hepatitis A in the United States. After vaccine licensure in 1995, hepatitis A incidence declined substantially: 3,366 hepatitis A cases were reported nationally in 2017 (1). During July 1, 2016–February 7, 2020, state health departments publicly reported >31,000 outbreak-associated cases, primarily affecting persons who use drugs and persons experiencing homelessness, in the largest person-to-person hepatitis A outbreaks in the postvaccine era (2). More than 18,900 (61%) outbreak-associated patients have reportedly been hospitalized in these outbreaks (2). As these unprecedented outbreaks continue, we sought to estimate the average direct medical costs per hepatitis A–related hospitalization, which can be used to guide investment in outbreak prevention efforts.

We analyzed data from the 2017 Healthcare Cost and Utilization Project National Inpatient Sample (NIS). NIS, a 20% stratified sample of discharges from US community hospitals, is the largest publicly available all-payer inpatient database in the country (3). We considered a hospitalization to be hepatitis A–related if it included codes B15.0 or B15.9 from the International Classification of Diseases, 10th Revision, Clinical Modification, as any of the 30 listed diagnosis codes. We converted the total hospital charges into cost estimates (in 2017 US dollars) by multiplying total charges with 2017 hospital-specific cost-to-charge ratios (4), then estimated the average cost of hospitalization, SD, and 95% CI on the basis of the NIS survey sampling design. We multiplied the average costs by the number of patients hospitalized for outbreak-associated hepatitis A to generate an estimate of the preventable economic burden of hospitalizations in the ongoing person-to-person outbreaks (2).

We examined hepatitis A-related hospitalizations in the 2017 NIS dataset for evidence of associated liver transplantation (procedure codes 0FY00Z0, 0FY00Z1, and 0FY00Z2 from the International Classification of Diseases, 10th Revision, Clinical Modification, listed as any of the 15 procedure codes). Because the unweighted number of hospitalizations associated with liver transplantation was <10, we included such hospitalizations in the analysis but did not report them as a separate category (5).

Overall, the average costs per hepatitis A–related hospitalization in the United States in 2017 were $16,232 (SD $602; 95% CI $15,052–$17,411). The average costs ranged from $12,921 (SD $1,443; 95% CI $10,091–$15,750) in the West North Central Census Division to $19,680 (SD $1,932; 95% CI $15,891–$23,467) in the Pacific Census Division.

During July 1, 2016–February 7, 2020, a total of 32 states reported >18,900 outbreak-associated hepatitis A hospitalizations resulting from the ongoing hepatitis A outbreaks (2). On the basis of results of our analysis as a multiplier, we estimate that hospitalization costs associated with these outbreaks have exceeded $306.8 million (SD $11.4 million) as of February 7, 2020.
Because the NIS reports hospital discharges rather than unique patients, we were unable to identify patients with multiple hospitalizations or estimate the per-person costs of hepatitis A inpatient care. We were also not able to separately report the costs associated with liver transplantation.

Even though using highly sensitive inclusion criteria might have introduced an element of cost overestimation in some patients incidentally diagnosed with hepatitis A while admitted for other conditions, our results almost certainly underestimate hospitalization costs associated with the ongoing hepatitis A outbreaks because NIS does not include hospital-based physician fees. Moreover, the national $306.8 million estimate does not account for outpatient visits, emergency department visits that did not result in an admission to the same hospital, lost productivity, out-of-pocket costs to patients or their informal caregivers, or public health costs associated with the hepatitis A outbreaks, further reinforcing the conservative nature of this estimate.

Given the high proportion of hospitalized patients during the ongoing hepatitis A outbreaks, we estimated the average hepatitis A-related hospitalization costs to highlight the preventable economic burden of these outbreaks on healthcare systems and state governments. Hepatitis A is a vaccine-preventable disease. Despite longstanding vaccination recommendations for adults at increased risk for hepatitis A virus infection or adverse consequences of infection, self-reported adult hepatitis A vaccination coverage with ≥2 doses was only 10.9% for persons ≥19 years of age in 2017 (6). Our findings underscore the importance of improving hepatitis A vaccination coverage among at-risk adults, in accordance with Advisory Committee on Immunization Practices recommendations (7).

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Crimée-Congo hemorrhagic fever (CCHF), the most widespread tickborne viral infection in humans, is a zoonotic disease caused by an orthonairovirus of the *Nairoviridae* family. Symptoms in humans vary from a nonspecific mild febrile syndrome to severe hemorrhagic disease that sometimes leads to death (1,2), and a wide range of animals are asymptomatic reservoirs (1). Corsica is an island of France located in the northwestern part of the Mediterranean Sea (Figure, panel A). Entomologic surveys have revealed that one of the main vectors of CCHF virus (CCHFV), the *Hyalomma marginatum* tick, is abundant on this island (1,3,4). Therefore, we performed a serologic cross-sectional survey to assess the prevalence of antibodies against CCHFV in domestic ruminants on Corsica. This work was approved by the French Ministry of Agriculture (Direction Départementale de la Cohésion Sociale et de la Protection des Populations de Corse-du-Sud and Haute-Corse and General Directorate for Food).

As part of national surveillance for animal diseases, veterinarians collected cattle, goat, and sheep blood samples during 2014–2016. In total, 3,890 animals (1,731 cattle, 1,035 goats, 1,124 sheep) were sampled from 269 farms, originating from 46% (137/298) of the municipalities with ruminant farming activities (3).

We tested the collected serum samples for the presence of CCHFV IgG using a double-antigen ELISA kit (ID Screen CCHF Double Antigen Multi-species, ID.Vet, https://www.id-vet.com) according to the manufacturer’s instructions (Appendix Figure, https://wwwnc.cdc.gov/EID/article/26/5/19-1465-App1.pdf). For this kit, the 95% CI for sensitivity is 96.8%–99.8%, and 95% CI for specificity is 99.8%–100% (5). To confirm ELISA results, we sent 35 ELISA-positive and 5 ELISA-negative serum samples to a Biosafety Level 4 laboratory (Laboratory Jean Mérieux, Lyon, France) to be analyzed by the World Health Organization and World Organisation for Animal Health national reference center for CCHFV (Institut Pasteur and Institut de Recherche Biomédicale des Armées, Paris, France). We used the pseudo-plaque reduction neutralization test (PPRNT) (6) to measure the neutralizing antibodies against IbAr10200 (same antigen used in ELISA) in triplicate. We included Hazara virus (same serogroup as CCHFV) and Dugbe virus (closely related virus, Nairobi sheep disease serogroup) to detect possible immune cross-reactions. We estimated overall and species-specific IgG prevalence against CCHFV using a β-binomial logistic regression model of data grouped by farm.

The overall estimated seroprevalence was 9.1% (95% CI 6.9%–11.9%); estimated seroprevalence in cattle was 13.3% (95% CI 10.2%–17.3%), goats 3.1% (95% CI 1.4%–7.0%), and sheep 2.5% (95% CI 1.0%–5.9%). CCHFV antibodies were detected across the island; 35.8% (49/137; 95% CI 27.8%–44.4%, estimated by exact binomial test) of the investigated municipalities had ≥1 positive ELISA test result. Because serum samples were not available from all municipalities, we used Voronoi polygons to draw regional boundaries and estimate the spatial distribution of seroprevalence across the island. Seroprevalence was high in the northwest corner of Corsica; however, most regions lacked evidence of seropositivity (Figure panel A). In areas corresponding to negative polygons, the probability of nondetection of positive serum samples was estimated assuming 3 levels of estimated serop-
revalence corresponding with the 10% quantile (2.8% seroprevalence), 25% quantile (5.0% seroprevalence), and 50% quantile (14.3% seroprevalence) (Figure, panel B) and by accounting for sample size. These data show that if seroprevalence in these regions is <5%, the probability of nondetection is high (Figure, panel B), and if the seroprevalence in these regions is >14.3%, the probability of nondetection is low. Therefore, the chance that we missed hotspots of transmission is highly unlikely.

Of 35 ELISA-positive serum samples tested, none showed neutralizing antibodies against Hazara and Dugbe viruses, and no ELISA-negative serum sample showed neutralizing antibodies against CCHFV, Hazara virus, or Dugbe virus (at lowest dilution 1:20; Appendix Table). Of 35 ELISA-positive serum samples, 23 had neutralizing antibodies against CCHFV at the 1:40 dilution, and 10 remained positive at the 1:80 dilution (including 2 positive at the 1:320 and 1:640 dilutions).

Our serologic survey results suggest CCHFV circulates in livestock on Corsica. Relative discrepancies between ELISA (35 positives) and PPRNT (23 positives) findings might result from their different target epitopes; the ELISA measures total immunoglobulin (neutralizing and nonneutralizing antibodies) and PPRNT just a subset (functional neutralizing antibodies) (7). Seroprevalence estimates were higher in cattle than smaller ruminants, probably reflecting that cattle in Corsica are more infested by Hy. marginatum ticks (3).

As of February 2020, CCHFV has not been detected in ticks on Corsica (8), and no clinical human case has been reported. The presence of a genetically close and less virulent strain in ticks on Corsica might help explain the lack of these findings. CCHFV was detected in ticks in Spain, where the first human cases were reported in 2016 (9), and in a tick collected on a migratory bird in Italy (10). Entomologic and epidemiologic investigations to identify the incriminated strain and characterize its spatial distribution are ongoing. This work will be essential to assess the risk for human CCHFV exposure and raise public health awareness on Corsica and in neighboring areas.

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References

Murine typhus, an undifferentiated febrile illness endemic worldwide in tropical and subtropical seaboard regions, where rats and rat fleas (Xenopsylla cheopis) are involved in the maintenance and transmission of the etiologic agent, Rickettsia typhi (1). Once prevalent in the United States, the disease was nearly eradicated following vector control practices of the 1940s using DDT (2). In 2012, murine typhus was identified in 2 patients from Galveston, Texas. The identification of cases in a city where murine typhus was perceived to have been eliminated prompted the investigation and identification of 12 patients from Galveston County in 2013 (3). Since then, murine typhus has been reported to the Galveston County Health District (GCHD) yearly (1 case in 2014, 8 in 2015, 2 in 2016, and 17 in 2017). Murine typhus has also increased in prevalence and distribution throughout Texas (4). To call attention to this increasingly prevalent disease, we
describe an increase of murine typhus reported to the GCHD in 2018.

In Texas, murine typhus is a reportable disease. Our data were collected as part of investigatory efforts of the GCHD to document typhus group rickettsioses (TGR) in Galveston County. The case definition for confirmed TGR includes a clinically compatible illness (acute onset of fever with ≥1 symptoms including headache, myalgia, anorexia, rash, nausea/vomiting, thrombocytopenia, and elevated hepatic transaminases) with 1 of the following: IgG seroconversion from acute- and convalescent-phase specimens, PCR amplification of *R. typhi*, immunohistochemical demonstration of the organism within tissue specimens, or culture isolation of *R. typhi*. We defined probable cases as those having an indirect immunofluorescence assay antibody titer of ≥1:128 during a compatible illness.

In 2018, a total of 40 cases (12 confirmed and 28 probable) were reported to the GCHD. All cases were considered as TGRs based on serology and were identified throughout the county, affecting persons from 8 cities (Dickinson, Friendswood, Galveston, Kemah, La Marque, League City, Santa Fe, Texas City) and 2 unincorporated areas (Appendix Figure). The city of Galveston reported the most cases (11, 28%). Although 1 case occurred in January, the others occurred during April–November, with the greatest numbers in June (8, 20%) and July (10, 25%). There was a male predominance (23, 58%) and the median age was 46.5 (range 4–87) years. Frequently reported signs and symptoms included fever (40, 100%), malaise (36, 90%), myalgias (34, 85%), headache (29, 73%), anorexia (22, 55%), nausea/vomiting (22, 55%), rash (20, 50%), and elevated hepatic transaminases (17, 43%) (Table). With the exception of more nausea/vomiting in probable cases, there were no differences in signs or symptoms between confirmed and probable cases. Thirty (75%) patients were hospitalized, and 2 (5%) required intensive care. Details of treatment were known in 35 cases (34 patients received doxycycline and 1 received ciprofloxacin). No deaths were reported. Flea exposure was reported frequently (22, 55%). Patients reported a variety of exposures to mammalian hosts for fleas (Table).

Murine typhus was once prevalent in the United States, with a peak of 5,401 cases in 1944. After eradication efforts using DDT were implemented in 1945, incidence plummeted (e.g., 98 cases in 1956) (2). In the decades that followed, parts of southern California and the most southern counties of Texas remained endemic. In these areas, a transmission cycle involving opossums (*Didelphis virginiana*) and cat fleas (*Ctenocephalides felis*) was suspected (1). After the recognized

<table>
<thead>
<tr>
<th>Feature</th>
<th>Confirmed cases, n = 12</th>
<th>Probable cases, n = 28</th>
<th>Total, n = 40</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>12 (100)</td>
<td>28 (100)</td>
<td>40 (100)</td>
<td>0.99</td>
</tr>
<tr>
<td>Malaise</td>
<td>10 (83)</td>
<td>26 (93)</td>
<td>36 (90)</td>
<td>0.57</td>
</tr>
<tr>
<td>Myalgia</td>
<td>11 (92)</td>
<td>23 (82)</td>
<td>34 (85)</td>
<td>0.65</td>
</tr>
<tr>
<td>Headache</td>
<td>9 (75)</td>
<td>20 (71)</td>
<td>29 (73)</td>
<td>0.99</td>
</tr>
<tr>
<td>Anorexia</td>
<td>7 (58)</td>
<td>15 (54)</td>
<td>22 (55)</td>
<td>0.99</td>
</tr>
<tr>
<td>Nausea/vomiting</td>
<td>3 (25)</td>
<td>19 (68)</td>
<td>22 (55)</td>
<td>0.02</td>
</tr>
<tr>
<td>Chills</td>
<td>1 (8)</td>
<td>8 (29)</td>
<td>9 (23)</td>
<td>0.23</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>0</td>
<td>5 (18)</td>
<td>5 (13)</td>
<td>0.30</td>
</tr>
<tr>
<td>Rash</td>
<td>7 (58)</td>
<td>13 (46)</td>
<td>20 (50)</td>
<td>0.73</td>
</tr>
<tr>
<td>Hepatic transaminase elevation†</td>
<td>5 (42)</td>
<td>12 (43)</td>
<td>17 (43)</td>
<td>0.99</td>
</tr>
<tr>
<td>Thrombocytopenia‡</td>
<td>6 (50)</td>
<td>5 (18)</td>
<td>11 (28)</td>
<td>0.06</td>
</tr>
<tr>
<td>Flea exposure§</td>
<td>5 (42)</td>
<td>17 (61)</td>
<td>22 (55)</td>
<td>0.32</td>
</tr>
<tr>
<td>Opossum exposure¶</td>
<td>6 (50)</td>
<td>20 (71)</td>
<td>26 (65)</td>
<td>0.28</td>
</tr>
<tr>
<td>Dog ownership#</td>
<td>5 (42)</td>
<td>18 (64)</td>
<td>23 (58)</td>
<td>0.30</td>
</tr>
<tr>
<td>Cat ownership#</td>
<td>6 (50)</td>
<td>7 (25)</td>
<td>13 (33)</td>
<td>0.15</td>
</tr>
<tr>
<td>Stray dog exposure#</td>
<td>2 (17)</td>
<td>4 (14)</td>
<td>6 (15)</td>
<td>0.99</td>
</tr>
<tr>
<td>Stray cat exposure#</td>
<td>4 (33)</td>
<td>16 (57)</td>
<td>20 (50)</td>
<td>0.30</td>
</tr>
<tr>
<td>Rodent exposure**</td>
<td>4 (33)</td>
<td>6 (21)</td>
<td>10 (25)</td>
<td>0.45</td>
</tr>
<tr>
<td>Raccoon exposure¶</td>
<td>2 (17)</td>
<td>3 (11)</td>
<td>5 (13)</td>
<td>0.63</td>
</tr>
</tbody>
</table>

*Fisher exact test was performed to compare the frequency of features in those with confirmed versus probable murine typhus. With the exception of more nausea/vomiting in the probable cases group, there were no significant differences between the groups.
†Defined as any aspartate aminotransferase or alanine aminotransferase level over the reference laboratories’ upper limit of normal.
‡Defined as <150,000 platelets/μL blood.
§Patients were asked whether fleas were present in their environment (i.e., yard, neighborhood, house, work) or if they had been bitten by fleas in the 3 weeks before disease onset.
¶Patients were asked whether other wild animals were present in their environment (i.e., yard, neighborhood, house, work). If they answered yes, they were asked to specify the type of animal.
#Patients were asked whether dogs or cats were present in their environment (i.e., yard, neighborhood, house, work). If they answered yes, they were asked whether these animals were owned or strays.
**Patients were asked whether rodents were present in their environment (i.e., yard, neighborhood, house, work).
The reemergence of murine typhus in Galveston, studies have demonstrated that 67% of opossums are seroreactive and 7% of C. felis fleas collected from these animals are infected with R. typhi (5). In contrast, only 0.3% of C. felis fleas collected from feral cats are infected (6). Although the detailed reservoir–vector dynamics involved with the increasing distribution of R. typhi are yet to be elucidated, the broad geographic range of opossums in North America and the ubiquity of cat fleas parasitizing opossums (7) cause concern for the spread of murine typhus to areas not yet endemic.

The increase in reported cases may be related not only to ecologic factors but also to increased recognition by physicians and enhanced surveillance. Indeed, murine typhus is well recognized at the University of Texas Medical Branch (UTMB), the largest healthcare system in Galveston County. Furthermore, murine typhus is now recognized as a cause of fever in local children. In a study performed at UTMB during 2012–2016, no pediatric patients had yet been identified (8). The later recognition in the pediatric age group was spurred in part by a pediatric case in the fall of 2017, which occurred after local education efforts. Two recently published case series of murine typhus in children may have also contributed (9,10). Finally, since September 2017, the GCHD has distributed several email advisories alerting local physicians of murine typhus.

The rise of murine typhus in Galveston is in step with the apparent increase in distribution across Texas. Awareness of this difficult to recognize undifferentiated febrile illness is paramount not only within Texas but also in other parts of the United States where opossums and cat fleas are distributed.

Acknowledgments
We thank David H. Walker for his thoughtful review and advice.

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Human Adenovirus 7d Strains Associated with Influenza-Like Illness, New York, USA, 2017–2019

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Human adenovirus 7d is a respiratory pathogen capable of causing acute respiratory disease of variable severity. Phylogenetic analysis of whole-genome sequences of 15 strains isolated from cases of influenza-like-illness during 2017–2019 demonstrated the circulation of 2 distinct clades of genomic variant 7d in colleges in New York, USA.

Human adenovirus genome (HAdV) type 7d (HAdV-7d) was first detected in the United States in December 2013 in Oregon, in association with acute respiratory disease (ARD) requiring hospitalization (1). In 2014, it was detected in more persons with ARD in Oregon and in Illinois in 2 adults with severe pneumonia (2). Circulation of this genome type, probably imported from East Asia, where its re-emergence was first documented in 2009 (3), has been detected since 2013 in other locations and settings in the United States in association with ARD of variable severity in children and adults, including in a long-term care facility in New Jersey (4); the US Marine Corps Officer Candidates School in Quantico, Virginia (5); and the University of Maryland (https://www.cnn.com/2018/11/21/health/university-of-maryland-death-adenovirus).

The recent increased detection of this distinct genomic variant of HAdV-7 in the United States and its association with severe disease manifestations has prompted public health laboratories to be more vigilant about detection of HAdVs in association with ARD. The Wadsworth Center of the New York State Department of Health (Albany, NY, USA), in collaboration with the Lovelace Respiratory Research Institute (Albuquerque, NM, USA), has monitored the prevalence of respiratory HAdVs detected in New York state since 2012. We characterized 15 HAdV-7 strains isolated from influenza virus-negative respiratory specimens collected from students with influenza-like illness at colleges in Tompkins, Albany, and Clinton counties in New York during the 2016–17, 2017–18, and 2018–19 influenza seasons. We used next-generation whole-genome sequencing and phylogenetic analysis to investigate possible epidemiologic connections among the New York college outbreaks and to monitor the dispersion of this reemerging variant within the United States.

Clinical specimens were initially tested for influenza viruses using a real-time reverse transcription PCR diagnostic panel and subsequently tested for HAdV as previously described (6). Samples testing positive for HAdV were processed for molecular typing by amplification and sequencing of hyper-variable regions 1–6 of the hexon gene (7) and for virus isolation by conventional culture with standard techniques. Intracellular genomic HAdV DNA was purified from infected A549 cells exhibiting cytopathic effect and used for initial genetic characterization by restriction enzyme analysis and for next-generation sequencing with Illumina MiSeq (Illumina, https://www.illumina.com), as previously described (6). We aligned genomic sequences generated in this study and reference sequences from GenBank in Geneious Pro R11 using MAFFT (https://www.geneious.com). We constructed a maximum-likelihood tree using MEGA6 (8). We also generated in silico digests of the genomic sequences in Geneious Pro R11. We annotated all sequences using VAPiD (9) and uploaded to GenBank (accession nos. MH921831–42, MK405661, and MN638755–56).

Initial digestion of viral DNA with endonucleases BanHI, BstEII, Hpal and subsequent in silico digestion of the corresponding complete genomic sequences identified all isolated strains as corresponding to genome type 7d (data not shown). Phylogenetic analysis of whole-genome sequencing demonstrated the co-circulation of 2 distinct clades of HAdV-7d strains in New York, even within the same county, in the sampled time period (Figure). The first clade comprised 11 strains isolated during March 2017–February 2019. Nine of these were genetically related to strains isolated in 2016–2017 in New Jersey (NJ/5644/2016 and NJ/6295/2017), and 2 were more closely related to a 2014 strain isolated in Oregon (OR/CDC2014012.949/2014). The second clade comprised 4 strains isolated during March and April 2017 that were closely related to strain DG01 isolated in 2011 in China and genetically similar to a sample isolated in 2017 from Virginia (VA/5677/2017). Both clades share a common ancestor, strain GZ6965, isolated in Guangdong, China, in 2011.
Our phylogenetic analysis provides strong evidence of ≥2 introductions of genomic variant 7d into New York state. Spatiotemporal analysis of a larger genomic sequence dataset with better representation of strains isolated in other US states, as well as in other countries, over an extended period is necessary to more accurately track the introduction of lineages and follow their dispersion.

As in our previous studies and those of others (4–6,10), these findings highlight the importance of HAdV as a causative agent of ARD in civilian communities and the value of college student populations for sentinel surveillance of HAdV activity. These data also provide another example of the power of whole-genome sequencing analysis for the epidemiologic investigation of HAdV-associated disease (9,10).

All HAdV-7d strains examined in this study were isolated from persons with influenza-like illness. Numerous recent publications report severe and even fatal cases of ARD in association with this genomic variant. All HAdV-7d strains sequenced thus far are indistinguishable by restriction enzyme analysis but not identical when examined at the whole-genome level. Characterization of more strains of diverse origins and associated disease and thorough mining of sequence data is needed to identify candidate determinants of virulence for HAdV-7. Host-related and other environmental risk factors are likely to contribute to the level of susceptibility, clinical presentation, and outcome of the associated disease.

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Risk for Transportation of Coronavirus Disease from Wuhan to Other Cities in China

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On January 23, 2020, China quarantined Wuhan to contain coronavirus disease (COVID-19). We estimated the probability of transportation of COVID-19 from Wuhan to 369 other cities in China before the quarantine. Expected COVID-19 risk is >50% in 130 (95% CI 89–190) cities and >99% in the 4 largest metropolitan areas.

In December 2019, a novel coronavirus, since named severe acute respiratory syndrome coronavirus 2, emerged in Wuhan, China (1), causing a respiratory illness that the World Health Organization has named coronavirus disease (COVID-19). On January 30, 2020, the World Health Organization declared the outbreak a public health emergency of international concern (2). By January 31, 2020, a total of 192 fatalities and 3,215 laboratory-confirmed cases had been reported in Wuhan; 8,576 additional cases were spread across >300 cities in mainland China, and 127 exported cases were reported in 23 countries spanning Asia, Europe, Oceania, and North America. The rapid global expansion, rising fatalities, unknown animal reservoir, and evidence of person-to-person transmission potential (3,4) initially resembled the 2003 SARS epidemic and raised concerns about global spread.

On January 22, 2020, China announced a travel quarantine of Wuhan and by January 30 expanded the radius to include 16 cities, encompassing a population of 45 million. At the time of the quarantine, China was already 2 weeks into the 40-day Spring Festival, during which residents and visitors make several billion trips throughout China to celebrate

¹These first authors contributed equally to this article.
the Lunar New Year (5). Considering the timing of exported COVID-2019 cases reported outside of China, we estimate that only 8.95% (95% credibility interval [CrI] 2.22%–28.72%) of persons infected in Wuhan by January 12 might have had COVID-19 confirmed by January 22. By limiting our estimate to infections occurring ≥10 days before the quarantine, we account for an ≈5–6-day incubation period and 4–5 days between symptom onset and case detection (Appendix, https://wwwnc.cdc.gov/EID/article/26/5/20-0146-App1.pdf) (2–4,6). The low detection rate coupled with an average lag of 10 days between infection and detection (7) suggest that newly infected persons who traveled out of Wuhan just before the quarantine might have remained infectious and undetected in dozens of cities in China for days to weeks. Moreover, these silent importations already might have seeded sustained outbreaks that were not immediately apparent.

We estimated the probability of transportation of infectious COVID cases from Wuhan to cities throughout China before January 23 by using a simple model of exponential growth coupled with a stochastic model of human mobility among 369 cities in China (Appendix). Given that ≈98% of all trips taken during this period were made by train or car,

**Figure.** Risks for transportation of coronavirus disease (COVID-19) from Wuhan, China, before a quarantine was imposed on January 23, 2020. A) Daily travel volume to and from Wuhan, given as a percentage of the Wuhan population. Gray shading indicates the start of Spring Festival season on January 10, 2020, a peak travel period in China. B) Estimated and reported daily prevalence of COVID-19 in Wuhan. The green line and shading indicate model estimates of cumulative cases since December 1, 2019, with 95% credible interval bounds, assuming an epidemic doubling time of 7.31 days (95% credible interval 6.26–9.66 days). Black dots indicate cumulative confirmed case counts during January 1–22, 2020 (10). Gray shading at right indicates the start of Spring Festival season. C) Probability that ≥1 COVID-19 case infected in Wuhan traveled to cities in China by January 22, 2020. The 131 cities with a risk threshold >50% are indicated in shades of orange; 239 cities below that threshold are indicated in shades of blue. Map generated by using Mapbox (https://www.mapbox.com).
our analysis of air, rail, and road travel data yields more granular risk estimates than possible with air passenger data alone (8).

By fitting our epidemiologic model to data on the first 19 cases reported outside of China, we estimate an epidemic doubling time of 7.31 days (95% CrI 6.26–9.66 days) and a cumulative total of 12,400 (95% CrI 3,112–58,465) infections in Wuhan by January 22 (Appendix). Both estimates are consistent with a similar epidemiologic analysis of the first 425 cases confirmed in Wuhan (4). Assuming these rates of early epidemic growth, we estimate that 130 cities in China have a ≥50% chance of having a COVID case imported from Wuhan in the 3 weeks preceding the quarantine (Figure). By January 26, a total of 107 of these 130 high-risk cities had reported cases. However, 23 had not, including 5 cities with importation probabilities >99% and populations >2 million: Bazhong, Fushun, Laibin, Ziyang, and Chuxiong.

Under our lower bound estimate of 6.26 days for the doubling time, 190/369 cities lie above the 50% threshold for importation. Our risk assessment identified several cities throughout China likely to be harboring yet undetected cases of COVID-19 a week after the quarantine, suggesting that early 2020 ground and rail travel seeded cases far beyond the Wuhan region under quarantine.

Our conclusions are based on several key assumptions. To design our mobility model, we used data from Tencent (https://heat.qq.com), a major social media company that hosts applications including WeChat (=1.13 billion active users in 2019) and QQ (=808 million active users in 2019) (Statista, https://www.statista.com); consequently, our model might be demographically biased by the Tencent user base. Further, considerable uncertainty regarding the lag between infection and case detection remains. Our assumption of a 10-day lag is based on early estimates for the incubation period of COVID-19 (4) and prior estimates of the lag between symptom onset and detection for SARS (9). We expect that estimates for the doubling time and incidence of COVID-19 will improve as reconstructed linelists and more granular epidemiologic data become available (Appendix). However, our key qualitative insights likely are robust to these uncertainties, including extensive prequarantine exportations throughout China and far greater case counts in Wuhan than those reported before the quarantine.

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We thank Henrik Salje, Dongsheng Luo, Bo Xu, Cécile Tran Kiem, Dong Xun, and Lanfang Hu for helpful discussions. Code for estimating epidemiological parameters and probabilities of case introductions, as well as aggregate mobility data, are available from GitHub (https://github.com/linwangidd/2019nCoV_EID). Aggregate data also are available (Appendix Table 3). Additional code and data requests should be addressed to L.A.M. (laurenmeyers@austin.utexas.edu).

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Dr. Du is a postdoctoral researcher in the Department of Integrative Biology at the University of Texas at Austin. He develops mathematical models to elucidate the transmission dynamics, surveillance, and control of infectious diseases.

References
In January 2020, we investigated a 2-family cluster of persons infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in the city of Zhoushan in Zhejiang Province, China. We attributed the infections to contact with an infected but potentially presymptomatic traveler from the city of Wuhan in Hubei Province. Our epidemiologic investigation was reviewed and approved by the Ethics Committee of the Zhoushan Centers for Disease Control and Prevention (CDC).

The initial 2 cases of SARS-CoV-2 infection (coronavirus disease [COVID-19]) in Zhoushan were diagnosed in 2 teachers (persons A and D) from the same department at a college that had sponsored an academic conference on January 5, 2020. A 45-year-old teacher from Wuhan (person W) arrived on January 5 for the conference and joined persons A and D on January 6 for dinner, where they ate from common serving plates. After returning to Wuhan on January 7, person W experienced the onset of fever, cough, sore throat, and malaise on January 8. He visited a local hospital where, according to the patient’s self-report, he was confirmed to have COVID-19 by a local office of the Chinese CDC. For person A and D, the only known potential exposures for SARS-CoV-2 were their dinner and conference attendance with person W (Figure).

On January 10, person A (a 29-year-old man) experienced the onset of fever, cough, and skin tingling and went to a local hospital for treatment. Laboratory tests at the hospital indicated leukopenia, and a real-time reverse transcription PCR (rRT-PCR) test for influenza A and B viruses was negative. The patient was given an antipyretic and some traditional medicines commonly used in China. After 3 days, his fever subsided, but his cough persisted. On January 15, the patient went to a different hospital, where routine blood test results were unremarkable but a chest radiograph revealed bilateral invasive lesions. He was prescribed amoxicillin and levofloxacin for 3 days. Because his cough did not improve, he was hospitalized for further evaluation. When the treating physician learned that the patient had had contact with a visitor from Wuhan before symptom onset, a throat swab specimen was sent for rRT-PCR testing for SARS-CoV-2 (1). On January 19, SARS-CoV-2 infection was confirmed at the laboratory of the Zhoushan CDC.

Person A lived with his 28-year-old wife (person B) and his 21-year-old sister (person C). The 2 women were confined at home for 14 days starting on the day of person A’s hospital admission. Because of their 10 days of contact with person A after his fever onset, their respiratory specimens were collected on January 20 by Zhoushan CDC staff for

We report a 2-family cluster of persons infected with severe acute respiratory syndrome coronavirus 2 in the city of Zhoushan, Zhejiang Province, China, during January 2020. The infections resulted from contact with an infected but potentially presymptomatic traveler from the city of Wuhan in Hubei Province.
SARS-CoV-2 testing. Person B was confirmed on January 20 to be positive for SARS-CoV-2 infection by rRT-PCR but had no symptoms. Person C remained negative for SARS-CoV-2 during her quarantine period.

On January 12, person D (a 42-year-old man) experienced onset of low-grade fever, cough, and myalgia. He did not seek medical attention, and he traveled on business to the city of Sanya in Hainan Province during January 16–18. In Sanya, he used over-the-counter medications for his dry cough, which did not improve. On January 19, after returning to Zhoushan, he was informed that his colleague (person A) had been diagnosed with laboratory-confirmed COVID-19; person D was then transported by ambulance to the local infectious diseases hospital for isolation and specimen testing. That night, his throat swab specimen was confirmed to be weakly positive for SARS-CoV-2 by rRT-PCR.

Person D’s 41-year-old wife (person E) and his 12-year-old son (person F) were confined at home starting the day of person D’s hospital admission (January 19). On January 21, Zhoushan CDC staff collected respiratory specimens from persons E and F to test for SARS-CoV-2 infection, which was later confirmed by rRT-PCR, despite their lack of symptoms.

On January 20, person W was interviewed by telephone by epidemiologists at Zhoushan CDC; he recalled having no prodromal or other symptoms on January 6, when he dined with persons A and D.

Our findings are subject to several limitations. The investigators conducted a telephone interview with the index patient (person W), who might have not recalled the full extent of his symptoms 14 days prior. Similarly, asymptomatic persons might have failed to report mild or subclinical symptoms. We cannot rule out other SARS-CoV-2 exposures to persons A and D in addition to their dinner and conference attendance with person W. Furthermore, we did not conduct convalescent serologic testing to provide additional evidence of the asymptomatic SARS-CoV-2 infections.

In summary, we identified 2 persons with confirmed cases of symptomatic COVID-19 after their exposure to a potentially presymptomatic person who was later diagnosed with laboratory-confirmed COVID-19. These 2 persons later transmitted SARS-CoV-2 to 3 family members, who did not report symptoms at the time their SARS-CoV-2 infections were detected.

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We thank all the persons involved in the response to this outbreak.
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Superbugs: The Race to Stop an Epidemic


Infectious disease professionals should not be put off by this book’s title. Sensationalist titles are de rigueur for books aimed at the general public. I do hope the title grabs that audience because the more lay readers who read this book, the better (great gift for families, friends, and especially young persons seeking meaningful careers). However, this book contains plenty of information for professionals, too. And it is a rattling good read that will greatly shorten a long flight.

Written by Matt McCarthy, a physician-scientist at Weill Cornell Medicine (New York, NY, USA), Superbugs is a vitally important tale of one arena in which the antimicrobial resistance crisis is playing out: New York City. McCarthy gives the book narrative drive by focusing on a clinical trial of a new antimicrobial drug (dalbavancin). He conducted the drug trial and only recently completed it, so the tale is personal and has a gripping immediacy. Using the trial as a hook, McCarthy discusses many aspects of antimicrobial resistance, from its origins (historic and within patients) to the history of antimicrobial drugs, medical ethics, medical malpractice, health economics, and perhaps most powerful of all the impact of antimicrobial resistance on individual patients and the physicians who care for them.

McCarthy is quite the storyteller—he even manages to wrestle drama and excitement out of the institutional review board process—but he is at his best describing the human impact of failing antimicrobial drugs. Healthcare professionals in the trenches with patients will find good therapy here. Professionals, like me, who work on antimicrobial resistance but do not see patients will be reminded why the work is so vital. And professionals who work on other aspects of infectious diseases and wonder whether antimicrobial resistance is overhyped, I implore you to read this important book.

Antimicrobial resistance is a complex, multifaceted problem. No magic bullets will make the problem vanish. It will be with us forever. McCarthy, like many, argues that much use of antimicrobial drugs is inappropriate (for example, for farming or viral infections), and of course we should avoid using them when they are unnecessary. I’d love to understand how much inappropriate use exacerbates the problem created by appropriate use of antimicrobial drugs. I know of no quantitative analysis. It is quite possible that medically appropriate use is the main driver of resistance evolution.

With a drug trial at the heart of the book, McCarthy argues the key solution lies in discovering new drugs. Yet, as he vividly points out, returns on investment are awful for antimicrobial development—and getting worse. Other avenues being explored—vaccines, phages, probiotics, combination therapies—seem as likely to break us out of unsustainable open-ended drug discovery treadmills. Whatever the answer, McCarthy’s book makes a strong case that we can make serious inroads on antimicrobial resistance if we focus scientific and clinical firepower on the problem. His stories of patients show that we really must.

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While walking along the bustling streets of Beijing, Chengde, Shenyang, Wuhan, or other Chinese cities during the Qing dynasty (1644–1911), people would regularly brush past bats, cranes, pheasants, peacocks, egrets, or ducks; slow their step so a lion, leopard, tiger, rhinoceros, or bear could hurry past; or yield to allow passage to a dragon, unicorn, or qilin (a chimera with horns, a dragon’s head, fish scales, an oxen’s tail, horse’s hooves, and multicolored skin). Of course, it was not those actual animals jostling their way through the crowded causeways but rather myriad Chinese statesmen, civic officials, military officers, and members of the imperial court, as well as their wives, all of whom indicated their rank and status by wearing embroidered badges featuring images of those creatures on their outer coats.

From the late 14th century until the early 20th century, these ornate rank badges (called buzì or Mandarin squares) featured fierce animals to denote military officials, various bird species to identify civic officials, and exotic and imaginary creatures to signify members of the imperial court. Art historian Mary Dusenbury writes, “Qing badges generally include an abbreviated cosmic diagram with an earth-mountain in the lower center, and a multitude of auspicious symbols filling up the surrounding space. In the center, the animal or bird looks up at a prominent red sun, symbol of the emperor.”

This month’s cover image is an 18th century Qing military rank badge that depicts a muscular leopard standing on a small piece of light brown, green-tinged
land amidst flowering plants and fruit trees. The surrounding sky is filled with swooping bats and tendrils of clouds. Stylized ocean waves rise and swell, and breakers reach out like fingers on a hand. What may be lingzhi mushrooms, which are used in traditional Chinese medicine and are also symbols of immortality, sprout from the junction of ocean with land. The small red sun disk in the upper left represents the emperor and provides a focal point for the leopard. The leopard itself symbolizes power, important for military officers. Combined, the various elements related to the sky, sea, and land denote the universe, and the bats indicate good fortune. The bold colors and textures of the finely woven threads stand out in contrast to the black background, and an intricately designed gold border wraps the edges of the badge.

According to the University of Michigan Museum of Art, “During the Ming dynasty, the leopard and tiger shared the third and fourth ranks and in the early years of the Qing dynasty the tiger was the third rank. From 1662 to the end of the Qing dynasty, the leopard solely represented the third military rank.”

Other changes in rank, which correspond to the start of Emperor Kangxi’s reign, included assigning the qilin and lion to represent the first and second military ranks, respectively—both ranks had previously been symbolized by lions. These woven or embroidered badges could be removed and replaced should the official or office be promoted to a higher rank. Although the design of the squares evolved from one dynasty to the next—for instance, Qing badges were smaller than Ming badges and featured decorative borders—the specific birds and animals used to denote rank remained more or less constant.

The birds and animals featured on the various rank badges (excepting, among others, dragons, unicorns, and qilin) may also serve as zoonotic reservoirs capable of transmitting viral pathogens that can cause respiratory infections in humans. For example, some of the birds that signify ranks among civic officials can transmit highly pathogenic avian influenza viruses. Bats, a mainstay on many badges because of their association with good fortune, are reservoirs for Hendra and Nipah viruses and for the severe acute respiratory syndrome (SARS) coronavirus. Coronaviruses are also found in many different species of animals besides bats, including swine, camels, and cattle.

The current COVID-19 pandemic is caused by a coronavirus named SARS-CoV-2. Many factors affect interactions among humans, animals, plants, and the environment, creating greater opportunities for novel pathogens, such as SARS-CoV-2 to emerge. Perhaps one’s rank and status might confer favor in some circles, but they offer no protection from human-to-human transmission of SARS-CoV-2 and other viral respiratory infections and illnesses.

Bibliography


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EMERGING INFECTIOUS DISEASES

Upcoming Issue

- Identifying and Interrupting Superspreading Events—Implications for Control of Severe Acute Respiratory Syndrome Coronavirus 2
- Genomic Epidemiology of 2015–2016 Zika Virus Outbreak in Cape Verde
- Risks Related to Chikungunya Infections among European Union Travelers, 2012–2018
- Pharmacologic Treatments and Supportive Care for Middle East Respiratory Syndrome
- Endemic Chromoblastomycosis Caused Predominantly by Fonsecaea nubica, Madagascar
- Zoonotic Alphaviruses in Fatal and Neurologic Infections in Wildlife and Nonequine Domestic Animals, South Africa
- Failures of 13-Valent Conjugated Pneumococcal Vaccine in Age- Appropriately Vaccinated Children 2–59 Months of Age
- Severe Acute Respiratory Syndrome Coronavirus 2 from Patient with Coronavirus Disease, United States
- Estimating Risk for Death from 2019 Novel Coronavirus Disease, China, January–February 2020
- Epidemiology of Coronavirus Disease in Gansu Province, China, 2020
- Temporary Fertility Decline after a Large Rubella Outbreak, Japan
- Characterization of Sporadic Creutzfeldt-Jakob Disease and History of Neurosurgery to Identify Potential Iatrogenic Cases
- Statin Use and Influenza Vaccine Effectiveness in Persons >65 Years of Age, Taiwan
- Unexpected Genetic Diversity and Emergence of New Non-CG258 High-Risk Clones among KPC-Producing Klebsiella pneumoniae, France
- Increased Risk for Carbapenem-Resistant Enterobacteriaceae Colonization in Intensive Care Units
- Antimicrobial Resistance in Salmonella enterica Serovar Paratyphi B Variant Java in Poultry from Europe and Latin America
- Multihost Transmission of Schistosoma mansoni in Senegal
- Distribution of Group A Streptococcus Pharyngitis and Acute Rheumatic Fever, Auckland, New Zealand, 2010–2016
- Ectoparasites and Vectorborne Zoonotic Pathogens of Dogs and Cats in Asia, 2017–2018

Complete list of articles in the June issue at http://www.cdc.gov/eid/upcoming.htm

Upcoming Infectious Disease Activities

EID advises readers to confirm information on the conference website before registering or making travel plans.

May 3–6, 2020
ASM Clinical Virology Symposium
West Palm Beach, FL, USA

June 18–22, 2020
American Society for Microbiology
ASM Microbe 2020
Chicago, IL, USA
https://asm.org/Events/ASM-Microbe/Home

Announcements
Email announcements to EIDEditor (eideditor@cdc.gov). Include the event’s date, location, sponsoring organization, and a website. Some events may appear only on EID’s website, depending on their dates.

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.
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Article Title
Food Safety and Invasive Cronobacter Infections during Early Infancy, 1961–2018

CME Questions

1. Your patient is a 2-week-old, formula-fed infant with *Cronobacter* bloodstream infection. According to the surveillance and case series by Strysko and colleagues, which of the following statements about clinical characteristics and outcomes of *Cronobacter* bloodstream infection or meningitis among infants reported to the CDC and in the literature from 1961 to 2018 is correct?

A. Only 20% of infants had meningitis
B. Compared with patients with isolated bacteremia, patients with meningitis were significantly more likely to have younger estimated gestational age (EGA) at birth
C. Most infants (67%) were neonates; 38% died
D. Immunocompromising conditions other than prematurity occurred in one-third of patients

2. According to the surveillance and case series by Strysko and colleagues, which of the following statements about epidemiological features of *Cronobacter* bloodstream infection or meningitis among infants reported to the CDC and in the literature from 1961 to 2018 is correct?

A. Most infections were transmitted during handling by nursery staff who used improper hand hygiene
B. In the final quarter of the study (2004-2018), reporting of *Cronobacter* infections had significantly decreased
C. One-fifth of cases reported recent consumption of powdered infant formula (PIF)
D. Among *Cronobacter* isolates identified from contaminated food or environmental samples, pulsed-field gel electrophoresis patterns were indistinguishable from corresponding clinical isolates 81% of the time

3. According to the surveillance and case series by Strysko and colleagues, which of the following statements about clinical and public health implications of epidemiology and outcomes of *Cronobacter* bloodstream infection or meningitis among infants reported to the CDC and in the literature from 1961 to 2018 is correct?

A. The findings highlight the need for safer infant-feeding options, especially in neonates, and for regulatory, engineering, and behavioral solutions to minimize PIF contamination risk
B. The study proves that the true incidence of invasive infant *Cronobacter* infections appears to have increased globally, despite the lack of mandatory reporting
C. Given the low incidence of *Cronobacter* overall, there is no reason to consider mandatory reporting
D. The study proves that immunocompromised infants are at high risk for invasive *Cronobacter* infection
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Article Title
Blastomycosis in Minnesota, USA, 1999–2018

CME Questions

1. You are seeing a 26-year-old woman with a 1-week history of cough, malaise, and intermittent fever. She was diagnosed with a viral upper respiratory infection 4 days ago and given supportive care, but her condition has grown slightly worse. What should you consider regarding the epidemiology of blastomycosis infection in the current study by Ireland and colleagues?
   A. The statewide annual incidence of blastomycosis was 23 cases/100,000 population
   B. The median patient age was 24 years
   C. Most patients were male
   D. There a decline in the number of cases reported over time

2. Which of the following statements regarding clinical characteristics of blastomycosis in the current study is most accurate?
   A. >50% of patients had joint pain and back pain
   B. Most patients were hospitalized
   C. Half of infections were disseminated
   D. The most common site of disseminated infections was the central nervous system

3. Blastomycosis is in the differential diagnosis for this patient, and you are concerned regarding a delay in the diagnosis of blastomycosis. What did the current study find regarding time to diagnosis of blastomycosis?
   A. The median time to diagnosis was 11 days
   B. The median time from symptom onset to first healthcare contact was 5 days
   C. Nonpulmonary cases had a shorter time to diagnosis
   D. Cases from endemic areas had a shorter time to diagnosis

4. The patient is diagnosed with blastomycosis, but she has no idea how she acquired the infection. What did the current study reveal about exposure history among patients with blastomycosis?
   A. Nearly 60% reported ≤ 1 outdoor activity in the 3 months before the onset of illness
   B. Occupational exposure to soil was a more common exposure compared with outdoor activity
   C. Nearly half of infected patients had a relative with blastomycosis
   D. Rates of exposure away from home were <10%