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November 2017

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On the Cover

Laurence Stephen Lowry (1887–1976)
***Going to Work*, 1943**
(detail). Oil on canvas, 179.9 in × 239.8 in/457 cm × 609 cm. ©Imperial War Museums (Art.IWM ART LD 3074), Manchester, Lancashire, England, UK.

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Legionnaires' Disease Outbreaks and Cooling Towers, New York City, New York, USA

Surveillance will determine whether a new law regulating cooling towers reduces the incidence of Legionnaires' disease.

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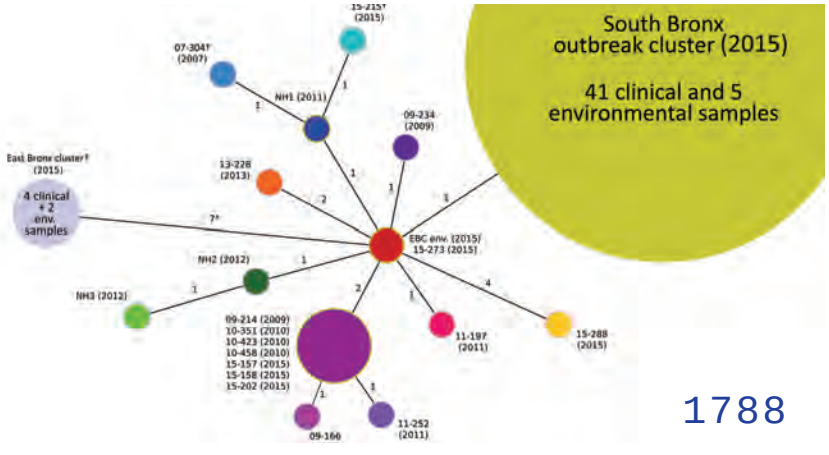

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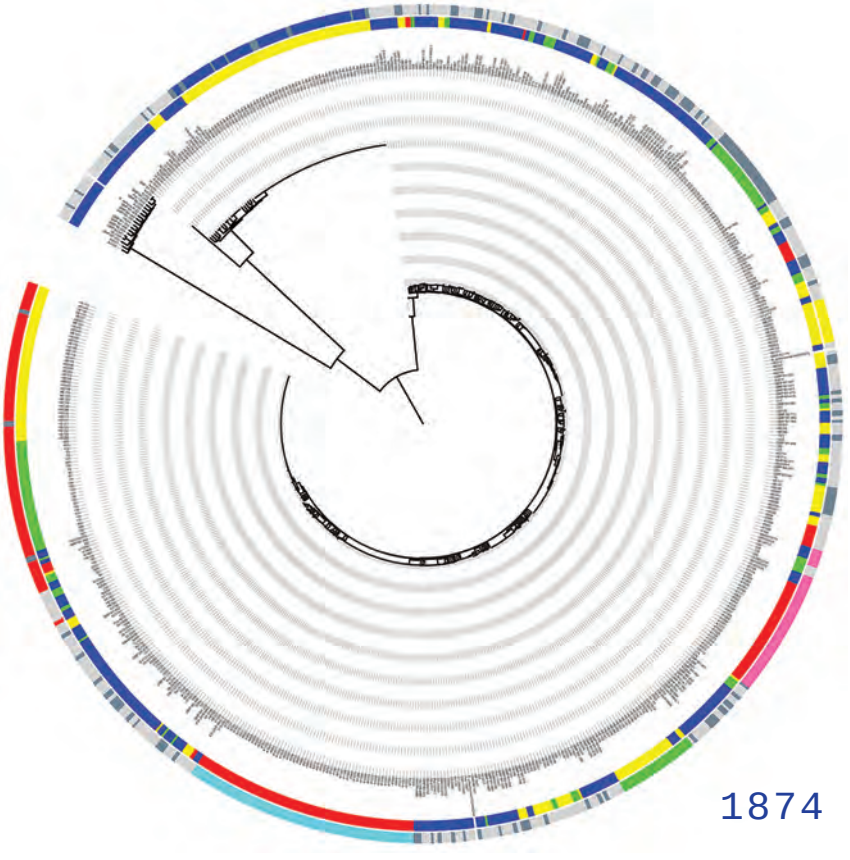

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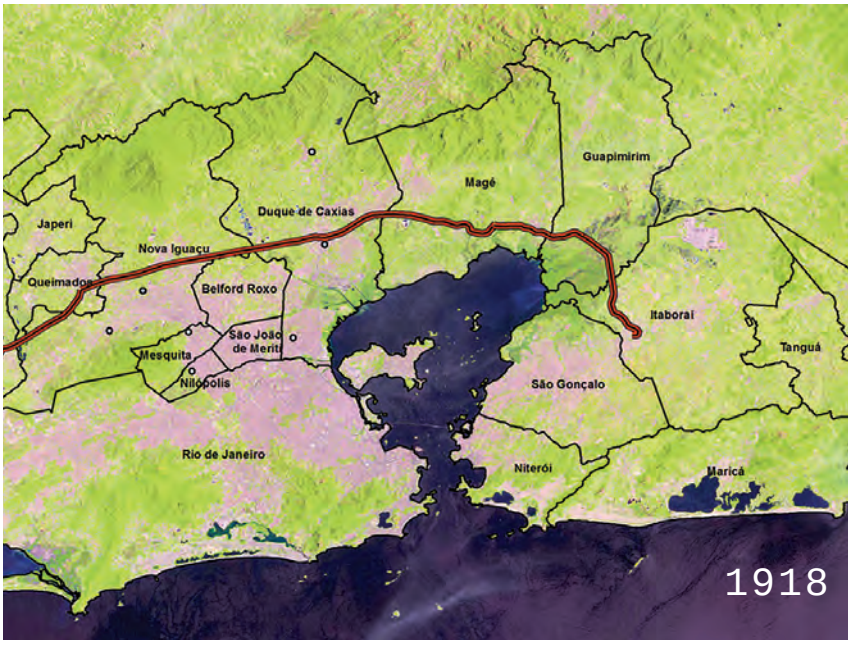

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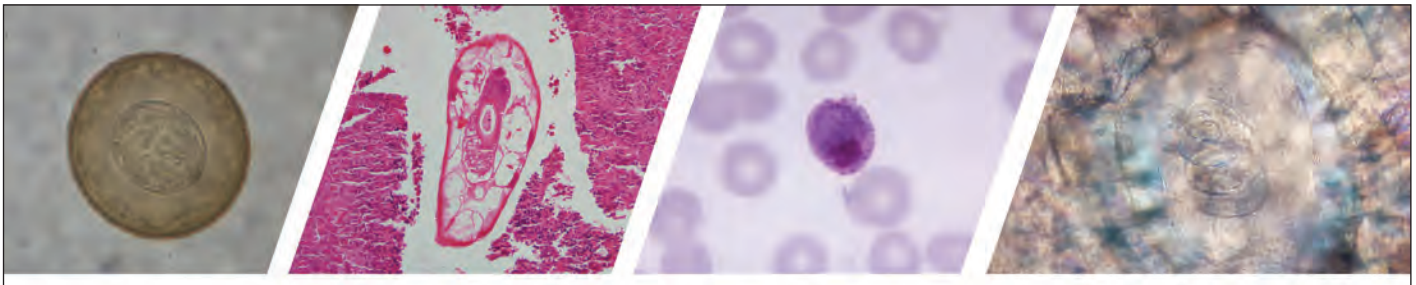

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Legionnaires' Disease Outbreaks and Cooling Towers, New York City, New York, USA

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Release date: October 12, 2017; Expiration date: October 12, 2018

Learning Objectives

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

1. Assess the epidemiological features of 6 community-associated Legionnaires' disease outbreaks occurring in New York City since 2006, based on a surveillance study
2. Evaluate the evolution of investigative methods used to study 6 community-associated Legionnaires' disease outbreaks occurring in New York City since 2006, based on a surveillance study
3. Examine the public health implications of 6 community-associated Legionnaires' disease outbreaks occurring in New York City since 2006, based on a surveillance study

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The incidence of Legionnaires' disease in the United States has been increasing since 2000. Outbreaks and clusters are associated with decorative, recreational, domestic, and industrial water systems, with the largest outbreaks being caused by cooling towers. Since 2006, 6 community-associated Legionnaires' disease outbreaks have occurred in New York City, resulting in 213 cases and 18 deaths. Three

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outbreaks occurred in 2015, including the largest on record (138 cases). Three outbreaks were linked to cooling towers by molecular comparison of human and environmental *Legionella* isolates, and the sources for the other 3 outbreaks were undetermined. The evolution of investigation methods and lessons learned from these outbreaks prompted enactment of a new comprehensive law governing the operation and maintenance of New York City cooling towers. Ongoing surveillance and program evaluation will determine if enforcement of the new cooling tower law reduces Legionnaires' disease incidence in New York City.

Legionnaires' disease (LD) is a pneumonia associated with human-made water systems and is classified as nosocomial (≈10% of cases), travel-related (≈20% of cases), or community-acquired (≈70% of cases) (*1*,*2*). LD is caused by bacteria from the genus *Legionella*, with *Legionella pneumophila* serogroup 1 (*Lp1*) being detected in up to 80% of cases (*3*). The incidence of LD has increased ≈4-fold in the United States since 2000 and ≈3-fold in Europe since 1995 (*4*,*5*). The reasons for this increase are unknown but might be partly a result of increase awareness of LD and the consequent increased testing for LD. LD outbreaks account for 4%–11% of cases; the remainder (i.e., those without a determined source of exposure) are classified as sporadic (*3*,*4*). In the United States, 62% of LD cases occur during June–October (*6*), a period of generally warm weather when commercial air conditioning systems, including those with cooling towers (CTs), are in operation. An estimated 28% of sporadic LD cases may be caused by CT emissions (*7*).

The first LD outbreak ever detected was linked to a Philadelphia hotel CT in 1976 (*8*), and since then many of the largest LD outbreaks have also been associated with CTs (*9*–*14*). Detection of LD outbreaks is made difficult by the standard medical practice of treating community-acquired pneumonia without performing diagnostic testing (*15*). When *Legionella* is suspected, the urine antigen test provides a rapid result but might miss 26% of cases (*16*). This test only detects *Lp1* and does not enable comparisons with environmental isolates (*16*). *Legionella* is a fastidious organism that requires specialized media and handling to culture. Respiratory cultures obtained after the start of antimicrobial drug use are less likely to grow; therefore, underdiagnosis and underreporting of LD cases is suspected (*6*). In addition, outbreaks of LD associated with CTs probably have gone undetected or, owing to the infrequency of obtaining clinical isolates, have been detected but not linked to a suspected CT.

In 2015, two LD outbreaks occurred in the New York City borough (county) of the Bronx, 1 of which was the largest ever in New York City and the second largest community outbreak in US history. Both outbreaks were linked to CTs by molecular characterization of clinical and environmental isolates. This prompted enactment of comprehensive legislation to regulate and inspect CTs to prevent LD outbreaks (*17*). We describe the evolution of community LD cluster detection and investigation, through the review of 6 LD outbreaks in New York City during 2006–2015, and the recent legislation enacted to control this environmental hazard.

Methods

LD has been a reportable condition to the New York City Department of Health and Mental Hygiene (DOHMH) since 1994. Every case is investigated, by chart abstraction and patient or proxy interview using standardized questionnaires, to determine whether the exposure could be associated with a healthcare facility (nosocomial), another type of building (e.g., a correctional facility, group home, hotel, shelter, residence, or workplace), or travel. Cases not belonging to these 3 categories are considered sporadic unless a link in space or time is recognized. LD cluster detection methods using surveillance data changed during the study period; during 2006–2013, the historical limits method (*18*) alone was used, and during 2013–2015, the historical limits method was modified to reduce bias (*19*). This method compares LD cases in the last 4-week period with data from the preceding 5 years and was applied at 3 geographic resolutions (citywide, borough, and neighborhood).

In addition, analyses using building identifiers (BINs) were added in January 2013 to identify LD events of public health concern. The BIN is a unique number assigned to every building in New York City, matched to patient address, and compared with a list of healthcare and other congregate facilities (*20*). The prospective space-time permutation scan statistic (SaTScan) was added in February 2014 and is used to detect LD clustering using either the home or work address that occurs within a flexible time window (*21*,*22*).

We defined a community outbreak of LD as cases meeting the Council of State and Territorial Epidemiologists definition (*23*) that were not associated with a healthcare facility or residential building and occurred in close space and time, as defined by either a markedly elevated incidence in ≥1 US postal code (ZIP code) area or by ≥1 cluster detection systems. Assignment of patients to outbreaks was defined by residential ZIP code area, work ZIP code area, and locations visited during the incubation period, as elicited during patient interviews. To identify community outbreaks, we reviewed DOHMH LD investigation reports, related files, and surveillance data for 2006–2015. The following outbreak characteristics were summarized: borough where the outbreak occurred, ZIP code areas in the outbreak zone, onset dates of cases, number of cases, LD incidence in outbreak zone compared with the rest of

New York City (using intercensal population estimate for each outbreak year by ZIP code area), number of deaths, the proportion of patients who were culture-positive for *Legionella*, environmental test results, link between environmental and clinical isolates, intervals from detection of the outbreak to environmental source decontamination, and whether an outbreak source was found.

Environmental sample *Legionella* testing was conducted by the New York State Department of Health Wadsworth Center (WC), the DOMHH Public Health Laboratory (PHL), and independent contractors. Criteria used to classify positive environmental results differed by laboratory. WC and PHL considered any culture growth as positive, whereas independent contractors used various CFU thresholds to define positive results (*24*,*25*). Culture and pulsed-field gel electrophoresis (PFGE) were performed by PHL, and real-time PCR (rPCR) and whole-genome sequencing (WGS) were performed by WC. Remediation was recommended whenever *Legionella* species known to be a risk to human health were identified. The study was determined to be public health surveillance and did not undergo institutional board review. Analyses were conducted using SAS version 9.2 (SAS Institute Inc., Cary, NC, USA) and SPSS version 22.0 (IBM Corp., Armonk, NY, USA).

Results

During January 1, 2006–December 31, 2015, a total of 2,262 confirmed LD cases were reported in New York City residents. Six community-associated LD outbreaks, comprising 213 total cases, 207 of which were in New York City residents (9.7% of all New York City cases), occurred during the study period (online Technical Appendix Figure, https://wwwnc.cdc.gov/EID/article/23/11/16-1584-Techapp1.pdf). Three outbreaks and 84% (174/207) of outbreak-associated cases occurred in Bronx residents. Cultures were positive for *Legionella* spp. for 14.5% (30/207) of New York City resident outbreak-associated cases (all *Lp1*) and 6.3% (130/2,055) of New York City resident non–outbreak-associated cases (90 *Lp1*, 2 *L. pneumophila 3* [*Lp3*], 4 *L. pneumophila 4* [*Lp4*], 3 *L. pneumophila 5* [*Lp5*], 1 *L. pneumophila 6* [*Lp6*], 3 *L. micdadei*, and 27 *L. pneumophila* of an undetermined serogroup).

Outbreak 1 was recognized in the spring of 2006, when an epidemiologist (D.C.) noticed that several LD cases occurred in residents of a large apartment complex (>100 buildings). Twenty-nine cases occurred in the outbreak zone, consisting of 5 ZIP code areas. LD incidence in the outbreak zone during June–October 2006 was 9.7 cases/100,000 persons compared with 1.1/100,000 for the rest of New York City (Table 1). Interviews of patients failed to identify a common exposure in >35% of respondents. No patients were culture-positive for *Legionella*, and no environmental sampling was conducted at the time. Four additional LD cases occurred in the outbreak zone in a 3-week period of May–June 2007. Environmental sampling was performed on 2 supermarkets (a mister and a CT), a department store CT, and a decorative fountain. The department store CT was culture-positive for *Lp5* and a supermarket CT for *Lp3*. No source for the original outbreak or subsequent cases was determined.

The only community outbreak of LD detected in the borough of Manhattan occurred in the summer of 2008 (outbreak 2). An epidemiologist (D.C.) identified 7 cases clustered in space and time, and all but 1 patient was either a resident of federally subsidized (n = 2) or supportive housing for formerly homeless persons (n = 4). Two buildings were associated with 2 cases each. The LD incidence in the 3-ZIP code outbreak zone was 9.8/100,000 compared with 0.4/100,000 for the rest of New York City. Potable and hot water systems in 3 buildings, an irrigation system, a supermarket misting system, and 2 supermarket CTs were tested for *Legionella*. *L. pneumophila* serogroup 1 was isolated from a supportive housing building, and *L. anisa* from another residential building, both from the hot water systems. Testing of the supermarket CTs identified *Lp*6 in 1 and *L. bozemanii* in the other. No clinical *Legionella* cultures were obtained, and no definitive source of the outbreak was identified.

In the winter of 2014–2015, outbreak 3 was detected by the historical limits method, which signaled for the Bronx and was subsequently focused by a BIN analysis signal that occurred 29 days later. The cases were associated with a large apartment complex that was unique in that it had its own electricity-generating power plant that used a CT. Eight LD cases occurred over 3 months for an outbreak zone (single ZIP code area) incidence of 18.8/100,000, whereas the rest of New York City had an LD incidence of 0.5/100,000. Of note, 2 previous LD cases had occurred in this apartment complex during 2012–2013; these are not included in outbreak or rate calculation.

The outbreak marked DOHMH's first use of rPCR to screen potential environmental sources. Four sites were sampled: the power plant CT, a mall CT, apartment building potable water, and water from a grocery store mister. Both *Lp1* and *Lp6* were identified in multiple water samples from the power plant CT by rPCR (29/30 samples) and culture (27/30 samples). The mall CT was positive for *Lp6* by rPCR (7/10 samples) and culture (1/7 samples). No *Lp1* was cultured from the potable water samples from the apartment complex; however, a consultant environmental service found *L. anisa* (2 samples from the same apartment). Samples from the supermarket mister were negative by rPCR and were not cultured. One patient isolate of *Lp1* was recovered and was shown by PFGE to be indistinguishable from an isolate from the power plant CT. The

Table 1. Community outbreaks of Legionnaires' disease, New York City, New York, USA, 2006–2015*

NYC borough (outbreak no.)	Outbreak dates	Outbreak zone ZIP codes	No. cases		Crude rate†		Median age, y (range)	No. deaths, all cases	No. patients *Lp* culture+ (zone residents)	Environmental testing results
			Outbreak total (zone residents)	Rest of NYC	Outbreak zone	Rest of NYC				
Bronx (1)	Jun–Oct 2006	10460, 10461, 10462, 10472, 10473	29 (29)	87	9.7	1.1	57 (38–91)	1	0	No sampling performed at time of outbreak
Manhattan (2)	Aug–Sep 2008	10018, 10019, 10036	7 (7)	36	9.8	0.4	64 (46–81)	0	0	Supportive housing potable water *Lp*1, residential building potable water *L. anisa*, supermarket CT *Lp*6, *L. bozemanii*
Bronx (3)	Nov 2014–Jan 2015	10475	8 (8)	41	18.8	0.5	58 (29–69)	0	1	Residential potable water (no *Lp*1), CT 1 (27/30 culture+ for *Lp*1 and *Lp*6), CT 2 (1/10 culture+ for *Lp*6), supermarket mister (no *Lp* by PCR)
Queens (4)	Apr–Jun 2015	11354, 11355	16 (14)	26	9.2	0.3	65 (50–99)	0	0	Potable water of 2 housing complexes (6 PCR+, *Lp*2 culture+), 1 CT PCR+ and culture+ for *Lp*1
Bronx (5)	Jul–Aug 2015	10451, 10452, 10454, 10455, 10456, 10459, 10474	138 (108)	48	25.7	0.6	55 (30–90)	16	26 (23)	All (55) identified CTs in outbreak zone (14 culture+ for *Lp*1)
Bronx (6)	Sep–Oct 2015	10461, 10462, 10469	15 (10)	18	5.0	0.2	56 (31–71)	1	4	All (50) identified CTs in outbreak zone (8 culture+ for *Lp*1)

*CT, cooling tower; *Lp*, *Legionella pneumophila*; NYC, New York City; +, positive.
†Cases/100,000 population.

power plant CT was remediated 40 days later and identified by PFGE as the source 53 days after the outbreak was first recognized (Table 2).

Outbreak 4 occurred in the spring of 2015 in a residential–commercial area in Queens. SaTScan analysis identified a cluster of 4 LD cases. The investigation included 3 LD cases in residents of 3 separate buildings of a public housing complex and 2 cases in residents of a nearby assisted-living residential building. The remaining case-patient residences were dispersed around the commercial center. Several CTs were identified in the outbreak zone, and, after visual inspection, environmental sampling was conducted at 1 CT and the residential buildings. All of the potable hot water samples from buildings in the public housing complex tested positive by rPCR and culture for *Lp2*. Potable water samples from the assisted-living facility were negative by rPCR and culture. The CT was positive for *Lp1* by rPCR and culture. Although no patient had *Legionella* infection confirmed by culture, 4 were positive from sputum samples by rPCR for *Lp1*. Because no molecular comparison of environmental and human *Legionella* isolates was possible, a definitive source of the outbreak was not identified.

The 2 Bronx LD outbreaks in 2015 included the largest outbreak in New York City (outbreak 5) and 1 in an area of the Bronx with a high density of CTs (outbreak 6). Outbreak 5 was detected by SaTScan in July and was defined by 7 ZIP codes areas (108/138 cases). Outbreak-associated cases were also found in all New York City boroughs, surrounding non–New York City counties, and in visitors from 3 other states. The incidence in the outbreak zone was highest of all the outbreaks at 25.7/100,000. The corresponding incidence in the rest of New York City was 0.6/100,000. A combined city and state effort identified 55 CTs in the outbreak zone. CTs were screened by rPCR for

Table 2. Timeline for investigations of community outbreaks of Legionnaires' disease, New York City, New York, USA, 2006–2015*

Outbreak no.	Date outbreak detected	Method of outbreak detection	Outbreak source	Date implicated CT initially sampled	Date implicated tower first reported with *Lp*	Date remediation of implicated CT began	Date CT linked to human case by DNA typing	Days from *Lp* detection to start of CT remediation	Days from detection to source identification
1	2006 Oct 2	Detected by epidemiologist	ND	NA	NA	NA	NA	NA	NA
2	2008 Sep 15	Detected by epidemiologist, then by historical limits method	ND	NA	NA	NA	NA	NA	NA
3	2014 Dec 1	Historical limits method, then BIN	Power plant CT	2015 Jan 7	2015 Jan 9	2015 Jan 10	2015 Jan 23	40	53
4	2015 May 7	SaTScan	ND	NA	NA	NA	NA	NA	NA
5	2015 Jul 17	SaTScan	Hotel CT	2015 Jul 29	2015 Jul 30	2015 Jul 31	2015 Aug 12	14	26
6	2015 Sep 25	Detected by epidemiologist, then by SaTScan	Worksite CT	2015 Sep 26	2015 Sep 29	2015 Sep 29	2015 Oct 16	4	21

*BIN, building identifiers; CT, cooling tower; *Lp*, *Legionella pneumophila*; NA, not applicable; ND, not determined; SaTScan, space-time permutation scan statistic.

the presence of *Legionella*, and those that were positive for *Lp1* were cultured. Twenty-two CTs were found by rPCR to have *Lp1*, and 14 were *Lp1* culture-positive. Twenty-three (21%) of the 108 outbreak zone resident case-patients were culture-positive for *Lp1*, and the isolates were indistinguishable by PFGE and WGS to an isolate obtained from a hotel CT. No human isolate matched to another CT. The hotel CT was remediated 14 days later and identified by WGS as the outbreak source 26 days after the outbreak was recognized (Table 2).

Outbreak 6 was recognized in a section of the Bronx several miles from outbreak 5 after the latter had ended. Two cases were identified by an epidemiologist (R.S.) who recognized that a workplace of a case-patient and residence of another case-patient were on the same city block. SaTScan signaled 2 days later, identifying 10 cases in the cluster, and a total of 15 cases were included in the outbreak (Table 1). DOHMH was in the process of compiling a registry of CTs at the time and identified a high concentration in the outbreak zone (64 registered CTs). Four patient isolates grew *Lp1* and were indistinguishable by PFGE and WGS from the isolate obtained from a workplace CT. Fifty CTs were screened for *Lp1* by rPCR; 12 were positive, and 8 subsequently found to be culture-positive. The workplace CT was remediated 4 days later and identified as the outbreak source by WGS 21 days after the outbreak was recognized (Table 2).

Discussion

The ability of DOHMH to detect and respond to community LD clusters has evolved over time. Improvements to the cadre of cluster detection tools has given DOHMH the confidence that that even small LD clusters will be detected, as in outbreak 4. Only 2 outbreaks were detected in the first 8 years of the study period, before many of the cluster detection methods were implemented or improved, whereas 4 were detected in the past 2 years, all of which signaled by 1 or more of the cluster detection systems. Cluster detection systems have shown great utility in detecting LD increases, but they are not without cost. Although we now recognize and respond to smaller LD clusters, additional investigation resources are required. After outbreak 6, case-patient work addresses were added to SaTScan, and a new daily proximity analysis, able to identify 2 or more cases occurring within 0.2 miles and 30 days of each other, was implemented in January 2016.

The ability to identify and test CTs during community outbreaks of LD also has evolved. When clusters of cases could not be linked to a building's water system, investigators used "shoe leather epidemiology" to identify other possible sources. Decorative fountains, supermarket misters, visible CTs, and other potential sources were discovered by walking through neighborhoods and interviewing area residents. Beginning in 2015, CTs were identified based on a list of buildings that had applied for tax credits from decreased sewer use, and a New York City Department of Buildings CT list was populated from building construction permits. However, as was made clear from outbreaks 5 and 6, these lists were incomplete. The creation of a CT registry would facilitate identification of potential sources during a suspected community LD outbreak; however, the paucity of clinical cultures for environmental source comparison remains a limitation. The use of rPCR became routine in 2015 and has allowed DOHMH to rapidly screen a large number of potential sources to

identify colonized CTs and request immediate remediation. For the outbreaks in which an environmental source was successfully identified, the time elapsed from the beginning of the investigation to source remediation decreased from 40 to 4 days, and the time required to identify the source decreased from 53 to 21 days. We attribute the decrease to several factors, including improved cluster detection, laboratory capacity, identification of CTs, and the experience gained from investigations involving teams of epidemiologists, laboratorians, and environmental health engineers.

DOHMH routinely communicates information about seasonal and emerging diseases to the medical community through an email listserv that includes all licensed physicians and other healthcare providers who have voluntarily subscribed. Repeated communications and media coverage during the study period regarding LD, particularly in the Bronx, likely sensitized the medical community to test patients with pneumonia for LD, as shown by the more than doubling of *Legionella* isolates obtained in outbreak-associated cases.

Although large outbreaks of LD are rare in the United States, public health officials struggle with identifying sources for the bulk of LD cases classified as sporadic. The focus has been on CTs because of their ubiquitous presence and direct discharge of potentially *Legionella* contaminated mist into the atmosphere. In New York City, 75% of the 5,886 registered CTs are located in the borough of Manhattan, but the Bronx has the second fewest (288 CTs [5%]). The concentration of CTs does not appear to predict whether or where an LD outbreak will occur. Other factors, such as CT design, maintenance, and elevation will need to be evaluated. We note, for example, rooftop CTs are, on average, at higher elevations in Manhattan than in the Bronx. The median elevation of CTs in Manhattan is 14 floors high, with 50% of the CTs located 7–46 floors high. In comparison, the median CT elevation in the Bronx is only 4 stories high, with 50% of the buildings being 2–8 stories. Higher elevation of CTs in Manhattan might present a lower risk for disease transmission, a result of greater particle dispersion, evaporation, or bacterial death; alternatively, the diffusion of contaminated mist from CTs at higher elevations might render outbreaks more difficult to detect.

Poverty probably contributes to the burden of LD because of patient susceptibility to infection, delayed access to medical care, and the maintenance of CTs, all of which play a role in LD outbreaks (*26*,*27*). The Bronx is fourth largest of the New York City boroughs by population, third by population density, and has the highest proportion of residents living in poverty (*28*–*30*). In poorer neighborhoods, the prevalences of concurrent conditions (e.g., diabetes and HIV) and smoking are elevated (*31*–*33*). In addition, building owners in poorer neighborhoods might lack the fiscal resources to hire staff or access training related to CT maintenance and implement a water safety plan for their CT.

Only 3 community-associated outbreaks, all within the last 2 years of the study period and in the Bronx, were successfully linked to a CT. In the 3 outbreaks for which no link was made, clinical isolates were not obtained. Because DOHMH does not receive negative *Legionella* culture reports, we are unable to assess how well our guidance is followed or the culture success rate. These factors remain challenges to LD source identification, control, and prevention efforts. When the first outbreak in this series occurred, no centralized registry of CTs existed. In August 2015, the New York City Council enacted a law requiring the registration, inspection, maintenance, and annual certification of CTs and other aerosol-producing engineering devices with rules promulgated by DOHMH (*17*). The rules require the creation of CT maintenance plans with routine monitoring of water quality (pH, biocide residual, and conductivity), weekly heterotrophic plate counts, weekly inspections of equipment by maintenance staff, and *Legionella* culture at least every 90 days during CT operational periods. On the basis of monitoring and sample results, specific minimum corrective actions must be made to control risk for *Legionella* amplification.

Many countries, including the United Kingdom and 9 other nations in Europe, have enacted legislation to register and regulate CTs, but no standard approach exists, and few countries perform active oversight of compliance (*34*). In the absence of oversight, compliance with regulations is often low, despite established industry standards, such as those issued by the American Society of Heating, Refrigerating, and Air-Conditioning Engineers and the Cooling Technology Institute (*35*,*36*). In New York City, unannounced inspections and financial penalties are expected to improve compliance. After the new law's enactment, during May 9, 2016–May 31, 2017, DOHMH had inspected 3,909 (79%) of registered CT systems. Samples from 46 systems, comprising 1 or more CTs, were found with *Legionella* exceeding 1,000 CFU/mL. Remediation in all instances was performed in accordance with the new regulations. In the absence of the regulations, it is likely that no samples would have been collected and tested for *Legionella*. DOHMH would have been unaware of the potential hazards, and remediation would not have occurred.

New York City is a densely populated metropolis with infrastructure that varies from individual homes to skyscrapers. Our experience with LD investigations might not be typical of other jurisdictions, and generalizing conclusions from a series of 6 outbreaks is difficult. Because the *Legionella* urinary antigen test primarily detects *Lp1*, outbreaks caused by other strains might have occurred and were missed. However, the ubiquitous nature of *Legionella* in the environment and the rising incidence of LD

nationally highlight the urgent need to shift from relying on the alarm bell of human disease to primary prevention strategies designed to limit *Legionella* colonization and dispersal from human-made aerosol-generating devices. New York City's new CT regulations will provide a test case to evaluate whether strict CT maintenance reduces exposure to *Legionella* and reverses trends in LD incidence and outbreaks.

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R.F. and D.W. conceived the study, wrote the manuscript, had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis and interpretation. D.C., S.B., C.B., L.A., R.S., A.E., Y.L., I.R., T.P., A.K., P.L. contributed to the investigations reported by collecting, analyzing and interpreting the data. All authors read and approved the final version to be published and had input in revising the manuscript for intellectual content and style.

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Pregnant Women Hospitalized with Chikungunya Virus Infection, Colombia, 2015

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Release date: October 13, 2017; Expiration date: October 13, 2018

Learning Objectives

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

- Assess clinical findings associated with chikungunya virus (CHIKV) infection during pregnancy
- Distinguish the common laboratory abnormality associated with CHIKV infection during pregnancy
- Evaluate clinical outcomes associated with CHIKV infection during pregnancy
- Analyze symptoms of CHIKV infection after pregnancy

CME Editor

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In 2015 in Colombia, 60 pregnant women were hospitalized with chikungunya virus infections confirmed by reverse transcription PCR. Nine of these women required admission to the intensive care unit because of sepsis with hypoperfusion and organ dysfunction; these women met the criteria for severe acute maternal morbidity. No deaths occurred. Fifteen women delivered during acute infection; some received tocolytics to delay delivery until after the febrile episode and prevent possible vertical transmission. As recommended by a pediatric neonatologist, 12 neonates were hospitalized to rule out vertical transmission; no clinical findings suggestive of neonatal chikungunya virus infection were observed. With 36 women (60%), follow-up was performed 1 year after acute viremia; 13 patients had arthralgia in ≥2 joints (a relapse of infection). Despite disease severity, pregnant women with chikungunya should be treated in high-complexity obstetric units to rule out adverse outcomes. These women should also be followed up to treat potential relapses.

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Chikungunya virus (CHIKV) is an alphavirus of the family *Togaviridae* that was isolated for the first time in Tanganyika (now Tanzania) in 1952 (*1*). CHIKV is transmitted to humans by several species of mosquito; *Aedes aegypti* and *Ae. albopictus* are the main vectors. Since 2004, the geographic distribution of the virus has expanded, which has led to major epidemics in Asia and Africa (*2*).CHIKV appeared as an emerging infection in the Americas in late 2013, with ≈1.1 million reported cases (*3*). In Colombia, by the 51st week of 2016, a total of 19,525 cases of CHIKV had been reported; 19,091 (97.8%) had symptoms suggestive of chikungunya, but only 206 (1.1%) had been confirmed positive for the virus by laboratory tests (*4*). Acute infection with CHIKV is characterized by high fever, asthenia, headache, emesis, rash, myalgia, and arthralgia (*1*,*5*). Infected persons usually recover spontaneously within several days or a week (*6*), but arthralgia can persist for months or even years (*7*).

Knowledge about CHIKV infection has been derived mostly from a large outbreak that occurred in Réunion Island in 2005. Therefore, data on the effects of this infection on maternal outcomes are limited, with no clear evidence that pregnant women infected with CHIKV have more obstetric complications. In a prospective study by Fritel et al., pregnant women with CHIKV infection were more likely to be hospitalized than nonpregnant women (*8*). Robillard et al. identified the first case of CHIKV vertical transmission (*9*), changing the perspective on perinatal infection. When maternal infection occurs at the end of pregnancy, serious, even life-threatening fetal and neonatal complications, such as meningoencephalitis and disseminated intravascular coagulation, can occur (*8*,*10*–*14*).

Information on the clinical presentation of chikungunya in pregnant women in Latin America is limited (*15*–*20*). The case descriptions in this report provide valuable information for the future management of suspected CHIKV infections during pregnancy, including information on the timing of delivery and appropriate level of care, which can help improve neonatal outcomes. The objective of this study was to describe the clinical features of the acute and chronic phases of CHIKV infection in pregnant women who were hospitalized in Fundación Valle del Lili, a fourth-level hospital. This hospital takes care of 1,400 births per year and serves as a reference facility for obstetrics cases of high clinical complexity for the southwest region of Colombia.

Materials and Methods

Type of Study

This investigation was a descriptive observational study of a series of cases. Pregnant women of any age who were hospitalized in the Unit of Obstetric High Complexity (UOHC) with CHIKV infection confirmed by reverse transcription PCR (RT-PCR) during January 1–December 31, 2015, were eligible for inclusion. All pregnant patients with RT-PCR–confirmed infections were admitted to the UOHC, regardless of their clinical condition, so they could be better observed to rule out or manage possible adverse outcomes. We conducted a retrospective review of patient medical records to collect information on demographics, medical history, clinical findings, and laboratory results, as well as to review information recorded by the epidemiologic surveillance committee. This project was approved by the Biomedical Research Ethics Committee of the Fundación Clínica Valle de Lili according to ethics act number 25 of December 7, 2015.

Laboratory Testing

To confirm the diagnosis of CHIKV infection, we performed RT-PCR in real time using the commercial LightMix kit Chikungunya-virus Light Cycler (TIB MOLBIOL, Adelphia, NJ, USA). We performed all CHIKV RT-PCR tests in the clinical laboratory of the Fundación Valle del Lili. Specific CHIKV genotypes were not identified in this study. However, Laiton-Donato et al. identified the genotypes responsible for the CHIKV epidemic in Colombia during 2014–2015 and found only the Asian genotype (*21*). We ruled out dengue virus infection by measuring blood for specific antibodies and nonstructural protein 1 antigen with the commercial SD BIOLINE Dengue Duo kit (Standard Diagnostic, Inc., Yongin, South Korea).

Case Definitions

We stratified CHIKV infection into 2 phases: acute (first 10 days of disease) and chronic (after day 10 of disease) (*22*). We used the definition from the International Sepsis Definitions Conference in 2001 to standardize all cases of sepsis, severe sepsis, and septic shock (*23*). To assess severe acute maternal morbidity (SAMM) in pregnant women requiring intensive care, we decided to use the World Health Organization's established criteria (*24*,*25*). To assess the chronic phase of disease, we contacted all patients by telephone 1 year after acute viremia. We used a survey used in previous studies (*16*,*26*) that included questions regarding the criteria for classification of rheumatoid arthritis of the American College of Rheumatology and European League of Rheumatism 2010 (*27*). The survey included questions on joint pain duration, morning stiffness, joint swelling, muscle pain, and joint redness. Qualified study authors (S.L.O. and J.S.B.) conducted the survey by telephone call (online Technical Appendix Table, https://wwwnc.cdc.gov/EID/article/23/11/17-0480-Techapp1.pdf).

Data Collection

We reviewed electronic medical records to obtain information about pregnant women with CHIKV infection and newborns whose births were cared for at the Fundación Valle del Lili. We retrieved the following data: demographics, symptoms and duration of symptoms before hospitalization, findings from physical examination, findings from laboratory tests, and progress during hospitalization. We entered all information collected into an electronic database.

Data Analysis

We performed a univariate analysis in which the distribution of numerical variables was evaluated by the Shapiro-Wilk test. We summarized data by using averages and SDs or medians and interquartile ranges (IQRs), as appropriate. We expressed qualitative variables as proportions and performed statistical analysis with the Stata program (StataCorp LLC, College Station, TX, USA).

Results

We identified 60 patients with confirmed CHIKV diagnoses; all of these patients were hospitalized January–September 2015. No patients hospitalized during October–December 2015 were confirmed to have CHIKV infection. All 60 patients with confirmed CHIKV diagnoses were hospitalized at the OHCU to rule out sepsis and other viral infections, such as dengue. Mean patient age was 26.4 ± 5.6 years. Five (8.3%) women were in their first trimester (<12 weeks), 17 (28.3%) were in their second trimester (12–28 weeks), and 38 (63.3%) were in their third trimester (>28 weeks). Fifteen (25%) women pregnant in their third trimester completed pregnancy during the hospitalization, and 9 (15%) women required treatment in the intensive care unit (ICU).

Clinical Findings

The most frequent clinical findings at the time of consultation at the emergency department were arthralgia (55/60, 91.6%), followed by fever (21/60, 35%) (Table 1). Other reported clinical manifestations were headache, rash, and myalgia. The interval between the onset of CHIKV symptoms and admission to the OHCU was 1.4 ± 0.5 days for patients in their first trimester, 1.4 ± 0.9 days for patients in their second, and 2.4 ± 1.9 days for patients in their third. We observed hemorrhagic manifestations among women in their third trimester (Table 1). Laboratory results showed leukopenia (<4,000 cells/μL) in 35 (58.3%) women and thrombocytopenia (<150,000/μL) and elevated transaminase levels (>32 μL) in 10 (16.7%) women. Thirty-one (51.7%) women had electrolyte abnormalities: 24 (77.4%) hyperchloremia (>107 mmol/L), 2 (6.5%) hyponatremia (<135 mmol/L), and 5 (16.1%) hypokalemia (<3.5 mmol/L). The median viral load for CHIKV was 1.76×10^5 (IQR 1.17×10^4 to 2.95×10^6) copies/mL. For all patients, test results for dengue viruses (nonstructural protein 1, IgM, and IgG) were negative.

We stratified the observed obstetric complications by the trimester in which the acute CHIKV infection occurred. Among women in their first trimester, 1 had a spontaneous abortion; among women in their second trimester, 3 had preeclampsia and 1 was admitted to the ICU because of CHIKV sepsis; and among women in their third trimester, 8 had CHIKV sepsis. The average hospital stay was longer for the women with CHIKV sepsis in their third trimester (4.3 ± 2.7 days) than for pregnant women without CHIKV (2.4 ± 1.2 days). The obstetric pathologies observed during the third trimester were premature rupture of membranes (3/38, 7.9%), intrauterine growth restriction (2/38, 5.3%), preeclampsia (6/38, 15.8%), preterm delivery (1/38, 2.6%), and postdelivery hemorrhage (3/38, 7.9%).

Table 1. Patient characteristics and clinical signs and symptoms of disease of 60 pregnant women with chikungunya virus infection, Colombia, 2015

		Trimester		
		First, n = 5	Second, n = 17	Third, n = 38
Patient age, y, average ± SD	26.4 ± 5.6	27.8 ± 7.5	26.8 ± 5.8	26 ± 5.3
Gestational age of fetus, wks, average ± SD	28.5 ± 8.8	13 ± 5.8	20.6 ± 5.6	34 ± 3.7
Reason for consultation, no. (%)				
Arthralgia	55 (91.7)	5 (100)	17 (100)	33 (86.8)
Fever	21 (35)	0 (0)	5 (29.4)	16 (42.1)
Pruritus	4 (6.7)	0 (0)	0 (0)	4 (10.5)
Headaches	9 (15)	1 (20)	4 (23.5)	4 (10.5)
Rash	9 (15)	4 (80)	5 (29.4)	0 (0)
Signs and symptoms during hospitalization, no. (%)				
Fever	11 (18.3)	0 (0)	0 (0)	11 (28.9)
Polyarthralgia	56 (93.3)	5 (100)	16 (94.1)	35 (92.1)
Headaches	45 (75)	4 (80)	12 (70.6)	29 (76.3)
Rash	52 (86.7)	5 (100)	13 (76.5)	34 (89.5)
Myalgia	46 (76.7)	4 (80)	13 (76.5)	29 (76.3)
Low back pain	28 (46.7)	3 (60)	6 (35.3)	19 (50)
Emesis	3 (5)	0 (0)	1 (5.9)	2 (5.3)
Nausea	11 (18.3)	2 (40)	2 (11.8)	7 (18.4)
Epistaxis	2 (3.3)	0 (0)	0 (0)	2 (5.3)
Gingivorragia	3 (5)	0 (0)	0 (0)	3 (7.9)

Intrapartum Period

Fifteen (39.5%) of the 38 patients who sought treatment for chikungunya fever in the third trimester gave birth during the hospitalization: 10 by vaginal delivery and 5 by cesarean section (because of unsatisfactory fetal orientation in the womb). The mean gestational age of their newborns at birth was 38.5 ± 1.08 weeks. For these 15 women, the average number of days from onset of symptoms to labor was 6.3 ± 1.9 days. Vital signs were monitored every 2 hours and platelets and hemoglobin every 24 hours, and no severe thrombocytopenia or anemia occurred. These patients required resuscitation with intravenous crystalloid fluids, with a cumulative fluid balance of 328 ± 657 mL during the obstetric event. Three (20%) women with regular uterine activity had fevers, so the doctors performed tocolysis with nifedipine for 2.3 ± 0.94 days to delay labor and prevent birth during the period of intrapartum fever.

Table 2. Criteria for severe acute maternal morbidity among 9 pregnant women with chikungunya virus infection who were admitted to intensive care, Colombia, 2015*

Criteria	No. (%) patients
Organ dysfunction†	7 (77.8)
Hepatic	2 (22.2)
Renal	3 (33.3)
Vascular	2 (22.2)
Clinical diagnosis	9 (100)
Severe preeclampsia	3 (33.3)
Severe postpartum hemorrhage	1 (11.1)
Sepsis	9 (100)
Interventions in critical care	9 (100)
Admission to intensive care unit	9 (100)
Transfusions of >3 units of red blood cells	2 (22.2)

*The World Health Organization's definition for severe acute maternal morbidity was used (*24*,*25*).
†Hepatic dysfunction was defined as hyperbilirubinemia (bilirubin >100 μmol/L or 6 mg/dL). Renal dysfunction was defined as oliguria <400 mL that did not resolve after administration of fluids or diuretics. Vascular dysfunction was defined as hypovolemia requiring transfusion or use of vasoactives.

Intensive Care Unit

Nine (15%) patients required ICU admission, 8 (89%) of which were in their last trimester of pregnancy. Although women in the third trimester comprised 63.3% of the study population, they comprised almost all of the ICU admissions. Seven (78%) of the 9 ICU patients did not have other comorbidities, and 2 (22%) had reported previous cardiac arrhythmias. All 9 women had sepsis, 7 (78%) had the criteria for severe sepsis, and none had septic shock. Bacteria cultures were performed with these patients' blood samples, and all were negative, ruling out nosocomial bacterial infection. Complications developed in some patients, with postdelivery hemorrhage occurring most frequently (11.1%). At admission to the ICU, lactic acid and base deficit were measured. Of the 9 patients sent to the ICU, mean lactic acid level was 2.6 ± 2.39 mmol/L and base deficit was –5 ± 1.4 mmol/L; 6 (66.7%) patients had hyperlactatemia.

Upon admission to the ICU, all 9 patients received resuscitation with crystalloids (30 mL/kg in 500 mL boluses every 15–30 min), resulting in a fluid balance of 960.3 mL at the end of resuscitation. Three women had hypertension, and 6 had clinical signs of tissue hypoperfusion. The mean APACHE II (Acute Physiology and Chronic Health Evaluation II) score was 10.44 ± 4.71 points, and death was the prognosis for 15% of these patients. All patients had SAMM criteria (Table 2); the main dysfunctions found were renal (33%), vascular (22%), and hepatic (22%). Although the patients had SAMM criteria, no maternal deaths were reported.

Newborn Infants

Of the 15 infants born during their mothers' acute CHIKV infection, 12 were hospitalized as recommended by the pediatric neonatologist to observe their clinical progress and to rule out vertical transmission. The average duration of hospitalization of newborns was 4 days. Because this event was the department of obstetrics and neonatology's first experience managing an outbreak of CHIKV in pregnant women, the hospital did not have a protocol to care for newborns born from mothers with CHIKV infections. A decision was made to perform RT-PCR only with newborns of mothers having viremia near the time of delivery (50% of the 12 hospitalized newborns), and all were negative for the CHIKV genome. At physical examination, 5 neonates had no abnormalities, and 1 neonate had a short neck with no internal anatomic abnormalities or problems with mobility. For this neonate, a karyotype was performed, leading to the diagnosis of Turner syndrome. Regarding the laboratory results of the other neonates, 2 (16.7%) of 12 had leukocytosis and 1 (8.3%) of 12 had lymphocytosis. No abnormalities were found with renal, liver, or platelet function tests. No neurologic or cardiovascular abnormalities were observed.

Postdelivery Follow-up

One year after delivery, we called study participants to perform follow-up of the chronic phase of disease. Only 36 patients (60%) could be contacted; the other 24 patients did not or were not willing to answer our calls. Twenty-three women had no residual symptoms. However, 13 patients (36% of the women who responded to the survey) experienced arthralgia in ≥2 joints. Of these women, 8 had joint swelling, 7 had erythema, and 4 had myalgia. Nine had an inflammatory pattern that included morning stiffness. The presence of these symptoms indicates these women were experiencing a relapse of acute disease. Joint pain reoccurred 72.6 ± 74.15 days after acute CHIKV infection and persisted for 186.9 ± 85.78 days. Five women required follow-up with a rheumatology specialist.

Discussion

Our cohort included 60 pregnant women with a diagnosis of CHIKV confirmed by RT-PCR. To enable observation of the behavior of the disease and the obstetric outcomes, all patients with confirmed CHIKV infection were hospitalized, regardless of clinical stability and severity of the infection. In 2015, the Fundación Clínica Valle del Lili did not have an established protocol for the management of pregnant women with CHIKV infection. The most common signs of illness in the patients admitted to the UOHC were polyarthralgia, exanthema, myalgia, headache, and fever. Thiberville et al. conducted a prospective study during the epidemic on Reunión Island that included 54 adults with CHIKV infection (*28*). This study indicated the same signs described in our series. Our analysis also confirms that the clinical manifestations do not vary between nonpregnant adults and pregnant women (*29*).

Previous studies of the nonpregnant population indicated that CHIKV can cause illnesses and deaths associated with sepsis (*30*–*32*). Our study reports 9 pregnant women with CHIKV-induced sepsis, and all required ICU treatment. Seven (77.8%) of those women had severe sepsis. Target-guided resuscitation was performed with crystalloids and vasopressors under strict surveillance of water overload. Among patients with SAMM, the APACHE II score was 10.4 ± 4.7 points, and 15% had a prognosis of death; however, no deaths occurred. These findings suggest that CHIKV infection during pregnancy can cause severe sepsis with organ dysfunction and tissue hypoperfusion.

CHIKV infection appears to have clinical presentations that differ by trimester of pregnancy. Another report related maternal infection with vertical transmission and pregnancy loss (*33*), although these occurrences were uncommon. In our cohort, spontaneous abortion occurred once; however, two thirds of our patients (38/60) were in their third trimester. This high concentration of women in late pregnancy might be explained by the common practice of low-complexity obstetrics centers of Colombia referring high-complexity obstetrics candidates (pregnant women at term or during labor with acute febrile syndrome) to Fundación Valle del Lili's UOHC.

Chikungunya is a potential risk for neonates born to symptomatic women (*11*,*14*). The most common clinical signs in newborns were fever, irritability, rash, hyperalgesia syndrome, diffuse limb edema, meningoencephalitis, and bullous dermatitis (*8*,*11*). With the Réunion Island outbreak of 2005, Gérardin et al. found that the risk for vertical transmission increased to 50% when maternal viremia was present during delivery (*11*). Furthermore, Ramful et al. found that the risk for mother-to-child transmission increased when the acute infection was documented during the intrapartum period (*12*). These findings suggest that the intrapartum period is the most critical time for vertical transmission. In 2016, a multicenter study occurring in 3 Latin America countries (El Salvador, Colombia, and Dominican Republic) showed vertical transmission rates ranging from 27.7% to 48.3% (*20*).

In our cohort, we could not rule out the transmission of CHIKV infection in all newborns but report that none had clinical signs of congenital infection. We could test only 50% of the newborns with RT-PCR, and all were negative. We delayed labor to prevent birth during the early febrile phase. The average interval from the onset of maternal symptoms to delivery of 6.3 ± 1.4 days might have been enough time for the passive transfer of antibodies to occur to prevent symptomatic infection in the newborn. We strictly monitored these patients for the presence of fever, thrombocytopenia, or intrapartum hemodynamic decompensation; 20% of the patients with intrapartum fever required tocolysis to delay labor. We have used this approach to prevent dengue maternal complications and to reduce dengue vertical transmission at Fundación Valle del Lili. Our findings suggest delaying the birth as long as possible in patients with acute febrile CHIKV infections is necessary, as long as there are no obstetric contraindications. In favor of this perspective, in the Leglet et al. study, 118 of 151 pregnant women with CHIKV infection had a clearance of their viremia before completing gestation, and no cases of vertical transmission were reported (*34*).

We found that the median viral load for CHIKV was 1.76×10^5 (IQR 1.17×10^4 to 2.95×10^6) copies/mL. In 2006, Panning et al. conducted a study with patients who returned to Europe from the Indian Ocean with a diagnosis of CHIKV and reported a mean viral load of 9.85×10^7 copies/mL (*35*).

Although this type of study cannot establish causality, we found a high incidence of preeclampsia (15.7%) in patients with CHIKV. In Colombia, preeclampsia is the leading cause of maternal illness and death; in 2014, a total of 10,499 cases of maternal illnesses were reported, with hypertensive disorders being the main associated cause (60.5% of cases) (*36*). Likewise, hypertensive disorders are one of the main reasons for consultations with the Fundación Valle del Lili (24% of all consultations per year).

The chronic phase of CHIKV has been poorly described in the obstetric population. In our study, 36 women (60%) were followed up 1 year after seeking treatment for acute CHIKV infection, and 13 patients (36%) were found to have episodes of arthralgia in ≥2 joints (relapsed infections). These findings are consistent with the study by Rodriguez et al., which characterized the chronic phase of CHIKV in an adult population in Colombia and reported that 50% of patients had chronic rheumatologic disorders with an inflammatory pattern (*16*). This observation is consistent with that from our report, in which 5 patients (13.9%) required care by a rheumatology service. Similarly, Rodríguez et al.

reported that a higher proportion of chronic pain might occur with some groups, such as women and the elderly (*16*). These findings emphasize the need to further characterize the degree of disability caused by rheumatologic symptoms in this population.

Our findings have methodologic limitations. First, this study involved a small retrospective cohort without a control population, so assessing causality and risk factors was not possible. Second, the hospital used was a reference center for the most serious cases in the region, generating a selection bias in recruited patients and explaining the high incidence of preeclampsia in this series. Third, electronic medical records of hospitalized women were used, which led to a restricted nongeneralizable sample. Fourth, because follow-up of the chronic phase of the disease was 1 year after onset of symptoms, memory bias could have affected our results. Finally, vertical transmission could have been underestimated given that not all neonates were hospitalized and, of those hospitalized, only half had RT-PCR to evaluate for CHIKV infection.

In conclusion, chikungunya is an emerging disease in the Western Hemisphere. All personnel in charge of obstetric populations should be aware of the behavior of CHIKV infection during pregnancy. This study suggests that CHIKV might cause cases of sepsis with hypoperfusion and organ dysfunction. Although the number of neonates potentially exposed was low, good perinatal outcomes without vertical transmission of infection justifies high-complexity obstetric center care of pregnant women, particularly those with signs and symptoms of CHIKV infection near term or at the time of delivery. Finally, the presence of residual pain in our patients suggests the need for follow-up throughout the first year after infection.

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Legionnaires' Disease Outbreak Caused by Endemic Strain of *Legionella pneumophila*, New York, New York, USA, 2015

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During the summer of 2015, New York, New York, USA, had one of the largest and deadliest outbreaks of Legionnaires' disease in the history of the United States. A total of 138 cases and 16 deaths were linked to a single cooling tower in the South Bronx. Analysis of environmental samples and clinical isolates showed that sporadic cases of legionellosis before, during, and after the outbreak could be traced to a slowly evolving, single-ancestor strain. Detection of an ostensibly virulent *Legionella* strain endemic to the Bronx community suggests potential risk for future cases of legionellosis in the area. The genetic homogeneity of the *Legionella* population in this area might complicate investigations and interpretations of future outbreaks of Legionnaires' disease.

Legionella spp. are ubiquitous in nature, live in soil and water, and frequently inhabit human-made water distribution systems, hot water tanks, decorative fountains, and cooling towers (*1*,*2*). Persons with underlying health conditions, such as chronic lung disease, or those with compromised immunity are at increased risk for contracting Legionnaires' disease (LD) (also referred to as legionellosis). Signs and symptoms typically include fever, cough, and chest pain; LD is fatal in ≈5%–10% of cases (*3*,*4*). Transmission of *Legionella pneumophila* is believed to occur mainly through exposure to contaminated aerosols and not from other infected persons; to date, only 1 case of human-to-human transmission has been documented (*4*,*5*).

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LD was initially detected in 1976, when an outbreak of illness occurred during a meeting of the American Legion in Philadelphia, Pennsylvania, USA; 221 cases were identified, and 34 infected persons died (*6*). The outbreak, which remains the largest community-associated outbreak of LD in United States, was later linked to the cooling system of the hosting hotel, and a bacterium classified as *L. pneumophila* serogroup 1 was subsequently isolated from 4 persons (*7*,*8*).

In the summer of 2015, a large community-associated LD outbreak affected persons who resided or traveled through a large area in the South Bronx region of New York, New York, USA. During July 2–August 3, a total of 138 adults with LD were linked to the outbreak; 128 patients required hospitalization, and 16 deaths occurred (Figure 1). A joint laboratory investigation to find the source of this outbreak was performed by the New York City Department of Health and Mental Hygiene and the Public Health Laboratory (NYC PHL), the Wadsworth Center (WC) of the New York State Department of Health (Albany, NY, USA), and the Centers for Disease Control and Prevention (CDC; Atlanta, GA, USA).

Pulsed-field gel electrophoresis (PFGE), real-time PCR, sequence-based typing (SBT), and whole-genome sequencing (WGS) were used to characterize human and environmental *L. pneumophila* isolates from the investigation. Epidemiologic data and water testing by PCR quickly led to identification of a cooling tower located on the roof of a South Bronx hotel as a potential source of this outbreak (*9*). However, *L. pneumophila* isolates recovered from a sample taken later during the outbreak from a homeless shelter located in the vicinity of the South Bronx hotel and other facilities within the outbreak zone were found to have PFGE and SBT patterns identical to that of the outbreak strain, raising the possibility that the South Bronx hotel might not have been the only source of an aerosolized

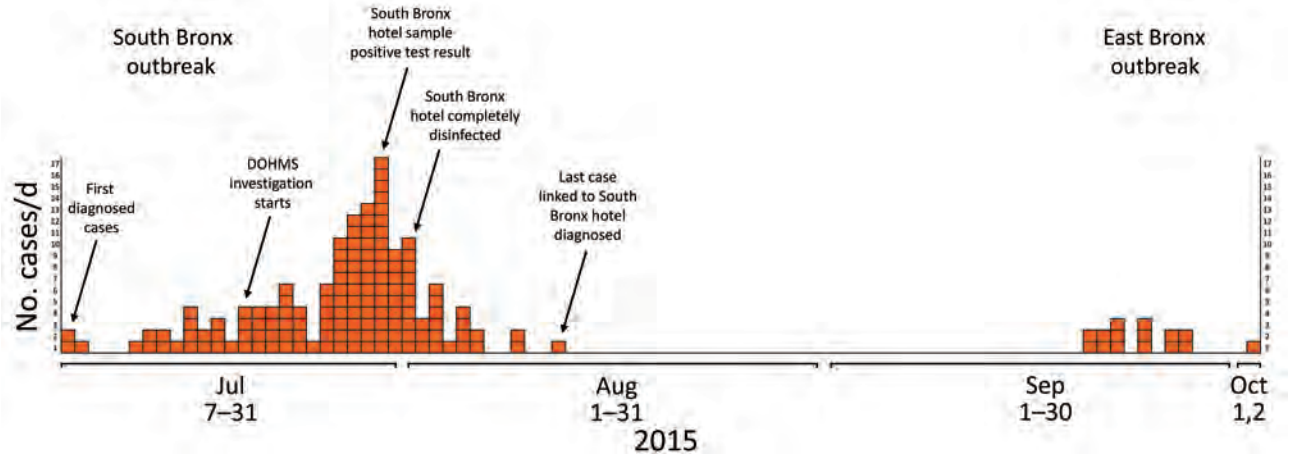

Figure 1. Legionnaires' disease outbreak caused by an endemic strain of *Legionella pneumophila*, New York City, New York, USA, 2015. Timeline shows all diagnosed cases linked to the July 2015 South Bronx and August 2015 East Bronx outbreaks. Each orange square represents the time at which a person was given a diagnosis of the disease. Annotations of some of the key actions taken by the authorities are listed above their corresponding days. DOHMS, Department of Health and Mental Hygiene.

Legionella species associated with cases of legionellosis. Our finding of highly related *L. pneumophila* isolates in multiple environmental samples and from past LD outbreaks suggests the presence of a potentially pathogenic endemic strain in the Bronx community.

Methods

Water Samples and Clinical Isolates

Initially, water and swab samples were collected by the New York City Department of Health and Mental Hygiene and split between the WC and the NYC PHL. Later in the outbreak, water and swab samples were also collected by the New York State Department of Health and submitted to WC. Samples were processed as described (*9*), except for a subset of samples, including swab samples and visibly complex samples, that were not concentrated by centrifugation but tested directly. Clinical isolates were received by the NYC PHL and forwarded to WC. A subset of water and clinical isolates was sent to CDC for SBT analysis or sequencing by using the RSII Platform (Pacific Biosciences, Menlo Park, CA, USA). SBT was performed according to the European Society of Clinical Microbiology and Infectious Diseases (Basel, Switzerland) Study Group for *Legionella* Infections Scheme (*10*,*11*).

Extraction of DNA

Nucleic acid extraction was performed for water and swab samples by using a modified Masterpure DNA Isolation Kit procedure (Epicentre, Madison, WI, USA) (*12*). In brief, for each extraction, a 1.2–1.5 McFarland suspension of the isolate in sterile water was centrifuged for 10 min at 7,500 rpm. A volume of 950 μL of supernatant was removed, leaving 50 μL. A total of 300 μL of 2× tissue and cell lysis buffer containing 1.5 μL of proteinase K was then added to each sample. Each extraction incorporated a negative extraction control that consisted of 50 μL of sterile water. The DNA was resuspended in 100 μL of 10 mmol/L Tris. Concentrations of the DNA were quantified by using the Qubit ds DNA HS Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instrctions, with the Qubit 2.0 fluorometer before WGS. Updates to the protocol include the addition of an internal inhibition control.

PCR Screening

We tested processed samples for *Legionella* DNA using real-time PCR with a newly validated and more comprehensive procedure than that previously published (*12*,*13*) to rapidly screen samples for prioritizing culture efforts. This assay detects and differentiates *Legionella* spp., *L. pneumophila*, and *L. pneumophila* serogroup 1 and uses an internal control to assess for inhibitory substances in the sample.

Culture of Water Samples

Samples in which *L. pneumophila* serogroup 1 was detected were processed and cultured at WC and NYC PHL by using standard methods. Isolates were identified as *L. pneumophila* serogroup 1 by using direct fluorescent antibody testing or real-time PCR. All *L. pneumophila* serogroup 1 isolates were initially typed by using digestion with *Sfi*1 and pulsed-field gel electrophoresis (PFGE) as described (*14*).

WGS

DNA sequencing was performed by using the MiSeq platform (Illumina, San Diego, CA, USA) at the WC Applied

Genomic Technologies Core and the RSII platform (Pacific Biosciences) at CDC. Individual sample libraries were prepared by using a Nextera XT protocol (Illumina) for sequencing. PacBio-compatible libraries were constructed by using 8 μg of sheared genomic DNA (≈15 kb) prepared by using the SMRTbell Template Prep Kit 1.0 (product number [PN] 100–259–100; Pacific Biosciences) according to the manufacturer's protocol (PN 100-092-800-06), the PacBio Binding Calculator version 2.3.11, the DNA Polymerase Binding Kit P6 version 2 (PN 100-372-700), and the MagBead Kit (PN 100-133600).

Sequencing runs were performed with a 2-kb DNA internal control (PN 100-356-500), 240-min movie time, and stage start with a DNA Sequencing Reagent Kit 4.0 (PN 100-356-400). Final library size was confirmed by using the Agilent Tapestation 2200 and the Genomic DNA ScreenTape (5067–5365 and 5067–5366). Hierarchical Genome Assembly Process version 3 was used to construct the complete *L. pneumophila* genome sequences (*15*). The expected genome size was set to 3.4 Mb and target genome coverage parameter was set to 15×. The minimum subread length value was adjusted to decrease genome coverage to the recommended 100×–150× for microbial genomes (*16*). Genome closure was performed by identifying and trimming nucleotide overlap at the ends of the single assembled contig sequences with Gepard version 1.3 (*17*), and the reformatted genome sequence was used as input for the RS-ReSequencing protocol in the SMRT analysis portal to construct the polished genome sequence.

To confirm nucleotide accuracy, we aligned paired-end Illumina data for each sequenced isolate to its respective PacBio polished sequence by using Bowtie version 2.1.0 (*18*). We used Samtools version 0.1.18 (*19*) and FreeBayes version 0.9.21 (*20*) to identify nucleotide discrepancies between the 2 types of data. We resolved any discrepancies with the Illumina dataset and used VCFtools version 0.1.11 (http://vcftools.sourceforge.net/) to construct the final consensus sequence by using both data types (*21*). We deposited the closed genome sequence of the South Bronx outbreak strain F4469 in GenBank (accession no. CP014760). All raw Illumina reads used in this study are available in BioProject (accession no. PRJNA345011) (https://www.ncbi.nlm.nih.gov/bioproject/).

Bioinformatics Analysis

We mapped raw reads to the South Bronx outbreak strain F4469 by using BWA MEM version 0.7.5a-r405 (*22*). Single-nucleotide polymorphisms (SNPs) were called by using Samtools/BCFtools version 0.1.19–44428cd (*19*), a minimum of Q20 for mapping quality and basecall quality, 10× minimum depth, and 95% of allele read agreements. Positions where ≥1 of the samples were found to have a mutation was manually verified and used to build a SNP alignment. Positions with ambiguous calls in any of the samples were discarded. We imported the resulting alignment into PHYLOVIZ (*23*) and built a minimum spanning tree by using the GoeBURST full minimum spanning tree algorithm (https://github.com/apetkau/microbial-informatics-2014/tree/master/labs/mst). Presence of plasmids was verified by performing de novo assemblies of different isolates in SPAdes version 3.7.0 (*24*) and compared by using Mauve (*25*). Recombination events were determined by finding regions of enriched SNP density by using a probability density function calculation and Fastgear (*26*). Blast ring genome comparisons were made by using BRIG (*27*).

Results

WC tested 289 cooling tower water samples from 183 cooling towers by real-time PCR during this outbreak investigation. A total of 162 (88.5%) cooling towers were positive for *Legionella* species DNA. *L. pneumophila* DNA was detected in 87 (47.5%) cooling towers; 52 (28.4%) cooling towers were positive *L. pneumophila* serogroup 1, and 21 (11.5%) showed negative or inconclusive results.

On the basis of the amount of DNA present, which was determined by the initial PCR screening, 26 cooling tower water samples were cultured during July 28–August 14, 2015. We identified 10 culture-positive cooling towers from which 15 *L. pneumophila* serogroup 1 isolates were subjected to PFGE. In addition, culture at NYC PHL identified isolates from a homeless shelter cooling tower that were identical by PFGE and included in this analysis. These *L. pneumophila* serogroup 1 PFGE results showed 7 PFGE patterns (Figure 2). Of these isolates, PFGE showed that those from the South Bronx hotel and the homeless shelter were identical to clinical isolates. SBT analysis showed that these isolates also had the same sequence type (i.e., 731).

WGS was used in real time during the course of the South Bronx LD outbreak as a confirmatory method and to provide additional insight on the source. The investigation also subsequently used WGS to help clarify whether the outbreak strain could have been present at other locations during the outbreak or at other times in the past. A total of 156 isolates of *L. pneumophila* serogroup 1 were available from culture performed at the WC, NYC PHL, and hospital laboratories (115 environmental and 41 clinical isolates from 26 patients, of which 35 were respiratory and 6 were postmortem specimens from 3 patients). These isolates were sequenced and analyzed by using an in-house bioinformatics pipeline developed at the WC.

Most (106/115) of the environmental *L. pneumophila* serogroup 1 isolates sequenced did not closely match any of the clinical *L. pneumophila* serogroup 1 isolates suspected to be part of this outbreak, and differed by several thousand SNPs over the 3.4-Mb genome of the South Bronx

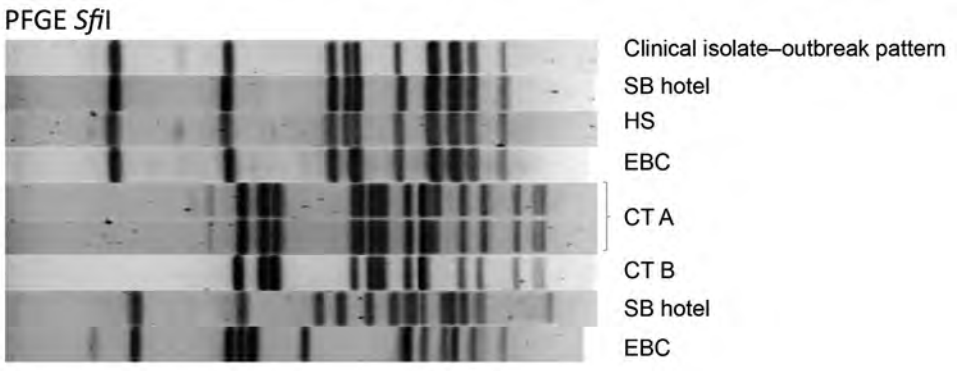

Figure 2. Pulsed-field gel electrophoresis (PFGE) of case-patient and environmental isolates from a Legionnaires' disease outbreak caused by an endemic strain of *Legionella pneumophila*, New York City, New York, USA, 2015. One clinical isolate with the outbreak PFGE pattern (Clinical isolate-outbreak pattern) shows matches to cooling tower (CT) isolates from the South Bronx Hotel (SB), a homeless shelter (HS), and East Bronx College (EBC). Molecular typing patterns of *L. pneumophila* serogroup 1 isolates from cooling tower from the SB hotel, HS, and EBC were indistinguishable from 26 clinical isolates associated with the Legionnaires' disease cluster in the South Bronx. Other samples that were not linked to the outbreak (CT A, CT B, SB hotel EBC) had major differences in patterns when compared with the outbreak pattern.

hotel strain F4469 used as a reference. Five *L. pneumophila* serogroup 1 isolates recovered from the South Bronx hotel and 41 clinical *L. pneumophila* serogroup 1 isolates from 26 patients linked to this outbreak were identical (no SNP differences among them) (Figure 3). Eight other *L. pneumophila* serogroup 1 clinical isolates (15–144, 15–157, 15–158, 15–202, 15–209, 15–215, 15–273, and 15–288) obtained during the same outbreak period had the same PFGE and SBT types as the outbreak isolates. However, these 8 isolates did not meet the epidemiologic case definition (*9*), and WGS showed that they contained 1–5 SNP differences compared with the South Bronx hotel isolate.

Four environmental isolates (3 isolates from the same homeless shelter and 1 from an East Bronx College) obtained during the investigation of the South Bronx outbreak (Figure 2) were nearly identical to the South Bronx hotel isolate, each differed by only 1 or 2 SNPs from the South Bronx hotel isolate and from one another. All 3 isolates from the homeless shelter had the same unique SNP that was absent from all clinical and environmental isolates linked to the South Bronx hotel. Moreover, the East Bronx College isolate, which was obtained from a site several kilometers from the South Bronx hotel, was identical by WGS to 1 South Bronx clinical isolate (15–273). Five other clinical isolates (15–288, 15–215, 15–157, 15–158, and 15–202) had SNP profiles that were closer to the isolate obtained from the East Bronx College than to the isolate obtained from the South Bronx hotel. Together, these observations suggest that 1) the South Bronx hotel cooling tower, and no other cooling towers, was most likely the source of the South Bronx outbreak; and 2) cases not epidemiologically linked with the outbreak might have originated from other environmental sources.

We also completed WGS for 10 historical clinical isolates of *L. pneumophila* serogroup 1 DNA from New York, New York, and included 3 genome sequences from a previously published study (*14*) that reported identical or similar PFGE patterns and sequence types with those of the South Bronx hotel outbreak strain. These genomes differed by ≤5 SNPs from those of the South Bronx hotel isolates. The oldest *L. pneumophila* serogroup 1 isolate, dating back to 2007, had only 3 SNP differences, indicating that the isolate that caused the current outbreak had been present in the Bronx for ≥8 years. Two clinical isolates and 1 environmental isolate (NH1, NH2, and NH3) obtained during an outbreak in a Bronx nursing home in 2011–2012 were also found to be closely related to the South Bronx hotel isolate (≤3 SNP differences), which indicated that this isolate caused ≥1 previous outbreaks of LD. WGS comparison of 4 other clinical isolates (09–214, 10–351, 10–423, and 10–458) from 2009 and 2010 showed an SNP profile that was identical to that of 3 of the clinical isolates from 2015 (15–157, 15–158, and 15–202) not epidemiologically linked to the South Bronx hotel–associated outbreak. These 2015 clinical isolates differed by 3 SNPs from the South Bronx hotel isolate, which suggested that patients might have been infected by an independent source that was not identified. In addition to SNPs, other genomic differences, such as the presence of plasmids or large indels, were also detected in some of the genomes analyzed.

WGS analysis of 6 *L. pneumophila* serogroup 1 isolates from a second, late summer outbreak in 2015 in the East Bronx neighborhood (15 cases, 4 clinical isolates, and 2 environmental isolate sequences) that was not suspected to be linked with the July outbreak, was confirmed to be unrelated (1,038 SNP differences) when compared with the South Bronx hotel outbreak strain. However, closer examination of locations of the SNPs showed that most differences were highly clustered in a few genomic locations, rather than being randomly dispersed throughout the genome, and might have been the result of recombination events. Only 8 SNP differences remained when these

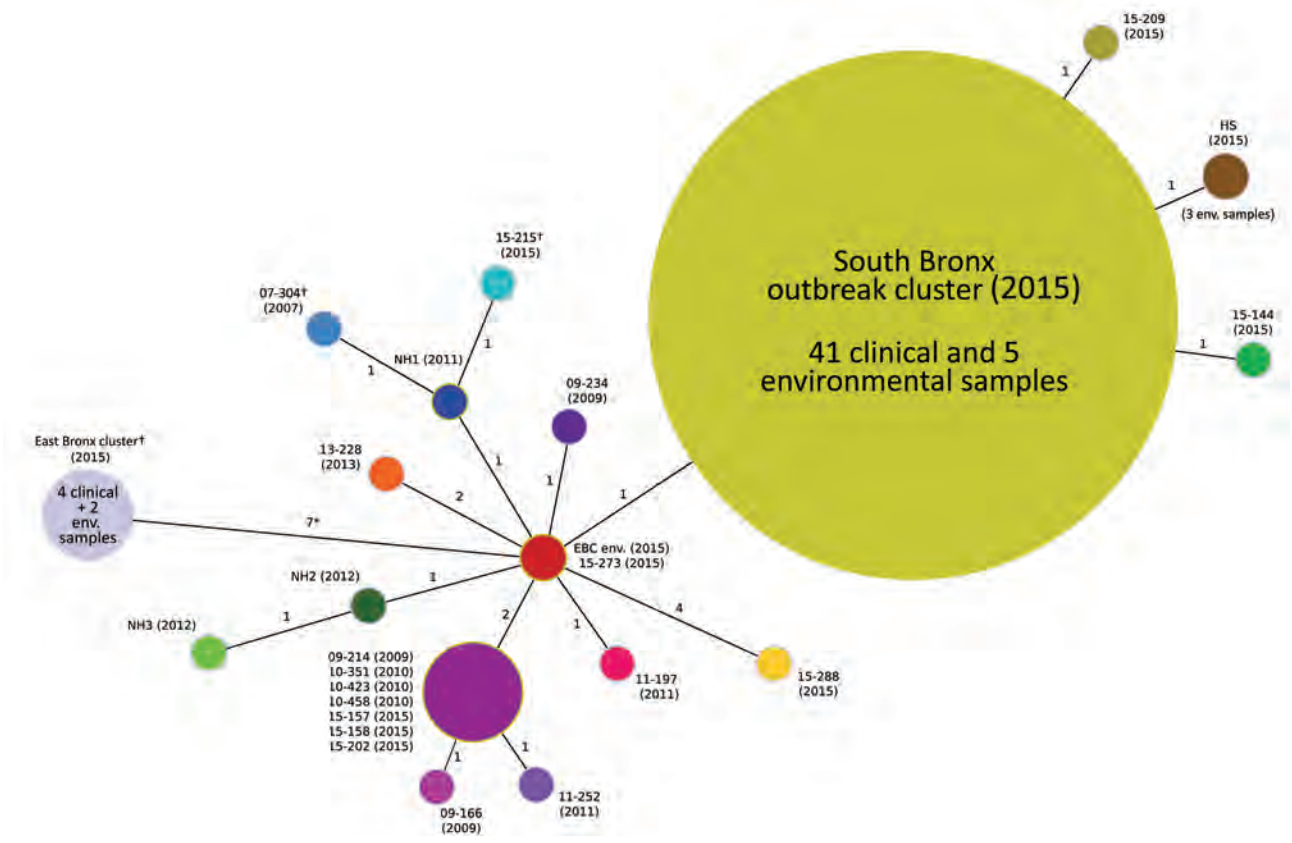

Figure 3. Minimum spanning tree of 77 isolates related to the 2015 South Bronx Legionnaires' disease outbreak caused by an endemic strain of *Legionella pneumophila*, New York City, New York, USA, 2015. The tree was created by using single-nucleotide polymorphism (SNP) differences found across all isolates. Sizes of circles are proportional to number of isolates having identical genomic backgrounds, numbers adjacent to lines indicate number of polymorphism differences between each node, and numbers in parentheses indicate years. Strains 07-304 and 15-215 contain the same plasmid and differ greatly from the plasmid present in all East Bronx isolates. These plasmids have only partial identity with known plasmids in other *Legionellaceae*. *East Bronx outbreak samples contain 1,030 additional SNP differences caused by the presence of suspected homologous recombination events that were omitted from the final SNP analysis; †Plasmids are present in these isolates. EBC, East Bronx college; env., environmental; HS, homeless shelter; NH, nursing home.

recombination locations were omitted. Clustering of SNPs in an otherwise isogenic background suggests that the East Bronx and South Bronx strains only recently diverged after horizontal gene transfer events. WGS was the only method powerful enough to discriminate between South Bronx hotel and all the other environmental isolates, including the homeless shelter, and confirmed the South Bronx hotel cooling tower as the source of this outbreak.

Discussion

This outbreak investigation represents a large-scale testing effort by the NYC PHL, WC, and CDC public health laboratories. As reported by Weiss et al. (*9*), the environmental and epidemiologic investigation provided a comprehensive set of samples and specimens for laboratory testing.

Large outbreaks of LD can occur in areas of high population density that are near human-made reservoirs and mechanisms of aerosolization, such as cooling towers (*28*–*30*). Preventing or controlling such outbreaks in urban areas is further complicated by the presence of multiple potential reservoirs, which present substantial challenges when attempting to determine the exact point source. In a metagenomics survey of air samples obtained in New York, New York, *Legionella* was the predominant genus identified in samples collected on the rooftop of an office building overlooking midtown Manhattan (*31*). Further complicating epidemiology studies, it has been shown that aerosols containing *L. pneumophila* are capable of infecting persons residing at a distance of ≥6 km from the contaminated source (*32*).

Our findings of similar *L. pneumophila* strains at multiple locations and over extended periods is consistent with results of these studies and further suggest that *L. pneumophila* is capable of long-term survival in multiple reservoirs over large areas in an urban environment.

Our findings also suggest that cooling towers colonized with *L. pneumophila* might contaminate other sites located nearby, leading to the possibility for an endemic strain to reestablish colonization after elimination of the organism at any single presumed source. This analysis warns us that because of the particular biologic and ecologic nature of *L. pneumophila*, reliance solely on 1 source of evidence (epidemiologic approaches or molecular data) might be insufficient to identify exact sources of legionellosis outbreaks.

Our extensive sampling and WGS of cooling tower isolates has shown that many cooling towers were colonized with a diverse and heterogeneous *Legionella* population, most of which have not caused detectable human disease. In the specific case of the South Bronx hotel cooling tower, 2 different *L. pneumophila* serogroup 1 strains were obtained (among 10 isolates recovered), including the strain responsible for the 2015 outbreak. This finding showed that populations of virulent clones can coexist among a wide variety of nonoutbreak strains not associated with known disease, and for which virulence has not been assessed.

It is still uncertain what triggered the LD outbreak in New York, New York, in 2015, but several factors might have contributed. Improper maintenance of cooling towers or excessive mist generated during operation could have created ideal conditions for *Legionella* spp. to multiply and aerosolize (*33*,*34*). In addition, a new *Legionella* subpopulation could have acquired, through mutation or recombination, new beneficial phenotypic capabilities (such as increased resistance to cleaning agents), better survival to desiccation, or enhanced aerosolization capability (*35*–*37*). The low level of heterogeneity seen between the historical and 2015 isolates is consistent with results from a similar study of a persistent *L. pneumophila* serogroup 1 outbreak–associated strain in Alcoy, Spain, where it was estimated that mutation rates for *L. pneumophila* in cooling towers can be as low as ≈0.15 SNPs/genome/year (or 1 mutation across the entire genome every 6.7 years) (*38*). This estimation raises the possibility that *L. pneumophila* can persist unchanged for extended periods in a dormant state until it is reactivated by favorable environmental conditions.

Genome analysis of the South Bronx outbreak strain identified several variable regions, many of which are associated with virulence factors, when compared with 5 previous outbreak-associated *L. pneumophila* strains (Figure 4). Two regions, 1 containing genes encoding an F-type IVA secretion system and 1 encoding *Legionella* U-box type E3 ligase/effector proteins, are also present in the 1976 Philadelphia 1 and Paris strains but absent from the other strains analyzed. The South Bronx outbreak strain F4469 also harbors an expanded isoform of the repeats in structural toxin gene (*rtxA*), similar to that found in the Corby and Alcoy strains. Finally, 2 genomic islands, 1 containing

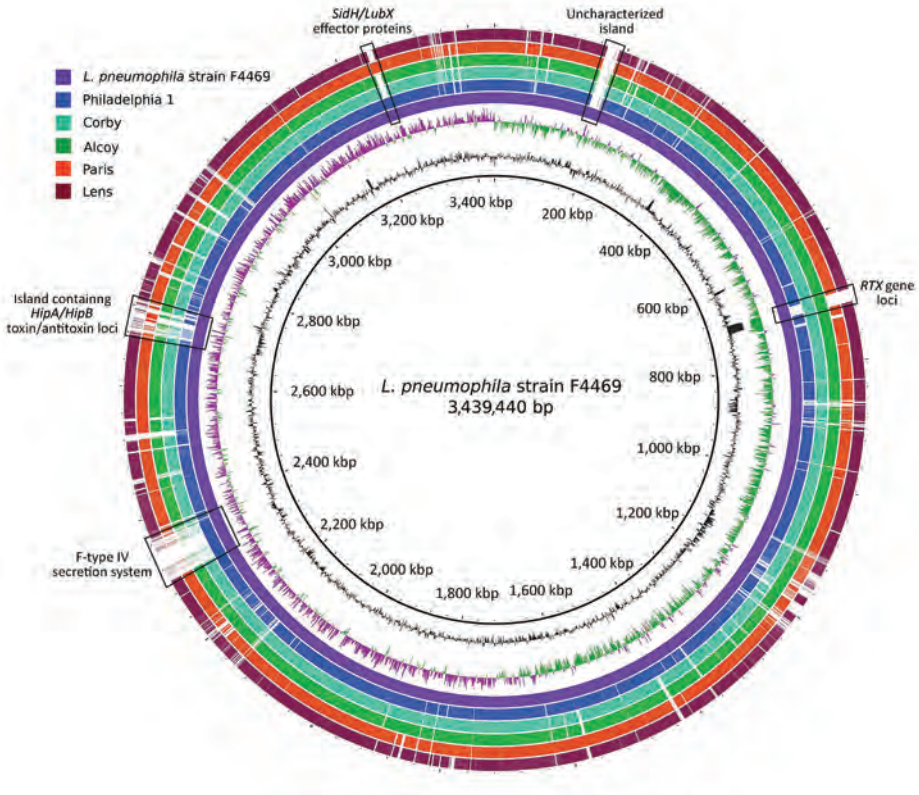

Figure 4. Blast ring genome comparison of *Legionella pneumophila* strains from investigation of Legionnaires' disease outbreak caused by an endemic strain of *L. pneumophila*, New York City, New York, USA, 2015. Comparison is shown between South Bronx outbreak strain (F4469) and other sequenced strains (Philadelphia 1, Corby, Alcoy, Paris, and Lens). The 2 innermost circles indicate G + C content and G + C skew, respectively, of the outbreak strain genome. Gaps in outer circles indicate genome areas in strain F4469 that are either absent or of low identity in compared genomes. Most of these regions are composed of virulence factor–associated genes, such as an F-type IVA secretion system, effector protein genes, toxin/antitoxin loci, and genes with unknown functions. *Hip*, hippurate hydrolysis gene; *Lub*, *Legionella* U-box gene; *RTX*, repeats in structural toxin gene; *Sid*; substrate of macrophage killing/defective organelle trafficking transporter gene.

the hippurate hydrolysis A and B gene toxin–antitoxin system, as well as other hypothetical genes, and 1 containing mostly uncharacterized genes, were found to be unique to the South Bronx strain. BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) searches on these 2 regions found matches with partial homology with other *L. pneumophila* strains in the National Center for Biotechnology (Bethesda, MD, USA) nonredundant database. Further laboratory investigations will be required to determine the role of these islands, if any, to pathogenicity of this strain.

Our analysis showed the presence of *L. pneumophila* strain F4469 in the Bronx since 2007 at multiple locations associated with different outbreaks and sporadic LD. Although it is unclear what caused the identified cooling tower to contribute to so many cases, our findings suggest that a persistent and pathogenic endemic strain exists and might pose a risk for future outbreaks. Conventionally, cooling towers are believed to be seeded by municipal water distribution networks, and although this factor might be true, in a densely populated area such as New York, New York, cross-contamination between towers is a real possibility. This contamination can potentially lead to reestablishment of *L. pneumophila* in cooling towers after decontamination and cause long-term persistence of endemic strains in communities. Therefore, strict protocols regarding tower operation, maintenance, and cleanup, such as those mandated by recent New York State and New York City legislation, might help to minimize risks associated with locally circulating *L. pneumophila* strains (*39*,*40*).

Acknowledgments

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P.L. developed the bioinformatics pipeline, performed data analysis, and wrote the article; E.N., K.A.M., Y.Z., D.W. and T.P. contributed to laboratory testing and reporting; and S.H., A.T., Y.L., J.K., R.F., D. Weiss, J.K.V., H.A.Z, J.L.R, S.S.M., B.H.R., and J.W.M. participated in the investigation and contributed to the manuscript.

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Symptom- and Laboratory-Based Ebola Risk Scores to Differentiate Likely Ebola Infections

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Rapidly identifying likely Ebola patients is difficult because of a broad case definition, overlap of symptoms with common illnesses, and lack of rapid diagnostics. However, rapid identification is critical for care and containment of contagion. We analyzed retrospective data from 252 Ebola-positive and 172 Ebola-negative patients at a Sierra Leone Ebola treatment center to develop easy-to-use risk scores, based on symptoms and laboratory tests (if available), to stratify triaged patients by their likelihood of having Ebola infection. Headache, diarrhea, difficulty breathing, nausea/vomiting, loss of appetite, and conjunctivitis comprised the symptom-based score. The laboratory-based score also included creatinine, creatine kinase, alanine aminotransferase, and total bilirubin. This risk score correctly identified 92% of Ebola-positive patients as high risk for infection; both scores correctly classified >70% of Ebola-negative patients as low or medium risk. Clinicians can use these risk scores to gauge the likelihood of triaged patients having Ebola while awaiting laboratory confirmation.

The 2014–2016 West Africa Ebola virus epidemic, unparalleled in spread for this disease, quickly overwhelmed the health systems of the 3 most affected countries (*1*). Ebola virus disease (EVD) can be difficult to initially identify even in a well-functioning health system because its early symptoms can closely mimic those of other common illnesses, such as malaria, typhoid, viral illness, and gastroenteritis (*2*,*3*). Thus, in an already weakened health system, the task of quickly but correctly identifying and isolating Ebola patients before laboratory test results are available is particularly challenging. This fact can result in missed opportunities to isolate infectious patients (through incomplete screening sensitivity) and expose non-Ebola patients to nosocomial infection (through incomplete specificity).

Currently, the most common laboratory test to identify EVD relies on a reverse transcription PCR (*2*), which is not a rapid point-of-care (POC) test but instead requires substantial laboratory infrastructure. In the West Africa outbreak, patient blood samples were typically sent to offsite laboratories set up through the international response. Although the test itself can be done in hours, the round trip from a health facility to the laboratory often took ≥3 days, especially during the peak of the epidemic (*4*). Although a few rapid POC EVD diagnostics were developed and field-tested during this outbreak, they are not yet ready for widespread commercial use (*5*,*6*).

In lieu of rapid POC EVD tests to identify EVD-positive cases, a standardized EVD case definition from the World Health Organization (WHO) was used during the epidemic as the primary tool for initially identifying potential EVD patients (*7*). Because false negatives for EVD put patients and their communities at great risk, this case definition is broad (high sensitivity/low specificity) (*8*). A broad case definition is also useful for epidemic surveillance. Patients meeting the case definition, which is based on symptoms and potential exposure, were sent to holding centers for EVD testing and isolation. However, the broad case definition meant that negative and positive EVD patients were mixed together, often for days, until their test results were available and treatment facilities had beds for the positive patients. Although some holding centers tried to separate suspect patients based on wet (i.e., diarrhea or vomiting) versus dry symptoms, this crude separation can expose Ebola-negative patients, particularly those with wet symptoms, to a higher risk of nosocomial infection.

Thus, an Ebola risk score that rapidly further differentiates the likelihood of Ebola infection beyond the case definition could be beneficial. Risk scores based on noninvasive information, such as demographic characteristics and symptoms, are practical tools for diagnostic prediction models because the predictors are comparatively simple to ascertain. Such risk scores are especially useful in

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resource-limited settings because a range of persons, from community health workers to clinicians, can quickly obtain the necessary patient information (*9,10*). Symptom-based risk scores have been used to identify patients at higher risk of various diseases (e.g., pulmonary tuberculosis, gastric cancer) or outcomes (e.g., predicting mortality in sick children) in resource-limited settings (*11–13*). However, symptom-based risk scores are limited by the accuracy of symptom reporting and the predictive power of those symptoms (*14,15*). Additional variables, such as laboratory tests, can improve the accuracy of risk scores, at the cost of less versatility (*16,17*). One solution has been to develop risk scores with optional additional variables to improve accuracy when those variables can be ascertained (*13*). As rapid POC laboratory devices such as the Piccolo Xpress (Abaxis, Inc., Union City, CA, USA) and i-STAT (Abbott Point of Care, Princeton, NJ, USA) analyzers have become more common even in low-income settings (*18*), adding optional laboratory tests to risk scores has become feasible.

We analyzed data from the Kerry Town Ebola treatment center (ETC) in Sierra Leone to develop 2 Ebola risk scores. The first uses symptom data and the second incorporates biochemistry laboratory tests to improve prediction accuracy. The goal of these risk scores is to supplement the broad WHO case definition by further separating triaged patients on the basis of their likelihood of being EVD positive.

Methods

Study Design and Patient Population

This research consisted of a retrospective cohort study of deidentified data on patients from November 5, 2014, through March 31, 2015, at the Kerry Town ETC in Sierra Leone. This 80-bed ETC, based in the Western Area Rural district, was operated by Save the Children International in partnership with the United Kingdom and Sierra Leone governments.

The patient population at the ETC consisted of patients with suspected or confirmed EVD, mostly from the nearby Western Area Urban and Western Area Rural districts. The ETC featured dry and wet wards for suspected Ebola patients (suspect wards) without a prior EVD test result and treatment wards for those confirmed to have EVD. Patients already confirmed to have EVD at previous holding centers were admitted directly to the confirmed wards. Suspected patients who met the admission criteria at triage were admitted to suspect wards while awaiting their EVD test results. All confirmed and suspected patients received on-site EVD tests, with results in <24 hours from admission. Suspected patients who tested positive were transferred to the confirmed wards; those testing negative were discharged or retested for up to 3 days before being discharged. All ETC patients with information recorded on basic demographic details and baseline symptoms were included in this study. The Sierra Leone Ethics and Scientific Review Committee and the London School of Hygiene and Tropical Medicine in the United Kingdom granted ethical approval for this study.

Data Collection

We used only data routinely collected for patient care on standardized clinical record forms. Referred patients often arrived with forms from their previous holding centers and/or a standardized case investigation form containing demographic and epidemiologic characteristics, as well as symptoms at admission (*7*).

We transcribed data from the paper clinical records into electronic format using Excel version 14.5.2 (Microsoft, Redmond, WA, USA). We then imported these data into Stata version 12 (https://www.stata.com) for statistical analyses.

Data Input and Cleaning

The outcome measure was EVD, confirmed by the on-site Public Health England laboratory using a reverse transcription PCR. For potential predictors of positive EVD test outcomes, we investigated 14 commonly recorded symptoms and analyzed these as binary variables. We recorded a symptom as present if it was checked as present on the case investigation, triage, or baseline admission forms. In an additional analysis, we included among the potential predictors 13 biochemistry laboratory tests performed by the on-site UK Ministry of Defense laboratory using a Piccolo Xpress device, which can yield rapid results (≈12 minutes) at the point of care. For the purpose of our analysis, we converted the test results into categorical variables based on low and high abnormal test ranges.

We performed multiple imputation by chained equations (*19*) for missing laboratory results based on the missing at random assumption. We used predictive mean matching with 20 iterations and included all the symptoms, the outcome variable, age, and sex as factors for the imputation.

Model Building

We built our symptom-based predictive model as follows. First, we performed univariable logistic regressions of each symptom against EVD outcome. We retained any symptom that had a p value of <0.40 for further analysis. We chose this lenient cutoff to balance the poor performance of diagnostic prediction models when relying solely on p values (*20*) against the need to reduce the number of symptom combinations being investigated. We used 10-fold cross validation to assess the best out-of-sample fit for all combinations of symptoms retained in the analysis after the univariable

analysis. We chose to use cross-validation because commonly used stepwise procedures for model selection have come under widespread criticism for several reasons, including overfitting, p value exaggeration, and biased coefficient estimates (*21*,*22*). Moreover, we were interested in selecting symptom combinations with better out-of-sample performance for use outside our patient dataset.

We used standard methods for cross-validation (*23*). First, we randomly partitioned the dataset into 10 equal subsamples. We then ran multivariable logistic regressions of each symptom combination against EVD outcome on 90% of the data (i.e., the training dataset), and used the results to predict the probability of EVD for each observation in the remaining 10% of the dataset (i.e., the validation dataset). For each symptom combination, we created receiver operating characteristic (ROC) curves and calculated the area under the curve (AUC) for each of the 10 validation datasets. The AUC is a standard measure of accuracy of test performance, with 1 indicating a perfect model and 0.5 indicating no better than random guessing (*24*). We took the median of the 10 AUCs as the overall goodness of fit for each candidate symptom combination model. We chose the best-fit model based on which model had the highest out-of-sample median AUC.

Development of Risk Score

We used previously established methods (*13*,*25*) to develop an easy-to-use Ebola symptom-based risk (ESR) score using the selected model. First, we ran a multivariable logistic regression of the model against EVD. We then assigned integer scores to individual symptoms based on their regression coefficients, with a score of 1 for coefficients <1 and a score of 2 for coefficients ≥1, while keeping the original sign. We calculated a patient's overall ESR score by adding the integer scores for the symptoms present in that patient. We then mapped the number of patients by true EVD status at each integer level of the ESR score. We evaluated the score by calculating the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for each level by designating all patients below that level as EVD negative and those at or above it as EVD positive. Finally, we determined low-, medium-, and high-risk categories by identifying risk thresholds that first maximized the number of true EVD positives in the high-risk category and minimized them in the low-risk category, and then best limited the true EVD negatives in the high-risk category.

Adding Laboratory Tests to the Ebola Risk Score

We performed additional analysis on patients with ≥1 non-Ebola laboratory result. We used univariable logistic regressions of the categorical laboratory test values against EVD outcome to retain laboratory tests with a p value of ≤0.40. For each possible combination of tests, we performed a multivariable logistic regression, this time adjusting for symptoms in the ESR score, and assigned an integer score to the laboratory tests using the same rule as that for the ESR score. We then used the net reclassification improvement (NRI) metric (*26*) and reclassification tables (*27*) to evaluate which laboratory test combinations best improved the symptom-based Ebola risk score. Of the 10 models with the highest NRI, we chose the model with the largest summed improvement in categorizing EVD-positive and EVD-negative patients within the high-risk category of the ESR score. We chose this metric to prioritize an improvement in sensitivity while not overly sacrificing specificity. We added the individual laboratory test integer scores to the ESR score for a final Ebola symptom- and laboratory-based (ESLR) score. To determine whether the ESLR score was an improvement over the ESR score, we visually compared their ROC curves and tested the statistical significance of the difference in AUCs (*28*).

Internal Validation

We performed internal validation of our risk scores using the bootstrap method to correct for overoptimism (*29*). We drew 1,000 bootstrap samples with replacement and calculated the AUC for the best model in each sample and the corresponding AUC in the full dataset. We then took the difference of these mean AUCs and corrected for overoptimism by subtracting this total from the AUC of our risk score. We performed this process separately for the ESR and ESLR scores.

Results

Of the 456 patients who were admitted to the Kerry Town ETC with suspected or confirmed EVD, we excluded 32 patients (7.0%): 31 had no baseline forms or minimal/no symptoms recorded, and 1 died before an EVD test could be conducted. Of the remaining 424 patients, samples from 252 tested positive for EVD and samples from 172 tested negative. Basic demographic characteristics and outcomes of the patients are provided in Table 1, and frequencies of clinical symptoms and the univariable logistic regression results by EVD status are shown in Table 2. The 10 symptoms with univariable p values ≤0.40 gave rise to 1,024 candidate symptom combinations for final model selection.

Using the cross-validation model selection, we obtained the best out-of-sample fit with a 6-symptom model comprising headache, diarrhea, difficulty breathing, nausea/vomiting, loss of appetite, and conjunctivitis. The median out-of-sample AUC for this model was 0.84 (interquartile range [IQR] 0.79–0.86). The validation analysis yielded a correction of 0.012, resulting in an internally validated AUC of 0.83 for the ESR score, which is considered excellent discrimination (*30*).

Table 1. Basic characteristics and outcomes of patients by EVD status at the Kerry Town ETC, Sierra Leone, 2014–2015*

Characteristic	EVD negative, n = 172	EVD positive, n = 252
Sex, no. (%)		
F	69 (40.1)	144 (57.1)
M	103 (59.9)	108 (42.9)
Median age, y (IQR)	27 (20–40)	25 (14–35)
Mode of arrival to facility, no. (%)		
Ambulance (referral)	95 (55.2)	242 (96.0)
Walk-in	77 (44.8)	10 (4.0)
Median days between onset of symptoms and admission (IQR)	3 (2–6)	3 (2–5)
Median length of stay at ETC, d (IQR)	1 (1–2)	6 (3–11)
Deaths, no. (case fatality ratio, %)	12 (7.0)	107 (42.5)

*ETC, Ebola treatment center; EVD, Ebola virus disease; IQR, interquartile range.

We found ≥1 non-EVD laboratory result for 309 patients. Of these, the percentage of missing laboratory results ranged from 5.1% for glucose to 15.2% for total bilirubin and aspartate aminotransferase. Visual inspection of the observed and imputed data suggested that the imputation worked well (online Technical Appendix Figures 1–13, https://wwwnc.cdc.gov/EID/article/23/11/17-0171-Techapp1.pdf). We excluded glucose and albumin because of p values >0.40 and thus retained 11 laboratory tests for consideration (online Technical Appendix Table 1).

According to the NRI and reclassification tables (online Technical Appendix Table 2), the best combination of additional laboratory tests was creatinine, creatine kinase (CK), alanine aminotransferase (ALT), and total bilirubin. The median out-of-sample AUC for the ESLR score was 0.91 (IQR 0.89–0.92). The internally validated AUC for the ESLR score was 0.90, which is considered outstanding discrimination (*30*).

Table 3 shows the multivariable model results, as well as the assigned integer scores for individual symptom and laboratory tests. An individual patient's ESR score could range from –3 to +5 and the ESLR score from –4 to +9. The trade-off between sensitivity and specificity for each score level was generally better for the ESLR than for the ESR score (Table 4). The difference in the AUC of the ROC indicated that the ESLR score was a significant improvement ($p<0.001$) over the ESR score (Figure 1).

We classified the ESR and ESLR scores as low risk if negative, medium risk if 0, and high risk if positive. Using the ESR score, we categorized 71.8% (95% CI 66.2%–77.4%) of EVD-positive patients as high risk and 14.3% (95% CI 10.0%–18.6%) as low risk (Figure 2, panel A). The ESLR score was more discriminant, with 91.9% (95% CI 87.8%–96.0%) of EVD positive patients considered high risk and 4.6% (95% CI 1.5%–7.7%) low risk using this score. Similar percentages of EVD-negative patients were categorized as low risk with the ESR (43.0%, 95% CI 35.6%–50.4%) versus ESLR (45.6%, 95% CI 37.2%–54.0%) scores (Figure 2, panel B). The ESR score performed better among EVD-negative patients classified as high risk, but not significantly so, with 23.8% (95% CI 17.4%–30.2%) for the ESR score compared with 29.4% (95% CI 21.7%–37.1%) for the ESLR score.

Discussion

We developed Ebola risk scores, based on reported symptoms and, optionally, complementary POC laboratory tests, to help categorize suspected Ebola patients into low-, medium-, and high-risk categories. These risk scores can be used after applying the case definition to further separate

Table 2. Patient clinical symptoms by Ebola status at the Kerry Town ETC, Sierra Leone, 2014–2015*

			Univariable logistic regression	
			Coefficient (95% CI)	p value
Fever†	165 (95.9)	230 (91.3)	–0.81 (–1.69 to 0.06)	0.068
Headache†	132 (76.7)	161 (63.9)	–0.62 (–1.06 to –0.19)	0.005
Fatigue	158 (91.9)	226 (89.7)	–0.26 (–0.94 to 0.42)	0.452
Joint/muscle pain†	146 (84.9)	182 (72.2)	–0.77 (–1.27 to –0.27)	0.003
Diarrhea†	68 (39.5)	165 (65.5)	1.06 (0.66 to 1.47)	<0.001
Bleeding	20 (11.6)	27 (10.7)	–0.09 (–0.71 to 0.52)	0.769
Difficulty breathing†	93 (54.1)	48 (19.1)	–1.61 (–2.04 to –1.18)	<0.001
Nausea/vomiting†	95 (55.2)	173 (68.7)	0.57 (0.17 to 0.98)	0.005
Abdominal pain	111 (64.5)	162 (64.3)	–0.01 (–0.42 to 0.39)	0.958
Hiccups†	39 (22.7)	42 (16.7)	–0.38 (–0.87 to 0.10)	0.124
Swallowing pain	56 (32.6)	90 (35.7)	0.14 (–0.27 to 0.55)	0.502
Loss of appetite/anorexia†	156 (90.7)	174 (69.1)	–1.47 (–2.05 to –0.90)	<0.001
Conjunctivitis†	44 (25.6)	122 (48.4)	1.00 (0.58 to 1.43)	<0.001
Rash†	6 (3.5)	16 (6.4)	0.63 (–0.33 to 1.59)	0.199

*ETC, Ebola Treatment Center; EVD, Ebola virus disease.
†$p \leq 0.40$ and thus retained to construct candidate symptom combinations.

Table 3. Factors included in ESR and ESLR scores to determine risk for infection in suspected Ebola patients*

Factor	Coefficient (95% CI) from multivariable model†	p value	Score value
Symptoms, for ESR and ESLR scores			
Conjunctivitis	1.44 (0.93 to 1.95)	<0.001	+2
Diarrhea	1.11 (0.60 to 1.61)	<0.001	+2
Nausea/vomiting	0.78 (0.24 to 1.31)	0.005	+1
Headache	−0.45 (−0.98 to 0.09)	0.103	−1
Difficulty breathing	−1.60 (−2.11 to 1.10)	<0.001	−1
Loss of appetite	−1.90 (−2.60 to −1.20)	<0.001	−1
Laboratory tests if available, for ESLR score only			
Alanine transaminase >48 U/L	3.83 (2.67 to 5.00)	<0.001	+2
Creatine kinase >380 U/L	1.78 (0.73 to 2.84)	0.001	+2
Creatinine >106 μmol/L	−1.15 (−2.21 to −0.09)	0.033	−1
Total bilirubin >27 μmol/L	−1.81 (−3.24 to −0.39)	0.012	−1

*ESLR, Ebola symptom- and laboratory-based risk; ESR, Ebola symptom-based risk.
†Laboratory tests have been adjusted for the symptom predictors. Coefficient values are before normalization.

suspected Ebola patients at triage based on their likelihood of having EVD.

The ESR and ESLR scores generally performed well. Of suspected patients whose specimens tested positive, >70% were categorized as high risk by the ESR score and >90% as high risk by the ESLR score. This result means that most true-positive patients could have been correctly separated from those who ultimately tested negative. About one quarter of EVD-negative patients were classified as high risk, but this trade-off was necessary to ensure that most true positives were correctly deemed high risk. Unfortunately, we do not have true diagnoses available for these non-Ebola patients to determine whether some illnesses were more associated with a high-risk classification than others. Compared with our results, we found low specificity (4.7%, 95% CI 1.5%–7.8%) and NPV (18.2%, 95% CI 6.8%–29.6%) when applying the symptom component of the WHO case definition (inexplicable bleeding or fever plus 3 of 10 symptoms) (*31*) to our patients. Both the ESR and ESLR scores can substantially improve upon these criteria. For example, using the threshold of ≥2 for the ESLR score resulted in 81.6% (95% CI 75.1%–88.1%) specificity and 80.4% (95% CI 73.8%–87.0%) NPV.

Although laboratory test results are generally of higher quality than self-reported symptoms, we included them

Table 4. Sensitivity, specificity, positive predictive value, and negative predictive value of ESR and ESLR scores to determine risk for infection in suspected Ebola patients*

Score	% EVD negative	% EVD positive	Sensitivity† (95% CI)	Specificity† (95% CI)	PPV† (95% CI)	NPV† (95% CI)
ESR score, range −3 to +5						
−3	8	0	100 (100–100)	0 (0–0)	59 (55–64)	NA
−2	16	4	100 (100–100)	8 (4–12)	61 (57–66)	100 (100–100)
−1	19	10	96 (94–98)	24 (18–31)	65 (60–70)	81 (70–91)
0	33	14	86 (81–90)	43 (36–50)	69 (64–74)	67 (59–76)
1	13	19	72 (66–77)	76 (70–83)	82 (76–87)	65 (58–71)
2	6	24	53 (47–59)	89 (84–94)	88 (82–93)	56 (50–62)
3	5	21	29 (23–35)	95 (92–98)	90 (84–97)	48 (43–53)
4	0	7	8 (5–11)	100 (100–100)	100 (100–100)	43 (38–47)
5	0	1	1 (0–3)	100 (100–100)	100 (100–100)	41 (36–46)
ESLR score, range −4 to +9						
−4	1	0	100 (100–100)	0 (0–0)	56 (50–62)	NA
−3	10	1	100 (100–100)	1 (0–2)	56 (51–62)	100 (100–100)
−2	16	1	99 (98–100)	10 (5–15)	59 (53–64)	93 (81–100)
−1	19	3	99 (97–100)	26 (19–34)	63 (57–69)	95 (88–100)
0	25	3	95 (92–99)	46 (37–54)	69 (63–75)	89 (81–96)
1	11	8	92 (88–96)	71 (63–78)	80 (74–85)	87 (81–94)
2	7	14	84 (79–90)	82 (75–88)	85 (80–91)	80 (74–87)
3	9	14	70 (63–77)	89 (84–94)	89 (84–94)	70 (63–77)
4	1	20	55 (48–63)	98 (95–100)	97 (94–100)	63 (57–70)
5	1	17	36 (29–43)	99 (97–100)	97 (93–100)	55 (48–61)
6	0	10	18 (13–24)	100 (100–100)	100 (100–100)	49 (43–55)
7	0	7	9 (4–13)	100 (100–100)	100 (100–100)	46 (41–52)
8	0	1	2 (0–4)	100 (100–100)	100 (100–100)	44 (39–50)
9	0	1	1 (0–3)	100 (100–100)	100 (100–100)	44 (39–50)

*ESLR, Ebola symptom- and laboratory-based risk; ESR, Ebola symptom-based risk; EVD, Ebola virus disease; NPV, negative predictive value; PPV, positive predictive value.
†Based on greater or equal to the score (e.g., sensitivity for a score of 0 means that those with a score of 0 or higher were considered to be EVD positive).

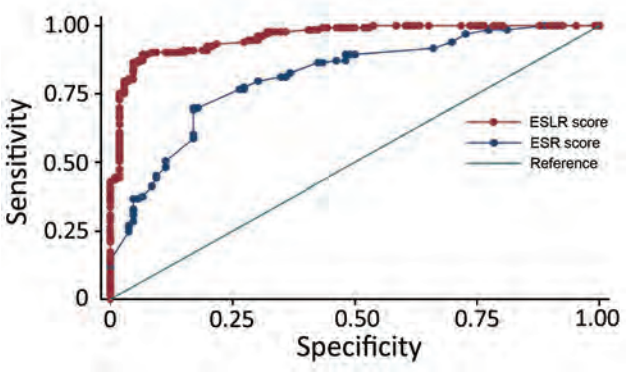

Figure 1. Receiver operating characteristic curves for ESR and ESLR scores to determine risk for infection in suspected Ebola patients. ESLR, Ebola symptom- and laboratory-based risk; ESR, Ebola symptom-based risk.

only as optional additions to our risk score, which permits better accuracy while retaining the versatility of using only the symptom-based score in places without necessary laboratory equipment. The improvement with laboratory tests can be substantial; in our study, 21.3% of EVD-positive patients were newly classified as high risk when using the ESLR score instead of the ESR score. With the rise of POC tests in low-resource settings, risk scores that rely on laboratory tests are becoming more feasible. For example, we included only laboratory tests that are available using the rapid POC Piccolo Xpress device, which was used at our site and by others during the West Africa Ebola outbreak (*32*,*33*).

Although risk scores have become a common tool for stratifying and predicting risk, we found only one previous study that developed a risk score for Ebola (*34*). That study, based on data from a Liberian ETC in 2014, was a notable first step for Ebola risk scores, but it did not include laboratory tests and had a smaller sample size. This difference may explain why our ESR and ESLR scores appear to have better sensitivity/specificity trade-offs.

We were constrained by the amount and quality of patient data because the data were collected during a challenging emergency response and only essential clinical, epidemiologic, and demographic information was collected for each patient. Thus, some data that may have improved our Ebola risk score, such as other symptoms or detailed exposure information, were unavailable. For example, we had to exclude exposure as a potential predictor because of questionable quality and large amounts of missing data for this variable.

Our study is based on a patient population at 1 treatment center; these patients may not be representative of the overall population of suspected Ebola cases in West Africa or in future Ebola outbreaks. Additionally, the distribution of patient characteristics may be different at a general medical facility or outside of an epidemic. The small correction factor from the internal validation exercise suggests that our scores would work similarly in a different epidemic study population. Ideally, our Ebola risk score should be externally validated against data from a future outbreak or, if made available, from this one. Although other common Ebola strains have similar reported symptoms to this Zaire strain (*35*), our scores should not be used for them without testing/validation. In general, the techniques presented here could be used to develop new risk scores for such strains or other hemorrhagic fevers. Given the good internal validation and the rare inclusion of high-quality POC laboratory tests, however, we believe that our work is a step toward having an accurate Ebola risk score.

Our risk scores cannot replace the WHO case definition or actual diagnostic testing. They can, however, help fill the gap between a broad case definition and an often-lengthy diagnostic process, which is valuable for several reasons. First, our risk scores can be used to more accurately separate likely negative from likely positive Ebola patients after initial screening with the WHO case definition.

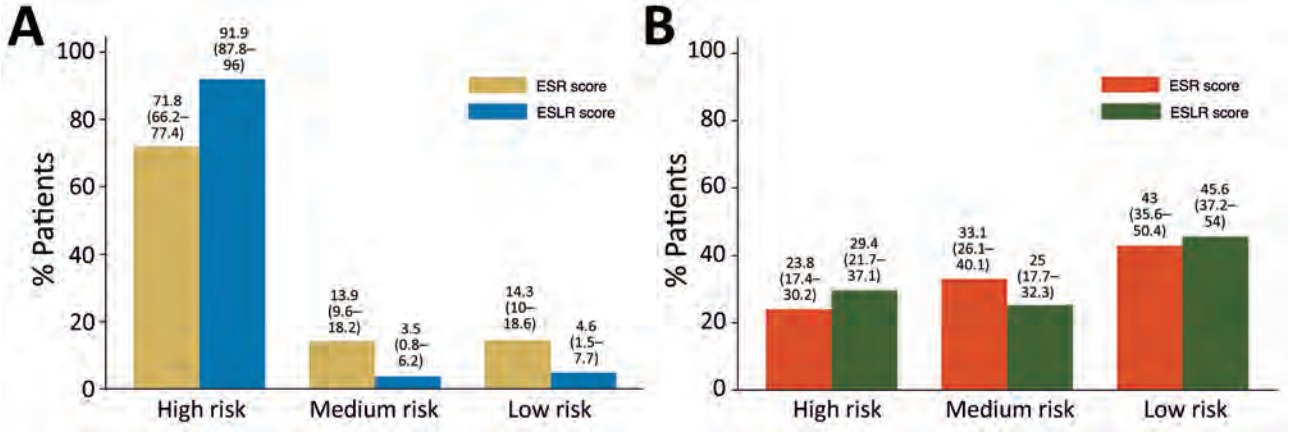

Figure 2. Suspected Ebola patients categorized as high-, medium-, and low-risk by ESR and ESLR scores, Kerry Town Ebola treatment center, Sierra Leone, 2014–2015. A) EVD-positive patients; B) EVD-negative patients. ESLR, Ebola symptom- and laboratory-based risk; ESR, Ebola symptom-based risk. Numbers in parentheses indicate 95% CIs.

For example, patients could be physically separated into low-, medium-, and high-risk suspect wards based on their risk score while awaiting Ebola test results. The higher-risk wards could then have further protections, such as additional barriers between patients or separation of wet and dry patients. This distinction is beneficial to both Ebola-negative patients and their communities because it reduces the risk of nosocomial infection that could be spread back to the community. Second, Ebola is a rapidly progressing disease, with typically only 6 to 16 days between onset of symptoms and death (*2*). Therefore, more accurately identifying likely positive cases while awaiting test results can mean earlier focus on and treatment of true positives during a response with limited resources. Third, with temporary Ebola holding and treatment centers now closed in West Africa, new cases there and elsewhere are likely to be screened at a wide range of places within the existing health system, from the community to local health centers to regional hospitals. When Ebola is rare, we still expect large numbers of patients to meet the WHO case definition because of the broad symptom list associated with EVD. An Ebola risk score can thus be useful in giving more precise information about risk to community health workers and clinicians. Finally, an advantage of using a risk score like ours is that, as the epidemic evolves, cutoffs for patient triage and categorization can be modified in real time (e.g., using Table 4) to reflect a changing emphasis on sensitivity vesus specificity.

Given the danger Ebola poses, classifying the risk of suspected Ebola patients is essential. Until a reliable rapid POC diagnostic for Ebola is readily available in low-resource settings, a flexible risk score that is easy to implement can be a useful tool for further triaging patients. Even though outbreaks of poorly understood but dangerous infectious diseases will continue in the future, developing such risk scores can help inform the difficult choices that healthcare workers must make during these emergencies.

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S.O., F.C., and K.W. contributed to designing the study. All authors contributed to data acquisition. S.O., A.S., and N.R. did data cleaning. S.O. performed the data analysis and wrote the first draft. All authors reviewed the paper, provided inputs, and approved the submission.

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Drug-Resistant Tuberculosis among Children, China, 2006–2015

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Microbial drug resistance has become a major public health concern worldwide. To acquire epidemiologic data on drug-resistant tuberculosis (DR TB) among children, a major cause of illness and death for this population, we conducted a retrospective study of 2006–2015 data from 36 TB prevention and control institutions in Shandong Province, China. A total of 14,223 new TB cases, among which children (≤18 years of age) accounted for only 5.5%, were caused by culture-confirmed *Mycobacterium tuberculosis*. Among children with TB, 18.9% had DR TB and 6.9% had multidrug-resistant TB. Over the past decade, the percentage of DR TB; multidrug-resistant TB; and overall first-line drug resistance for isoniazid, rifampin, ethambutol, and streptomycin among children increased significantly (at least 12%). Understanding the long-term trends of DR TB among children can shed light on the performance of TB control programs, thereby contributing to global TB control.

Tuberculosis (TB) is one of the leading causes of death worldwide (*1*). The World Health Organization (WHO) estimates that worldwide during 2015, this disease developed in 10.4 million persons and caused the death of 1.8 million (*2*). Children <15 years of age account for an estimated 10% of those affected by the global TB burden, corresponding to 1 million cases. Each year, ≈0.2 million children die of TB, which means that 2 children die of this disease every 5 minutes (*2*).

The attention given to TB in children has increased since the 2006 publication of the first edition of Guidance for National Tuberculosis Programmes on the Management of Tuberculosis in Children (*3*). However, expanding prevalence of TB globally, especially drug-resistant (DR) TB among children, is still a major cause of childhood illness and death (*4*). Control of TB among children is impeded by the challenges of presentation, diagnosis, reporting, and treatment; the absence of clear targets; and perceptions of low public health importance of TB (*5*). Difficulties with sputum collection and the paucibacillary nature of TB in children often make TB diagnosis difficult and drug-susceptibility testing (DST) inaccessible because DST is possible only after bacteriologic confirmation (*4*–*7*). Accordingly, children initially receive treatment for drug-susceptible TB (*7*). Because of its paucibacillary nature, childhood TB has been deemed less infectious and neglected by TB prevention and control institutions (*4*–*6*). Even some diagnosed and treated TB cases in children failed to be recorded in registers or reported to national TB programs (*8*).

Children with TB are especially susceptible to severe disease and death (*9*–*12*); even those with a favorable treatment outcome (cure or completion) or a latent infection could become a reservoir of disease relapse or reactivation (*13*). Although children metabolize drugs more rapidly than adults, guidance on drug regimens, dosages, appropriate monitoring, and duration of therapy for children is frequently extrapolated from adult data (*14*). With few drug options and limited experience, treatment for children with DR TB is complex. TB in children represents recent transmission and can be considered a sentinel of disease spreading throughout the community (*13*,*15*).

In this study, we compared baseline characteristics for children (≤18 years of age) and adults (>18 years of age) in Shandong Province, China, who had new cases of TB during 2006–2015. From a longitudinal perspective, we comprehensively assessed the burden of DR TB patterns among children.

Materials and Methods

Ethics

Ethics approval was obtained from the Ethics Committee of Shandong Provincial Hospital, affiliated with Shandong University, Shandong, China. Before analysis, patient records were anonymized and deidentified.

Study Population and Data Collection

This retrospective cohort study was conducted among 36 monitoring sites: 13 municipal-level local health departments, 21 county-level hospitals, and 2 province-level hospitals (Shandong Provincial Hospital and Shandong Provincial Chest Hospital). Monitoring site selection was based on convenience and reflection of a range of TB burdens and clinical capacities. New culture-confirmed TB cases

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that occurred in Shandong Province during 2006–2015 were consecutively collected from the China Information System for Disease Control and Prevention (http://www.chinacdc.cn/). In 2004, the Center for TB Control and Prevention of Shandong Province established the Katharine Hsu International Research Center of Human Infectious Diseases, the provincial health department where trained researchers collected and recorded patient information on a standard case report form. Since then, the Katharin Hsu Center has been responsible for laboratory quality assurance and TB surveillance in Shandong Province.

Mycobacterium tuberculosis was identified by culture; susceptibility to isoniazid, rifampin, ethambutol, and streptomycin was identified by DST. Information for all patients (age, sex, TB contact history, disease sites [pulmonary and extrapulmonary], and prior TB treatment history) was collected and recorded.

Laboratory Methods

Pulmonary samples were collected by expectoration, gastric aspiration, and sputum induction. Extrapulmonary samples (pleural fluid, spinal fluid, and lymph nodes) were collected by pleural tap, lumbar puncture, lymph node biopsy, fine needle aspiration, and other techniques (*3*).

All samples available from suspected sites of involvement were processed for smear and culture. Tissue samples were also examined for the presence of granulomas. To identify the presence of acid-fast bacilli, we used Ziehl-Neelsen staining for smear microscopy. Each sample was cultured on Lowenstein-Jensen culture medium. *M. tuberculosis* was identified according to combined growth characteristics, morphologic characteristics of the colony, and inhibition by p-nitrobenzoic acid (*16*). Samples containing nontuberculous mycobacteria were eliminated.

DST was performed by using the proportion method on Lowenstein-Jensen medium and the following drug concentrations: isoniazid (0.2 μg/mL), rifampin (40 μg/mL), ethambutol (2.0 μg /mL), and streptomycin (4.0 μg/mL) (*17*). Isolates with growth proportion for >1% on medium containing anti-TB drugs compared with the growth on drug-free medium were considered to be resistant to those drugs.

Laboratory Quality Control

The National Tuberculosis Reference Laboratory of the Chinese Center for Disease Control and Prevention and the Supranational Tuberculosis Reference Laboratory of the Public Health Laboratory Hong Kong were responsible for external quality assessment (EQA) (*16*). EQA for smear, culture, and DST in county- and district-level laboratories was conducted by the prefectural and provincial TB laboratories; EQA in the provincial reference laboratories and the National Tuberculosis Reference Laboratory was conducted by the Supranational Tuberculosis Reference Laboratory according to WHO guidelines (*17*). Blinded retesting of a random selection of ≈10% of isolates from each laboratory by a superior laboratory was essential.

Data Inclusion and Definitions

We included all patients with new TB cases and a positive *M. tuberculosis* culture for whom DST results, demographic information, and clinical information were obtainable. We excluded patients with nontuberculous mycobacteria infection and patients with HIV co-infection (in China, HIV-positive patients are immediately transferred to HIV-specialized hospitals).

Childhood TB was defined as TB in a patient ≤18 years of age. A TB isolate susceptible to all 4 of the tested first-line drugs was defined as drug-susceptible. MDR TB was defined as TB resistant to at least isoniazid and rifampin. TB contact was defined as contact with family members or schoolmates with TB long enough to enable long-term exposure (*18*). Bilateral disease means bilateral lesions (such as the tree-in-bud sign, bronchiectasis, cavitary pulmonary disease, and other inflammation signs) on radiologic images. A patient who had received anti-TB treatment for <1 month was classified as a new TB case-patient; a patient who had received anti-TB treatment for ≥1 month was classified as a previously treated TB case-patient (*17*).

Statistical Analyses

We analyzed the changes in proportions of the different resistance patterns over time by using the χ^2 test for trends and linear regression. From univariable analyses we obtained odds ratios (ORs) and 95% CIs, for comparison of specific characteristics between child and adult TB case-patients by Pearson χ^2 test. $p<0.05$ was considered to be significant. Statistical analyses were performed by using SPSS software, version 17.0 (SPSS Inc., Chicago, IL, USA) (*19*).

Results

Characteristics of Patients

We analyzed demographic, clinical, and laboratory information for 14,223 new TB case-patients in Shandong Province who had had culture-confirmed *M. tuberculosis* infection during the past decade. The mean ± SD age of these patients was 43.3 ± 19.6 years, and 784 (5.5%) patients were ≤18 years of age. Of the 784 children with TB, 597 (76.1%) were ≥15 years of age, 101 (12.9%) were ≥13 but <15 years of age, 86 (11.0%) were <13 years of age, and only 32 (4.0%) were <5 years of age.

Adults with TB were more likely than children with TB to be male (OR 2.13, 95% CI 1.84–2.44) and to have cavitary pulmonary disease according to chest radiographs (OR 1.38, 95% CI 1.17–1.61) (Table 1). Children with TB

Table 1. Sociodemographic and clinical characteristics of child and adult TB patients, Shandong Province, China, 2006–2015*

Characteristics	Age ≤18 y, no. (%), n = 784	Age >18 y, no. (%), n = 13,439	Total OR (95% CI), n = 14,223	p value
Male sex	458 (58.42)	10,078 (74.99)	2.134 (1.842–2.437)	<0.001
Extrapulmonary TB	154/784 (19.64)	2,274/13,439 (16.92)	0.833 (0.695–0.999)	0.05
TB contact†	39/784 (4.97)	396/13,134 (3.02)	0.594 (0.424–0.832)	0.002
Chest radiology				
Cavitary pulmonary disease	220/779 (28.24)	4,683/13,334 (35.12)	1.375 (1.172–1.614)	<0.001
Bilateral disease‡	218/401 (54.36)	2,929/5,051 (57.99)	1.159 (0.945–1.421)	0.16

*OR, odds ratio; TB, tuberculosis.
†Contact with family members or schoolmates with TB long enough for long-term exposure (*18*).
‡Bilateral lesions such as "tree-in-bud," bronchiectasis, cavitary pulmonary disease, and other inflammation signs seen on radiologic images.

were more likely to have extrapulmonary disease (OR 0.83, 95% CI 0.70–1.00) and to have had contact with a TB case-patient (OR 0.59, 95% CI 0.42–0.83) who was a family member or schoolmate.

Drug-Resistance Patterns

Among isolates from 784 new TB case-patients ≤18 years of age, the highest proportion of resistance was found for streptomycin (14.3%), followed by isoniazid (12.1%), rifampin (8.3%), and ethambutol (5.5%). MDR TB was found in 54 (6.9%) of these 784 children. Resistance to all 4 tested first-line drugs was found for 32 (59.3%) of the children with MDR TB. Among patients ≤18 years of age, 148 (18.9%) had cases resistant to ≥1 first-line drug and 52 (6.6%) had cases resistant to either isoniazid or rifampin (but not both). The proportion of overall ethambutol resistance and resistance to all 4 tested first-line drugs was significantly higher among children than adults (p = 0.001). The proportion of resistance to isoniazid or rifampin (but not both) was significantly lower among children than adults (p = 0.03) (Table 2).

Trends over Time

Among the 784 new cases of TB in children, the proportion that were DR TB increased from 14.7% in 2006 to 27.5% in 2015, a yearly increase of 1.3% ($R^2 = 0.58$; χ^2 test for trends: $\chi^2 = 7.231$, p = 0.007). Over the past decade, MDR TB increased yearly at a rate of 1.5% ($R^2 = 0.79$; χ^2 test for trends: $\chi^2 = 21.916$, p<0.001), from 1.3% to 15.4%. The percentage of a special type of MDR TB (resistance to all 4 tested first-line drugs) also increased 1.2% per year ($R^2 = 0.64$; χ^2 test for trends: $\chi^2 = 22.836$, p<0.001), from 0.0% to 13.2% (Figure 1). In addition, over the past decade, the estimated percentage of overall first-line drug resistance for isoniazid, rifampin, ethambutol, and streptomycin increased significantly (p<0.001 for isoniazid, rifampin,

Table 2. First-line drug resistance found for 14,233 new cases of TB, Shandong Province, China, 2006–2015*

Drug resistance	≤18 y, no. (%), n = 784	>18 y, no. (%), n = 13,439	p value
Any resistance to first-line drug	148 (18.88)	2,853 (21.23)	0.12
INH	95 (12.12)	1,884 (14.02)	0.14
RIF	65 (8.29)	1,112 (8.27)	0.99
EMB	43 (5.48)	449 (3.34)	0.001
SM	112 (14.29)	2,084 (15.51)	0.36
Resistance to 1 drug	67 (8.55)	1,323 (9.84)	0.23
INH	20 (2.55)	445 (3.31)	0.25
RIF	5 (0.64)	129 (0.96)	0.36
EMB	3 (0.38)	33 (0.25)	0.45
SM	39 (4.97)	716 (5.33)	0.67
Resistance to 2 drugs	27 (3.44)	683 (5.08)	0.04
INH + RIF	3 (0.38)	113 (0.84)	0.22
INH + EMB	2 (0.26)	30 (0.22)	0.70
INH + SM	17 (2.17)	459 (3.42)	0.06
RIF + EMB	1 (0.13)	4 (0.03)	0.25
RIF + SM	4 (0.51)	74 (0.55)	1.00
SM + EMB	0	3 (0.02)	NA
Resistance to 3 drugs	22 (2.81)	548 (4.08)	0.08
INH + RIF + EMB	2 (0.26)	15 (0.11)	0.24
INH + RIF + SM	17 (2.17)	468 (3.48)	0.05
INH + EMB + SM	2 (0.26)	55 (0.41)	0.77
RIF + EMB + SM	1 (0.13)	10 (0.07)	0.46
Resistance to at least INH/RIF	52 (6.63)	1,206 (8.97)	0.03
Multidrug resistant, overall	54 (6.88)	895 (6.66)	0.80
Resistance to 4 drugs	32 (4.08)	299 (2.22)	0.001

*EMB, ethambutol; INH, isoniazid; NA, not applicable; RIF, rifampin; SM, streptomycin; TB, tuberculosis.

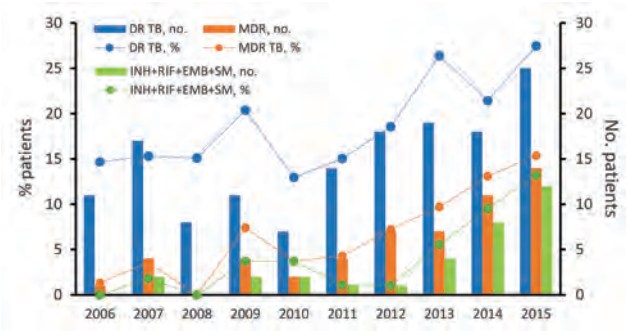

Figure 1. Trends for DR TB and MDR TB among children with primary cases of TB, Shandong Province, China, 2006–2015. The χ^2 and linear regression results are shown in Table 3. DR TB, drug-resistant TB; EMB, ethambutol; INH, isoniazid; MDR, multidrug-resistant; RIF, rifampin; SM, streptomycin; TB, tuberculosis.

and ethambutol; p = 0.01 for streptomycin) (χ^2 = 12.879, for isoniazid resistance, increasing at a yearly rate of 1.3% [R^2 = 0.64] from 6.7% to 22.0%; χ^2 = 26.743 for rifampin resistance, increasing at a yearly rate of 1.8% [R^2 = 0.84] from 1.3% to 18.7%; χ^2 = 24.972 for ethambutol resistance, increasing at a yearly rate of 1.4% [R^2 = 0.68] from 0.0% to 15.4%; χ^2 = 6.555 for streptomycin resistance, increasing at a yearly rate of 1.1% [R^2 = 0.47] from 10.7% to 23.1%) (Table 3, Figure 2).

Discussion

This 10-year retrospective cohort review of children with TB in the second largest province of China describes the clinical characteristics of TB in children and the epidemiology of DR TB among children. The major findings of this study are as follows: 1) among 784 new, treated cases of TB, an estimated 5.5% were in patients ≤18 years of age, among which children <15 years accounted for only 23.9%; 2) children with new cases of TB were more likely than adults to have extrapulmonary TB or a history of contact with TB patients; 3) ≈18.9% of TB cases in children were DR TB and 6.9% were MDR TB (over half of which were resistant to all 4 drugs tested; and 4) over the past decade, the percentage of DR TB, MDR TB, and overall first-line drug resistance among children has increased significantly.

The low sensitivity (*20*) of microbiological testing among children (sputum smear microscopy <5%, sputum culture 15%) may exacerbate the discrepancies between the number of expected cases and reported cases (*4*–*6*). Culture-confirmed TB in children <15 years of age accounts for only 1.3% of new TB cases in this study, far lower than the predicted proportion of childhood TB in China (>5%) (*5*). According to WHO data, TB incidence among young children <5 years of age was predicted to make up 58% (interquartile range 40–77) of that among total patients <15 years (*5*). Underreporting was more pronounced for the younger age group worldwide and especially in China (*5*). Young children <5 years of age accounted for only 4% of all TB cases in children during this study period. Children, especially young children <5 years of age, often have paucibacillary disease, and obtaining specimens is difficult, which prevents microbiological diagnosis (*21*,*22*), a vital element for patient selection in this study. The diagnosis of TB in children should be made cautiously by experts after thorough assessment of all evidence derived from a careful history, clinical examination, bacteriological confirmation, and relevant investigations (*3*). Unfortunately, in most low- and middle-income countries, the recommended contact investigations, including TB contact tracing for children suspected of having disease and contact screening for young children living close to a source case-patient, were rarely and inconsistently conducted (*18*). Without effective contact investigation, TB, especially DR TB, in children is rarely diagnosed and treated, which may worsen the situation. Although risks for severe disease and death are highest among children, TB in young children is the least likely to be confirmed bacteriologically (*20*). All these factors together suggest that effective diagnostic methods to microbiologically confirm TB, and regular contact investigations are urgently needed to refine future estimates of the incidence of TB and DR TB among children in China.

According to the most recent national DR TB survey in China, the reported proportions of new cases of DR TB and MDR TB were 34.2% and 5.7%, respectively (*16*); the proportions of new cases of DR TB and MDR TB among children in our study were 18.9% and 6.9%, respectively. Because drug resistance rarely develops for children during treatment (*23*), the high proportion of primary cases of MDR TB in children in our study may reflect recent transmission of MDR TB strains in Shandong. Previous

Table 3. Changes in proportions of different *Mycobacterium tuberculosis* resistance patterns, Shandong Province, China, 2006–2015*

Resistance pattern	χ^2	p value	R^2	X-coefficient	SE
Drug-resistant TB	7.231	0.007	0.58	0.013	0.117
Resistant to INH	12.879	<0.001	0.64	0.013	0.048
Resistant to RIF	26.743	<0.001	0.84	0.018	–0.019
Resistant to EMB	24.972	<0.001	0.68	0.014	–0.024
Resistant to SM	6.555	0.01	0.47	0.011	0.077
Multidrug resistant	21.916	<0.001	0.79	0.015	–0.015
Resistant to INH + RIF + EMB + SM	22.836	<0.001	0.64	0.012	–0.024

*EMB, ethambutol; INH, isoniazid; RIF, rifampin; SM, streptomycin; TB, tuberculosis.

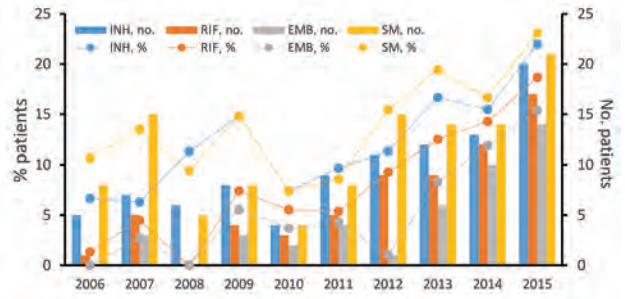

Figure 2. Overall first-line drug resistance for INH, RIF, EMB, and SM in primary cases of tuberculosis in children, Shandong Province, China, 2006–2015. The χ^2 and linear regression results are shown in Table 3. EMB, ethambutol; INH, isoniazid; RIF, rifampin; SM, streptomycin.

surveys reported that patients who had a history of prior contact with a TB patient were more likely to have MDR TB (*24*,*25*). In this study, more contact with TB was recorded among children than adults (p = 0.002). Because of the lack of standardized protocols for the therapy of childhood DR TB, children are empirically given the few formulations that are available for children and based on DST results, which are hard to access and often delayed (*26*,*27*). The fact that more than half of the new cases of MDR TB in children in this study (59.3%, 32/54) were resistant to all 4 tested first-line anti-TB drugs made the situation much worse. Reducing community-transmitted drug resistance and basing therapy on each patient's (children and source case-patients) DST results may enlighten future childhood TB control strategies (*6*,*28*).

The percentage of DR TB, MDR TB, and overall first-line drug resistance for isoniazid, rifampin, ethambutol, and streptomycin in primary cases of TB in children increased significantly over the study period. This finding indicates ongoing primary transmission of DR TB strains in China. Ongoing primary transmission of DR TB strains among children may cause catastrophic consequences. Other studies have reported that independent host factors that predispose to TB recurrence are malnutrition, smoking, HIV infection, and other immunosuppressive states (*29*). After the state of the host changes, even a person with a favorable treatment outcome (cure or completion) or a latent infection could become a reservoir for disease relapse or reactivation (*30*). To make things worse, the strongest risk factor for acquired DR and the highest risk for death is retreatment, as has occurred in Limpopo (South Africa) (*31*), Uganda (*32*), and Malaysia (*33*).

This study had some limitations. First, because we examined only 1 province on the eastern coast of China, the economic and regional disparities limited the generalizability of the results. Second, because we included only children with culture-confirmed TB, we did not analyze those who were treated on the basis of DR TB contact history or who had poor clinical response to therapy. Third, in this retrospective study, medical records provided little information on source cases, education, and living conditions; consequently, we failed to show the relationships between these factors and the DR TB epidemic. Last, the lack of genotyping (the standard for identifying the origin of resistant isolates) impeded us from correlating the mutations in the observed strains with the source strains in the environment in which the children lived.

In conclusion, primary cases of DR TB in children in Shandong Province, China, increased over the past decade. DR TB strains, especially MDR TB, are mainly transmitted by airborne infection from an adult source case-patient (*3*). To control the ongoing primary transmission of DR TB among children, especially among children in close contact with patients with diagnosed TB, more effective strategies are urgently needed. For more individualized anti-TB regimens for children, DST should be performed for both first- and second-line anti-TB drugs among children and their sources; regular contact investigations should also be performed. Moreover, ongoing reforms for financing TB diagnosis and treatment for children will be essential components of effective interventions for TB prevention and control in China.

If the global TB control strategy continues to pay less attention to the usually asymptomatic, paucibacillary, noncontagious childhood TB (*22*), the goal of achieving zero deaths from childhood TB by 2025 will be difficult to reach (*34*); control of TB in children and adults still faces huge challenges. Understanding the long-term trends of DR TB among children can shed light on the performance of TB control programs in China, thereby contributing to global TB control.

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Airborne Transmission of Highly Pathogenic Influenza Virus during Processing of Infected Poultry

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Exposure to infected poultry is a suspected cause of avian influenza (H5N1) virus infections in humans. We detected infectious droplets and aerosols during laboratory-simulated processing of asymptomatic chickens infected with human- (clades 1 and 2.2.1) and avian- (clades 1.1, 2.2, and 2.1) origin H5N1 viruses. We detected fewer airborne infectious particles in simulated processing of infected ducks. Influenza virus–naive chickens and ferrets exposed to the air space in which virus-infected chickens were processed became infected and died, suggesting that the slaughter of infected chickens is an efficient source of airborne virus that can infect birds and mammals. We did not detect consistent infections in ducks and ferrets exposed to the air space in which virus-infected ducks were processed. Our results support the hypothesis that airborne transmission of HPAI viruses can occur among poultry and from poultry to humans during home or live-poultry market slaughter of infected poultry.

Since 2003, approximately 850 human cases of Eurasian A/goose/Guangdong/1/1996 (Gs/GD) lineage H5N1 virus infection have been reported; case-fatality rate is 53% (*1*–*3*). Most human infections with highly pathogenic avian influenza (HPAI) subtype H5N1 virus have occurred following direct or indirect exposure to infected poultry in live-poultry markets (LPM) in developing countries (*1*–*3*). The main risk factors associated with human infections include visiting an LPM or performing activities with intensive contact with infected poultry, like slaughtering, defeathering, or preparing poultry for cooking (*3*,*4*).

Poultry-to-human avian influenza (AI) virus transmission can occur from 3 types of exposure: fomite-contact transmission, including contact with contaminated surfaces; droplet transmission, in which large (≥5 μm) particles contact a person's conjunctiva or respiratory mucosa; and droplet nuclei transmission (or aerosol transmission), in which a person inhales small (<5 μm) particles suspended in the air (*5*–*8*). The LPM setting plays a critical role in maintaining, amplifying, and disseminating AI viruses among poultry and from poultry to humans (*1*,*2*,*9*), with indirect evidence of potential transmission via fomites, as supported by the detection of AI viruses in the environment (*10*–*12*), and airborne exposure, supported by the recent isolation of influenza A viruses from air sampled at LPMs in China (*12*). Furthermore, viable AI viruses can be detected in the air where live poultry are kept and processing activities, such as slaughtering and defeathering, are performed (*12*).

Collective epidemiologic and surveillance data suggest that the slaughter of infected poultry is a major public health concern. In our study, we determined that viable airborne HPAI virus particles were generated during simulated processing of HPAI virus–infected poultry and that the airborne virus was transmitted to virus-naive poultry and mammals.

Materials and Methods

Viruses

Eurasian goose/Guangdong lineage H5N1 viruses were selected from human cases of influenza A(H5N1) virus, representing various years, hosts, countries, and clades (*1*,*3*) (Table 1). For experiment 1, we used 7 viruses (Table 1, all but Mong/05) for challenge in chickens, of which 4 that generated airborne virus particles were used in ducks. For experiment 2, we used Mong/05 and VN/04 viruses as challenge viruses. We propagated and titrated the viruses in embryonating chicken eggs (ECE) by standard methods (*13*).

Animals

For experiment 1, we obtained 9-week-old specific pathogen free (SPF) white Leghorn chickens (*Gallus domesticus* from the US Department of Agriculture Southeast Poultry Research Laboratory, Athens, GA, USA) and 8-week-old domestic Pekin ducks (*Anas platyrhynchos*

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Table 1. Information on Eurasian A/goose/Guangdong/1/1996 lineage (H5N1) virus isolates used in study of airborne transmission of highly pathogenic influenza virus during processing of infected poultry

Isolate	Abbreviation	Country	Host/source	Genetic clade	Accession nos.*
A/Vietnam/1203/2004	VN/04	Vietnam	Human	1	HM006756–63
A/chicken/Vietnam/NCVD-878/2011	VN/878/11	Vietnam	Poultry	1.1	Not available
A/chicken/West Java-Subang/29/2007	WJ/07	West Java	Poultry	2.1.3	EPI533441†
A/whooper swan/Mongolia/244/2005	Mong/05	Mongolia	Water fowl	2.2	GU186700–07
A/chicken/Egypt/102d/2010	Eg/10	Egypt	Poultry	2.2.1	HQ198270.1 HQ908480.1 KR732432.1 KR732440.1 KR732445.1 KR732492.1 KR732530.1
A/Egypt/N6658/2011	Eg/11	Egypt	Human	2.2.1	EPI372860–67†
A/chicken/Vietnam/NCVD-675/2011	VN/675/11	Vietnam	Poultry	2.3.2.1	KR732403 KR732406 KR732415 KR732468 KR732481 KR732506 KR732521 KR732536
A/chicken/Vietnam/093/2008	VN/08	Vietnam	Poultry	7.2	FJ538949.1 FJ538950.1 FJ842480.1

*Accession numbers from GenBank except as indicated. Accession numbers represent sequences from all available segments of influenza A virus.
†Accession numbers from GISAID (http://platform.gisaid.org).

domestica, from McMurray Hatchery, Webster City, IA, USA). All birds were serologically negative for influenza A virus infection by hemagglutinin inhibition (HI) test (*13*) before inoculation. For experiment 2, chickens and ducks were used as either infected or virus-naive exposed birds. Intravenous injection of sodium pentobarbital (100 mg/kg) was used to euthanize naive exposed survivors. Naive 3- to 5-month-old female domestic ferrets (*Mustela putorius furo*; Marshall BioResources, North Rose, NY, USA, and Triple F Farms Inc., Sayre, PA, USA) were used as the mammalian model for HPAI virus transmission to humans (*4*). Ferrets were anesthetized with an intramuscular injection of a mixture of ketamine (25 mg/kg), xylazine (2 mg/kg), and atropine (0.05 mg/kg) before nasal sample collection or euthanasia by intracardiac injection of sodium pentobarbital. Ferrets were H5-seronegative by HI test and virus neutralization test, and nasal wash samples were negative for virus isolation in ECE before exposure. All procedures were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee and the Institutional Biosecurity Committee.

Environmental Conditions in the Processing Enclosure

All experiments were conducted in Biosafety Level 3 animal facilities enhanced with additional biosafety features. The processing area was a high-efficiency particulate air (HEPA) enclosure (Class Biologically Clean Ltd., Madison, WI, USA) 1.5 m wide × 6.7 m long × 2.1 m high with unidirectional and single-pass airflow of 8.3 air changes/h (340 m^3/h) at 0.046 m/s from the processing area toward the air samplers or the naive animals (Figure 1). The mean temperature in the enclosure during the slaughter runs was 24.2°C ± 0.4°C; mean relative humidity was 81.0% ± 1.7%. We performed all procedures using adequate personal protective equipment: respiratory protection (HEPA-filtered powered air purifying respirators with full-shroud shield), closed-front gown, double gloves, and rubber boots.

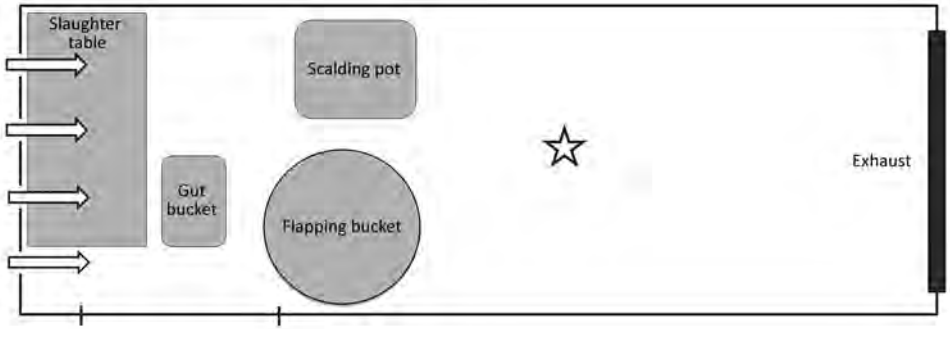

Figure 1. Processing area for study of airborne transmission of highly pathogenic influenza virus during processing of infected poultry. The star represents the location of the air sampler (experiment 1) or the naive hosts (experiment 2). The arrows indicate the airflow within the HEPA enclosure. The enclosure was 1.5 m wide × 6.7 m long × 2.1 m high, with 8.3 air changes/h (340 m^3/h) and a velocity of 0.046 m/s.

Air Sampling

The National Institute for Occupational Safety and Health (NIOSH) cyclone air sampler (model BC 251; NIOSH, Morgantown, WV, USA) collected particles and sorted them by their aerodynamic diameters into >4 μm, 1–4 μm, and <1 μm fractions at a flow rate of 0.0035 m^3/min (*14*). We mounted 2 stationary samplers 1.2 m above ground, 1 within the enclosure 80 cm downwind from the processing area (center to center) and the other outside the enclosure as negative control. Samplers were operated for the duration of each slaughter run plus 10 minutes; we sampled a total of 0.158–0.280 m^3 air per study trial, depending on the number of birds processed per trial.

Experimental Design

Experiment 1: Generation of Airborne HPAI Virus Particles during Simulated Processing of Infected Poultry

Each run (i.e., tested virus per bird species) was repeated at least twice for reproducibility. Chickens (10 for VN/04 and 5 for all other viruses) and ducks (5 per virus) were inoculated intranasally with $10^{5.3}$–$10^{6.5}$ mean egg infectious dose (EID_{50})/0.1 mL per virus and housed in negative-pressure isolators with HEPA-filtered ventilation. We moved chickens at 24 h after inoculation and ducks at 2.5 days after inoculation, which corresponded to times of peak shedding titers, to the processing enclosure while they were still asymptomatic. We anesthetized them by intramuscular injection of ketamine (10 g/kg) and xylazine (1 g/kg) and collected oral swab samples to confirm infection. The anesthetized birds were processed following 5 steps (total duration 6–7 min/bird) (*15*): 1) manual killing by severing the right jugular vein with a scalpel blade, causing bleeding and agonal involuntary muscle contractions (1 min); 2) scalding in a covered pot (52–53°C/2 min); 3) manual defeathering (2 min); 4) evisceration and removal of head, feet, and internal organs (1.5 min); and 5) cleanup of processing area with water (0.5 min). We rubbed the ducks with detergent before the scalding step to remove preening oils and facilitate defeathering. During the processing, air samplers were used as aforementioned. After each run, we disinfected all materials and surfaces within the enclosure, as well as the units holding the infected birds, with Virkon S 2% (DuPont, Wilmington, DE, USA). We tested swab samples for viable virus in ECE and titrated aerosol samples in ECE (*16*). The minimum detectable titer in ECE was 0.9 $\log_{10}$ EID_{50}/mL.

Experiment 2: Transmission of HPAI Viruses to Poultry and Ferrets during Simulated Processing of Infected Poultry

We performed 5 runs (Table 2). We inoculated chickens and ducks intranasally with $10^{5.9}$–$10^{6.1}$ EID_{50}/0.1 mL per virus (Table 2) and housed them in negative-pressure isolators. As in experiment 1, we anesthetized asymptomatic chickens and ducks, took oral and cloacal swab samples, and processed the birds using the 5-step method. During the processing, naive chickens, ducks, or ferrets (Table 2) were placed in cages at the same location and height as the air samplers in experiment 1 (with variations in experiment 2.1). After completion of each run, we placed the exposed animals in negative-pressure isolators and monitored them for clinical signs for 2 weeks. We collected oral and cloacal swab samples from exposed chickens at time of death and from exposed ducks at 3, 7, 10, and 14 days postexposure (dpe). We collected nasal wash samples and bodyweight measures from exposed ferrets at 3 and 7 dpe. We euthanized ferrets that had lost > 25% bodyweight or exhibited neurologic dysfunction. We performed necropsies on dead or euthanized exposed animals and collected tissues in 10% buffered formalin for hematoxylin/eosin and immunohistochemical staining (*17*). We titrated swab and nasal wash samples in ECE (*16*). At 14 dpe, we collected blood from the survivors for homologous HI and virus neutralization testing, then euthanized them.

Statistical Analysis

Using the D'Agostino-Pearson test, we determined that none of our parameters were normally distributed. We

Table 2. Experimental design and clinical outcome of animal hosts exposed to airborne highly pathogenic avian influenza (H5N1) viruses through simulated live-poultry market slaughter*

Virus	Intranasally infected birds processed (no.)	Duration of slaughter process, min	Naive exposed hosts (no.)†	Deaths of exposed hosts (mean time of death)	Virus detection in exposed hosts‡	Seroconversion in surviving exposed hosts§
Mong/05	Chickens (10)	60	Chickens (5)	5/5 (4.4 dpe)	5/5 at time of death¶	NA
VN/04	Chickens (10)	60	Chickens (5)	5/5 (4.0 dpe)	5/5 at time of death¶	NA
VN/04	Chickens (10)	60	Ferrets (4)	3/4 (8.3 dpe)	1/4 on 3 dpe (3.0)¶	0/1
VN/04	Ducks (5)	30	Ducks (5)	0/5	5/5 (1.6)	1/5
VN/04	Ducks (5)	30	Ferrets (3)	0/3	0/3	0/3

*dpe, days postexposure; EID, mean egg infectious dose; Mong/05, A/whooper swan/Mongolia/244/2005; NA, not available; VN/04, A/Vietnam/1203/2004.
†Exposed hosts placed 75–80 cm from the slaughter area.
‡No. positive/total no. Numbers in parentheses indicate mean virus titers ($\log_{10}$ EID_{50}/mL) determined by virus isolation in embryonating chicken eggs from oral and cloacal swab samples of exposed poultry or by nasal wash samples of exposed ferrets.
§Determined by hemagglutinin inhibition and virus neutralization tests when >12 dpe serum samples were available.
¶Virus antigen was detected by immunohistochemistry in tissues of 5/5 Mong/05-exposed chickens, 5/5 VN/04-exposed chickens, and 3/4 VN/04-exposed ferrets.

conducted 2-tailed Mann-Whitney test to determine significant difference in mean viral titers ($p<0.05$) using GraphPad Prism 6 (GraphPad Software, Inc., La Jolla, CA, USA).

Results

Experiment 1

Preslaughter swab samples were positive for virus in all asymptomatic birds with titers ≥1.5 $\log_{10}$ EID_{50}/mL. We isolated VN/04, VN/878/11, WJ/07, and Eg/11 viruses from air samples collected when processing virus-infected chickens, with highest virus quantity in >4 μm particles, moderate quantities in 1–4 μm particles, and no virus in <1 μm particles. We did not detect Eg/10, VN/67511, or VN/09 viruses in air samples (Figure 2, panel A). We used the 4 airborne viruses recovered from the chicken study in the duck slaughter experiment; we detected VN/04 and Eg/11 viruses in both >4 μm and 1–4 μm particles, and VN/878/11 virus in >4 μm particles. We did not detect airborne virus from slaughter of WJ/07 virus–infected ducks (Figure 2, panel B). We detected no virus from aerosol samplers located outside the enclosure.

Experiment 2

Experiment 2.1. Transmission of A/whooper swan/Mongolia/244/2005(H5N1) HPAI Virus to Naive Chickens Exposed During Simulated Processing of Infected Chickens

Swab samples were virus positive from asymptomatic Mong/05 virus–inoculated chickens. As a variation, for every 10 processed Mong/05 virus-infected chickens, we placed 5 exposed naive chickens 75 cm, 150 cm, or 300 cm from the slaughter area (all distances found in an LPM scenario) 1.2 m above ground, in a holding cage similar to those used in LPMs. Regardless of the distance from the processing area, all exposed chickens died between 3 and 6 dpe. All oral and cloacal swab samples collected at time of death were positive by virus isolation. We found lesions typical of those caused by HPAI and AI viral antigen in multiple internal organs of all exposed chickens, indicating infection after droplet/aerosol exposure (Table 2).

Experiment 2.2. Transmission of A/Vietnam/1203/04(H5N1) HPAI Virus to Naive Chickens and Ferrets Exposed during Simulated Processing of Infected Chickens

Swab samples were virus positive from asymptomatic VN/04 virus–inoculated chickens. Following the processing of infected chickens, all 5 exposed naive chickens died between 3 and 5 dpe, and all oral and cloacal swab samples we collected at time of death were virus positive (Table 2). Out of 4 exposed ferrets, 2 died, 1 on 6 dpe and the other on 7 dpe; another ferret was euthanized on 12 dpe. Neurologic disease, with lesions typical of those caused by HPAI and AI virus in multiple internal organs including the brain, developed in these 3 ferrets (Figure 3). The ferret that died on 7 dpe had positive nasal wash samples collected at 3 dpe (3.0 $\log_{10}$ EID_{50}/mL), and the ferret that was euthanized on 12 dpe seroconverted (Table 2). The survivor had no antibodies to AI or pathologic lesions and no virus in nasal wash samples, and it was the only ferret to gain weight; therefore, we considered it not infected. In summary, 3 of 4 naive ferrets became infected after droplet/aerosol exposure.

Experiment 2.3. Transmission of A/Vietnam/1203/04(H5N1) HPAI Virus to Naive Ducks and Ferrets Exposed during Simulated Processing of Infected Ducks

Swab samples were virus positive from asymptomatic VN/04 virus–inoculated ducks. Following the processing of infected ducks, exposed naive ducks and ferrets did not exhibit clinical signs nor did they die over the 2-week observation period (Table 2). We isolated virus from oral and cloacal samples of

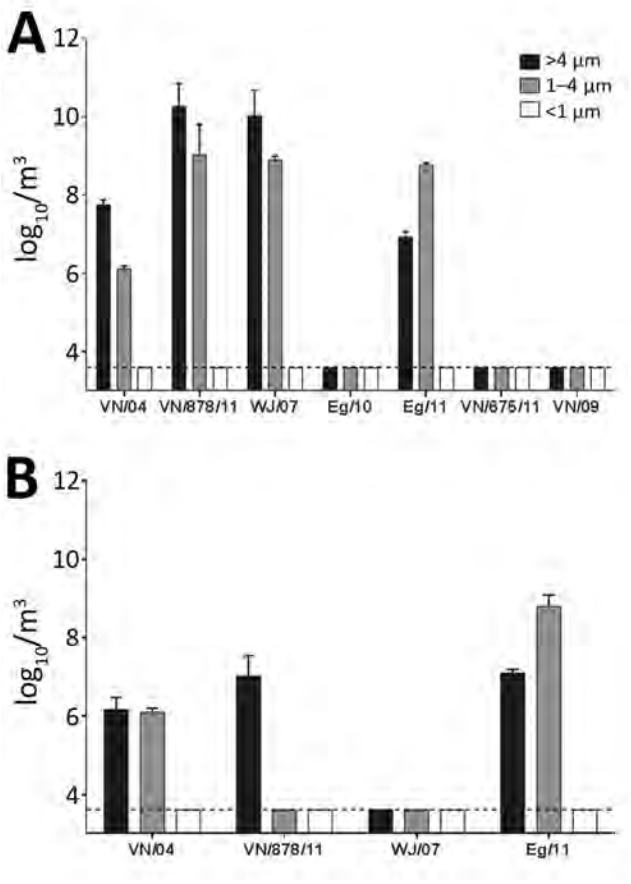

Figure 2. Highly pathogenic avian influenza virus isolation from air samples collected using cyclone air sampler dur-ing simulated slaughter of infected chickens (A) and ducks (B) in study of airborne transmission of highly pathogenic influenza virus during processing of infected poultry. Detection of virus was attempted in 3 dif-ferent airborne particle sizes. Error bars indicate virus recovery from >2 repeats per run. Dashed lines indi-cate limit of detection by virus isolation of 3.6 log10 mean egg infectious dose/m3 air sampled. Isolate names are as given in Table 1.

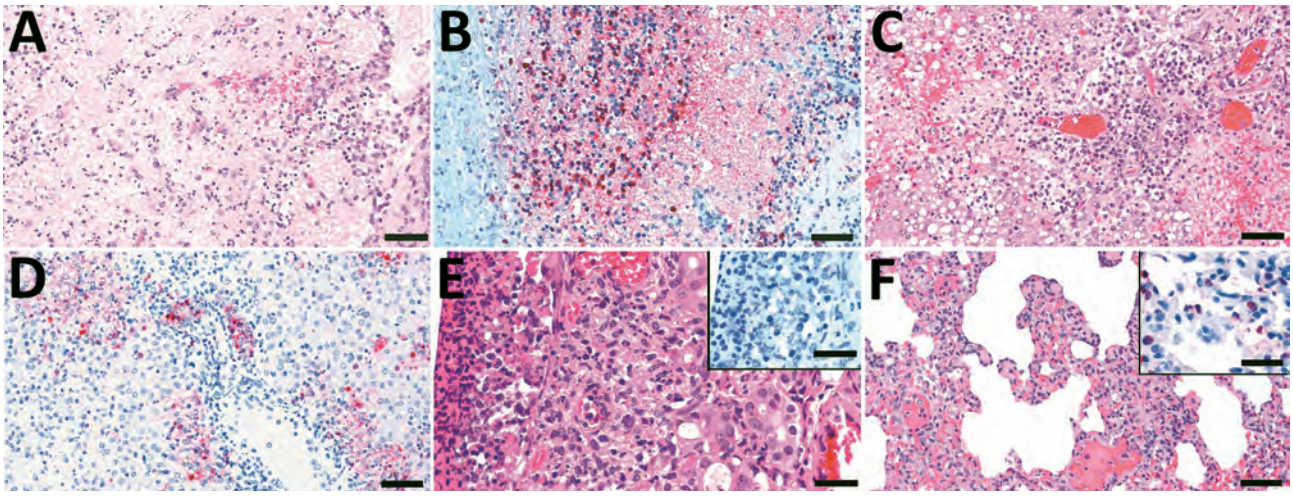

Figure 3. Histologic lesions and immunohistochemical detection of viral antigen in samples from ferrets exposed to live poultry market processing of highly pathogenic avian influenza A/Vietnam/1203/04 (H5N1) virus–infected chickens in study of airborne transmission of highly pathogenic influenza virus during processing of infected poultry. A) Olfactory bulb, 7 dpe, showing diffuse and severe neuropil malacia with mild cavitation and focal hemorrhages. Scale bar = 50 μm. B) Olfactory bulb, 7 dpe, showing viral antigen detected in neuropil, astrocytes, and neurons. Scale bar = 50 μm. C) Liver, 8 dpe, showing confluent coagulative necrosis of hepatocytes and bile duct necrosis with mononuclear cellular infiltrate in the portal triad. Scale bar = 50 μm. D) Liver, 8 dpe, showing viral antigen detected in hepatocytes, bile duct epithelia, and cellular debris. Scale bar = 50 μm. E) Nasal cavity, 7 dpe, showing moderate necrotic rhinitis with coagulative necrosis of mucous glandular epithelial cells; insert shows no viral antigen detected in mucosal membrane. Scale bars = 25 μm. F) Lung, 7 dpe, showing mild histiocytic interstitial pneumonia; insert shows viral antigen detected in type II pneumocytes. Scale bars = 25 μm. dpe, days postexposure.

exposed ducks; peak individual titers were 3.1 $\log_{10}$ EID_{50}/mL and mean titers were 1.6 $\log_{10}$ EID_{50}/mL on 3 dpe (Figure 4). All exposed ferrets gained weight and had negative nasal wash samples and were considered to be uninfected (Table 2). All exposed ducks and ferrets were seronegative at termination with the exception of 1 duck (HI titer of 8) (Table 2).

Discussion

The epidemiology of human influenza A (H5N1) infections suggests that LPM slaughter processing of infected poultry could provide sufficient exposure to cause transmission to humans (*1*–*4*). Zhou et al. showed that viable H5, H7, and H9 AI viruses with human zoonotic potential are detectable in the air of LPMs in China (*12*). Here we demonstrated that the processing of asymptomatic HPAI virus–infected poultry in high biocontainment laboratory facilities produced airborne HPAI virus particles, which are airborne transmissible to naive poultry and mammals.

The simulated slaughter of infected poultry generated viable virus predominantly in droplets (>4 μm) and aerosols (1–4 μm) but none in particles (<1 μm). Our findings align with those of previous studies that used air samplers in LPMs (*12*) and swine barns (*18*,*19*), and farm-to-farm dissemination studies to demonstrate airborne virus (*20*). Determining the particle size distribution has key implications for the control of influenza in humans through droplet and aerosol transmission. Infectious particles with aerodynamic diameters <4 μm (i.e., aerosols) can more easily reach the lower respiratory tract of humans, where AI viruses with binding specificity for α-2,3 receptors primarily replicate, than larger particles can (*21*). The recovery efficiencies we obtained in this study ($\leq$10 $\log_{10}$ particles per m^3 in >4 μm fraction) were higher than those from similar sampling methods in LPMs (*12*) possibly due to standardized high-dose challenge of all birds, optimized timing of slaughter, controlled environmental conditions, or other reasons. Human-origin viruses of clades 1 and 2.2.1 and avian-origin viruses of clades 1.1, 2.2, and 2.1.3 were detected in droplets and aerosols during the slaughter of infected chickens. However, other poultry-origin viruses (clades 2.2.1, 2.3.2.1, and 7.2) were not detectable (Figure 2). Three viruses (VN/04, VN/878/11, and Eg/11) generated consistent infectious droplets, aerosols, or both during the slaughter of infected ducks (Figure 2). Our results suggest that differences in the potential for incorporation of infectious HPAI viruses in airborne particles generated while processing infected poultry vary with the infected poultry species and specific HPAI virus. This study aimed to detect infectious virus; whether the viruses that were not detected or transmitted were not aerosolized, or whether they were present in airborne particles but were not infectious, warrants further study.

The processing of HPAI virus–infected chickens seems to be more effective at generating infectious droplets and aerosols than the processing of infected ducks.

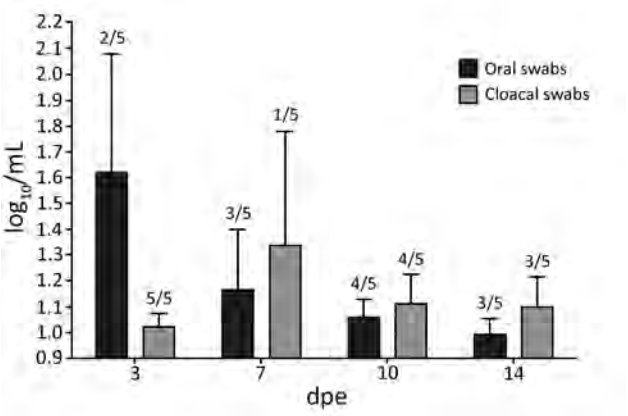

Figure 4. Virus titers in oral and cloacal samples of ducks exposed to simulated live poultry market slaughter of highly pathogenic avian influenza A/Vietnam/1203/04(H5N1) virus–infected ducks in study of airborne transmission of highly pathogenic influenza virus during processing of infected poultry. Shedding titers are expressed as $\log_{10}$ with error bars included. Numbers on top of the bars indicate the number of positive samples out of the 5 tested samples at each time point. The limit of detection was 0.9 $\log_{10}$ mean egg infectious dose/mL.

This finding may be due to greater infectivity, virulence, and pathogenicity (i.e., viral loads present systemically) in asymptomatic infected chickens than in ducks. Chickens are highly susceptible to HPAI viruses and in particular to Gs/GD lineage H5N1 viruses, which usually cause multiple organ failure associated with systemic virus replication and high mortality rates (*22*). By comparison, domestic ducks have shown moderate to high susceptibility to post-2002 Gs/GD H5N1 HPAI viruses (*23*). The lack of virus replication in duck endothelial cells and the absence of associated vascular damage has been identified as a key difference in pathogenesis between domestic ducks and chickens (*23*,*24*), which could determine not only the extent of replication for certain H5N1 HPAI viruses but also the quantities of virus found in different tissues. Previous reports have shown lower H5N1 virus titers in duck tissues than corresponding chicken tissues after intranasal inoculation (*25*–*27*). It is worth highlighting that age at infection can affect the pathogenicity of Gs/GD H5N1 HPAI viruses; VN/04 virus is more pathogenic and can replicate to higher titers (up to 4 $\log_{10}$ EID_{50}/mL difference) in 2-week-old ducks than in 5-week-old ducks (*23*,*28*). The great majority of pathogenicity studies in domestic ducks use 2- to 5-week-old birds, whereas our studies required older ducks to match the age of slaughter in LPMs. The use of older ducks could have reduced the infectivity, replication, and virulence of H5N1 HPAI viruses, limiting systemic virus replication and reducing the quantity of virus incorporation into airborne particles generated during slaughter. Another factor responsible for differences between chickens and ducks is that the tested viruses were of chicken, human, and swan origin; whether a duck-origin virus would have been more efficient at generating infectious aerosols during duck manipulation needs to be investigated. In addition, all birds were confirmed to be infected at the moment of slaughter, but virus quantification in swab samples was not attempted; whether differences in oropharyngeal virus replication could explain differences in aerosolization and transmission is worth pursuing in future studies.

The slaughter of H5N1 virus–infected chickens had variable efficiency in producing infectious airborne particles and was not associated with specific HA genetic clades. However, specific changes in the HA and other gene segments could play a relevant role in airborne transmission. Similarly, sequence polymorphisms in internal proteins, in addition to those previously described for HA, may regulate airborne transmission of HPAI virus strains in mammals (*4*,*29*). Furthermore, the processing of A/chicken/Chile/184240–1(4322)/2002(H7N3) HPAI virus–infected chickens did not produce airborne virus (D.E. Swayne, unpub. data), compatible with the lack of human cases during the outbreak in Chile (*30*). However, human infections with H7N9 low pathogenicity AI virus have frequently been reported in China since 2013 (*31*), with a clear link between human cases and LPM exposure (*1*,*2*,*9*). These data suggest that not only H5N1 HPAI viruses have the potential to generate transmissible particles but also some H7 AI viruses (*4*) and potentially H9N2 viruses (*4*).

The LPM setting offers a variety of live bird species, providing an ideal environment to introduce and maintain AI viruses in the poultry population (*9*). Although intranasal administration is considered a standard practice for the study of AI virus pathogenicity, it is not the natural route of infection by contact or airborne routes. To our knowledge, this study is the closest re-creation of airborne transmission in the home or LPM slaughter setting. Naive chickens and ferrets exposed to the slaughter of Mong/05 and VN/04 virus–infected chickens, respectively, became infected and died. This finding confirms that the slaughter of infected chickens is an efficient source of exposure not only to other birds but also to ferrets, which are the model for human influenza transmission. The pathogenicity observed in chickens exposed to airborne Mong/05 was consistent with that observed in previous studies of systemic disease after intranasal inoculation of Gs/GD HPAI viruses (*22*). Similarly, the high pathogenicity and systemic infection in ferrets exposed to airborne VN/04 is consistent with that found by previous pathogenicity studies with this and other HPAI viruses in intranasally inoculated ferrets (*32*–*35*). Overall, these data confirm that the natural airborne route produces comparable infections to those produced by the commonly used intranasal route (*5*,*36*,*37*). Ocular

exposure probably contributed to transmission because ocular mucosa represents a potential site for both replication and entry of airborne respiratory viruses (*38*–*40*).

In contrast, naive ducks and ferrets exposed to the same air space as the processing of VN/04 virus–infected ducks caused airborne infections in some of the animals. Although virus was isolated from swab samples of some exposed ducks, the lack of illness and death and lack of consistent seroconversion suggested that the slaughtering of infected ducks did not generate sufficient quantities of airborne viable virus to consistently produce infection in exposed ducks and failed to transmit virus to ferrets. Low levels of local replication at the mucosal level could have induced low levels of circulating antibodies in exposed ducks; therefore, systemic antibody titers may have been under the limit of detection. Collectively, these findings suggest that the processing of infected 8-week-old ducks may not be as consistent a source of airborne virus as processing infected chickens. One reason may be the age at slaughter: older ducks may not support such systemic virus replication as do chickens, lowering the quantities of generated airborne virus and, consequently, not reaching the minimum infectious dose required to efficiently infect naive adult ducks and ferrets. Another reason could be the lower number of slaughtered infected ducks (n = 5) compared with chickens (n = 10) per airborne exposure group, which implies a shorter exposure time for naive ducks.

In addition to the slaughter processes and the environmental conditions, time parameters were controlled to emulate field conditions (Table 2). Previous transmission studies in co-housed animals generally involve continuous exposure in which the recipient and donor animals are exposed to the same air space and sometimes fomites for 14 days (*41*). However, exposures of uninfected humans to others with seasonal influenza viruses are limited to a few hours (*42*), similar to HPAI virus exposure during slaughter or other manipulations of infected poultry. Each processing trial in our study lasted for $\leq$ 1 h because of the need to mimic time-limited exposure events (*41*). Because this limit of exposure probably decreased successful transmission events compared with other animal studies with longer exposure times, we believe that our experiments more appropriately reflect the transmissibility of airborne AI viruses to humans and emphasize the high risk that slaughtering infected poultry entails (*41*). Although all the steps in the slaughter procedure may contribute to virus aerosolization, defeathering is often identified as a main risk activity (*4,12*). Further research to determine the most contaminating steps will help develop efficient mitigating measures.

This study recreates generation and transmission of infectious influenza airborne virus particles by processing infected poultry in an experimental setting, matching time exposure events. We confirmed that the simulated slaughter of chickens infected with different clades of Gs/GD lineage H5N1 viruses generated infectious droplets and aerosols. Moreover, naive chickens and ferrets exposed to the same air space as the slaughter of infected chickens became infected and died, but the same could not be consistently confirmed following the slaughter of infected ducks. Further experiments investigating simple, feasible changes in slaughter methods to prevent or reduce infectious airborne particles during the slaughter process, and determining the effectiveness of such strategies on reducing virus transmission, are critical for preventing zoonotic HPAI (H5N1) virus infections of humans.

Acknowledgments

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Antimicrobial Nonsusceptibility of Gram-Negative Bloodstream Isolates, Veterans Health Administration System, United States, 2003–2013[1]

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Bacteremia caused by gram-negative bacteria is associated with serious illness and death, and emergence of antimicrobial drug resistance in these bacteria is a major concern. Using national microbiology and patient data for 2003–2013 from the US Veterans Health Administration, we characterized nonsusceptibility trends of community-acquired, community-onset; healthcare-associated, community-onset; and hospital-onset bacteremia for selected gram-negative bacteria (*Escherichia coli*, *Klebsiella* spp., *Pseudomonas aeruginosa*, and *Acinetobacter* spp.). For 47,746 episodes of bacteremia, the incidence rate was 6.37 episodes/10,000 person-years for community-onset bacteremia and 4.53 episodes/10,000 patient-days for hospital-onset bacteremia. For *Klebsiella* spp., *P. aeruginosa*, and *Acinetobacter* spp., we observed a decreasing proportion of nonsusceptibility across nearly all antimicrobial drug classes for patients with healthcare exposure; trends for community-acquired, community-onset isolates were stable or increasing. The role of infection control and antimicrobial stewardship efforts in inpatient settings in the decrease in drug resistance rates for hospital-onset isolates needs to be determined.

Despite advances in public health and medical care, bacteremia is still a major cause of illness and death (*1–3*). Bacteremia caused by gram-negative bacteria is a frequent cause of severe sepsis and septic shock (*4*,*5*) and poses serious therapeutic challenges. Treatment options are limited because of increased infections with multidrug-resistant, gram-negative bacteria in community and hospital settings. Understanding the epidemiology of infections with gram-negative bacteria is needed to improve guidelines providing empiric therapy recommendations and adequately allocate resources toward infection control and antimicrobial stewardship programs.

Descriptions of the epidemiology of gram-negative bacteremia have been limited by care settings, referral bias, small geographic regions, short study duration, or case identification by use of administrative code data (*6–10*). As modern healthcare systems become increasingly diverse and complex, large-scale studies are needed that accurately estimate the burden of antimicrobial resistance and chronologic trends of gram-negative bacteremia (*11–13*).

The Veterans Health Administration (VHA) is the largest healthcare system in the United States and has ≈8 million veterans enrolled (*14*). The VHA uses an integrated electronic health record and a nationwide data repository. Microbiological results from all VHA facilities have been added to the data repository, which enables identification of all cases of gram-negative bacteremia across the entire VHA system. These results enable examination of national trends in gram-negative bacteremia in a geographically dispersed and diverse population.

We previously reported incidence rates of community-onset and hospital-onset bacteremia caused by 3 species of gram-negative bacteria in a cohort of patients admitted to the VHA system during 2003–2013 (*15*). In this article, we expand on that study by reporting trends in antimicrobial nonsusceptibility (intermediate or resistant) among bacteremia isolates of 4 selected gram-negative bacteria (*Escherichia*

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coli, *Klebsiella* spp., *Pseudomonas aeruginosa*, and *Acinetobacter* spp.) in the national VHA healthcare system.

Methods

Study Population

We analyzed a retrospective cohort of all veterans who were admitted to acute-care units at VHA hospitals during January 2003–December 2013 and who had positive blood cultures for *E. coli*, *Klebsiella* spp., *P. aeruginosa*, or *Acinetobacter* spp. between 48 hours before admission and time of discharge. We used these 4 bacterial species a priori because studies consistently showed that these organisms represent most gram-negative bacteria causing bacteremia across various healthcare settings (*3*,*16*–*22*). If 1 patient had multiple positive blood cultures for the same species during 1 hospital admission, we included only the first isolate. To estimate the population at risk for community-onset bacteremia, we identified the number of veterans who had visited VHA clinics and emergency departments inside the catchment areas of acute inpatient care facilities by calendar year (outpatient denominator for community-onset bacteremia). To estimate the population at risk for hospital-onset bacteremia, we calculated patient-days in acute inpatient care units (inpatient denominator for hospital-onset bacteremia).

Data for 130 VHA acute-care hospitals in the 48 contiguous states, the District of Columbia, and Puerto Rico were used in this study (*23*). Using the US Department of Agriculture and Department of Health and Human Services Rural-Urban Commuting Areas System, we found that 107 facilities were located in urban areas, 22 in rural areas, and 1 in a highly rural area (*24*). These VA hospitals also serve as referral centers for >1,400 outreach clinics. Acute inpatient capacities were 10–260 beds. Total acute inpatient capacity was ≈10,000 acute-care beds, including 1,900 authorized intensive care unit beds (*25*). We did not include stays at mental health, rehabilitation, and nursing home care units. All but 3 included hospitals have on-site microbiology laboratories, and they are required to conduct quality control/quality assessment routinely per requirements of VHA-designated accreditation organizations and to use methods and equipment approved by the Food and Drug Administration (FDA; Silver Spring, MD, USA).

The institutional review board at the University of Iowa and the research and development committee of the Iowa City Veterans Affairs Health Care System approved this study. A waiver of informed consent was issued for this retrospective analysis.

Data Source

We obtained data through Veterans Affairs Informatics and Computing Infrastructure, which includes data extracted from the VHA integrated electronic medical record system. Susceptibility results in microbiology reports (susceptible/intermediate/resistant) were recorded in a standardized manner, and isolates were classified as nonsusceptible if they were reported as intermediate resistance or resistant. MICs or sizes of inhibition zones were not typically available.

Definitions

Bacteremia episodes were classified according to Centers for Diseases Control and Prevention (Atlanta, GA, USA) criteria as community-onset and hospital-onset. This classification was based on time of the first positive blood culture as a standard definition (*26*).

An episode of bacteremia was considered to be community-onset when the first positive blood culture was collected between 48 hours before and <48 hours after admission. For episodes of hospital-onset bacteremia, the first positive blood culture was collected ≥48 hours after admission.

We further categorized community-onset bacteremia episodes as community-acquired, community-onset and healthcare-associated, community-onset on the basis of healthcare exposure in the VHA before hospital admission. Episodes were classified as healthcare-associated, community-onset when the patient had been admitted to an acute-care facility ≤90 days before onset of bacteremia; was a resident of a nursing home or rehabilitation facility; was receiving renal replacement therapy; or received wound care or specialized nursing care in an outpatient setting or at home in the 30 days before onset of bacteremia (*19*). If the patient with community-onset bacteremia did not meet these criteria, the episode was classified as community-acquired, community-onset. Information for healthcare exposure outside the VHA before admission was available only if the VHA was the payer of care.

We categorized antimicrobial drugs listed on susceptibility reports into antimicrobial classes by using interim standard definitions for acquired resistance produced by an international panel of experts from the Centers for Disease Control and Prevention and the European Centre for Disease Prevention and Control (Solna, Sweden) (*27*). We provide included antimicrobial agents and antimicrobial classes for each organism (online Technical Appendix Tables 1–3, https://wwwnc.cdc.gov/EID/article/23/11/16-1214-Techapp1.pdf). Isolates were considered nonsusceptible if they were not susceptible to ≥1 agent in an antimicrobial class (*27*).

Measurements

We focused primarily on annual proportions and trends of antimicrobial nonsusceptibility rates for the 4 most clinically relevant antimicrobial classes (carbapenems, extended-spectrum cephalosporins, aminoglycosides, and fluoroquinolones) used in gram-negative bacteremia

management. We also evaluated incidence rates per 10,000 outpatients for community-onset bacteremia and incidence rates per 10,000 patient-days and per 1,000 admissions for hospital-onset bacteremia for each organism. To enable comparisons with previously reported studies, we calculated age-standardized incidence rates by using a direct method, used US population data for 2000 as a standard population for community-onset bacteremia (*28*), and reported incidence rates with 2 denominators for hospital-onset bacteremia.

Statistical Analysis

We summarized characteristics of the study population and incidence rates for all 3 categories by using descriptive statistics. We calculated 95% CIs by using the Clopper-Pearson exact method (*29*) for crude incidence rates. Proportions among acquisition categories were compared by using Fisher exact tests. To assess annual trends in proportions of nonsusceptible isolates, we used the Cochrane-Armitage χ^2 test for trend. All p values were 2-sided, and values <0.05 were considered statistically significant. All statistical analyses were performed by using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA).

Results

Study Population Demographics and Overall Incidence Rates

During 2003–2013, the VHA provided 53,576,096 person-years of outpatient care and 30,015,733 patient-days of acute inpatient care. We obtained demographic characteristics of the study population (Table 1). A total of 47,746 episodes of gram-negative bacteremia occurred during the study. Most (96.4%) patients were male, and community-acquired, community-onset cases accounted for 42.3% of cases of gram-negative bacteremia, followed by healthcare-associated, community-onset (29.2%) and hospital-onset (28.5%) (Table 2; online Technical Appendix Figure 1).

Overall incidence rates from the entire study were 3.77 episodes/10,000 person-years for community-acquired, community-onset gram-negative bacteremia, 2.60 episodes/10,000 person-years for healthcare-associated, community-onset gram-negative bacteremia, and 4.53 episodes/10,000 patient-days (2.40 episodes/1,000 admissions) for hospital-onset gram-negative bacteremia. Age-standardized incidence rates were 1.92 episodes/10,000 person-years for community-acquired, community-onset gram-negative bacteremia and 1.40 episodes/10,000 person-years for healthcare-associated, community-onset gram-negative bacteremia (online Technical Appendix Figures 2–4).

Trends for Antimicrobial Drug Susceptibilities

Susceptibility data were available for >96% of all organism–antimicrobial drug combinations. The only exception was carbapenem susceptibility of *Acinetobacter* spp., which was available for 90% of isolates tested.

E. coli

Carbapenem nonsusceptibility for *E. coli* was infrequent (0.3%) (Figure 1). However, nonsusceptibility to carbapenems increased for community-acquired, community-onset isolates (0 in 2003–2007 and 0.2% in 2008–2013, p<0.01 by test for trend). Nonsusceptibility for healthcare-associated, community-onset (p = 0.92 by test for trend) and hospital-onset (p = 0.82 by test for trend) isolates did not change.

Overall, 6.3% of *E. coli* isolates were nonsusceptible to extended-spectrum cephalosporins; an increasing proportion were nonsusceptible (4.1% in 2003–2007 and 7.9% in 2008–2013, p<0.01 by test for trend). Extended-spectrum cephalosporin-nonsusceptible isolates were more frequent for patients with previous or recent healthcare exposure (community-acquired, community-onset 3.9%; healthcare-associated, community-onset 9.0%, and hospital-onset 9.8%, p<0.01). The proportion of isolates that were nonsusceptible to extended-spectrum cephalosporin increased for all categories (community-acquired, community-onset; healthcare-associated, community-onset; hospital-onset, p<0.01 by test for trend).

The overall proportion of isolates that were nonsusceptible to aminoglycosides was 11.9%. Healthcare exposure was associated with higher rates of nonsusceptibility (community-acquired, community-onset 9.0%; healthcare-associated, community-onset 15.6%; hospital-onset 16.0%, p<0.01). We observed increasing nonsusceptibility rates during the study (10.5% in 2003–2007 and 12.9% in 2008–2013, p<0.01 by test for trend).

Incidence rates for fluoroquinolone-nonsusceptible isolates rapidly increased during the first half of the study

Table 1. Characteristics of acute-care patients, by year, Veterans Health Administration System, United States, 2003–2013

Characteristic	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
Outpatient care											
No. outpatients	4,357	4,532	4,623	4,705	4,719	4,784	4,945	5,083	5,193	5,281	5,355
Mean age, y, on Jan 1	61.5	61.7	61.7	61.8	61.6	61.4	61.1	61.0	60.9	60.7	60.6
Inpatient care											
No. hospital admissions	470	481	489	492	498	516	536	549	552	552	545
Average length of stay, d	6.0	5.8	5.6	5.5	5.4	5.3	5.1	5.0	5.0	4.8	4.8
No. patient-days	2,805	2,783	2,749	2,718	2,701	2,745	2,747	2,748	2,735	2,675	2,611

Table 2. Characteristics of patients with bacteremia caused by gram-negative bacteria, Veterans Health Administration System, United States, 2003–2013*

Characteristic	Total	*Escherichia coli*	*Klebsiella* spp.	*Pseudomonas aeruginosa*	*Acinetobacter* spp.
No.	47,746	24,557	14,270	6,929	1,990
Age, y, mean ± SD	69.1 ± 2.2	69.8 ± 12.3	68.5 ± 12.1	68.5 ± 11.9	65.7 ± 12.6
Sex, %					
M	96.40	95.30	97.30	97.90	97.30
F	3.60	4.70	2.70	2.10	2.70
Infection					
CA-CO	20,210 (42.3)	13,515 (55.0)	4,802 (33.7)	1,454 (21.0)	439 (22.1)
HCA-CO	13,932 (29.2)	6,857 (27.9)	4,242 (29.7)	2,262 (32.7)	571 (28.7)
HO	13,604 (28.5)	4,185 (17.0)	5,226 (36.6)	3,213 (46.4)	980 (49.3)
HO (ICU onset)	3,819 (8.0)	838 (3.4)	1,389 (9.7)	1,174 (16.9)	418 (21.0)

*Values are no. (%) unless otherwise indicated. CA-CO, community-acquired, community-onset; HCA-CO, healthcare-associated, community-onset; HO, hospital-onset; ICU, intensive care unit.

and then remained stable (23.7% in 2003–2007 and 32.4% in 2008–2013, p<0.01). Fluoroquinolone-nonsusceptible isolates were more frequent in patients with healthcare exposure (community-acquired, community-onset 21.6%; healthcare-associated, community-onset 37.6%; hospital-onset 37.9%, p<0.01), and nonsusceptibility rates increased in groups with these exposures (community-acquired, community-onset; healthcare-associated, community-onset; and hospital-onset, p<0.01 by test for trend).

Klebsiella spp.

Carbapenem nonsusceptibility was reported for 3.0% of *Klebsiella* spp. isolates (Figure 2). We observed a trend toward increasing nonsusceptibility rates (2.1% in 2003–2007 and 3.7% in 2008–2013, p<0.01 by test for trend). Healthcare exposure was associated with higher rates of carbapenem nonsusceptibility (community-acquired, community-onset 0.9%; healthcare-associated, community-onset 2.2%; hospital-onset 5.6%, p<0.01).

Overall, 11.8% of isolates had nonsusceptible results to extended-spectrum cephalosporins and a relatively stable proportion of nonsusceptibility rates (12.6% in 2003–2007 and 11.2% in 2008–2013, p = 0.07 by test for trend). However, nonsusceptibility rates increased for community-acquired, community-onset isolates (p = 0.04 by test for trend) but not for healthcare-associated, community-onset (p = 0.17) and hospital-onset isolates (p = 0.21). Proportions that were nonsusceptible to extended-spectrum cephalosporins were more frequent for healthcare-associated, community-onset (10.4%) and hospital-onset (20.2%) isolates than for community-acquired, community-onset isolates (3.9%, p<0.01).

Overall, 9.8% of isolates were nonsusceptible to aminoglycosides, and a higher proportion were nonsusceptible for patients with healthcare exposure (community-acquired, community-onset 3.6%; healthcare-associated, community-onset 8.5%; hospital-onset 16.7%, p<0.01). Overall, nonsusceptibility to aminoglycosides decreased (10.9% in 2003–2007 and 9.0% in 2008–2013, p<0.01 by test for trend). However, nonsusceptibility increased for community-acquired, community-onset isolates (2.9% in 2003–2007 and 4.1% in 2008–2013, p = 0.01 by test for trend) and decreased for hospital-onset isolates (18.6% in 2003–2007 and 14.8% in 2008–2013, p<0.01 by test for trend).

Fluoroquinolone nonsusceptibility for all isolates was 12.6%, and higher proportions were observed for patients with healthcare exposure (community-acquired, community-onset 5.3%; healthcare-associated, community-onset 11.6%; hospital-onset 20.1%, p<0.01). Among all isolates, the nonsusceptible proportion was stable during the study (p = 0.12 by test for trend). However, there were increasing nonsusceptibility rates for community-acquired, community-onset isolates (4.7% in 2003–2007 and 5.6% in 2008–2013, p = 0.04 by test for trend), and there were no changes observed for other healthcare-associated, community-onset (p = 0.41) and hospital-onset (p = 0.28) isolates.

P. aeruginosa

Antipseudomonal carbapenem nonsusceptibility was reported for 15.8% of all isolates (Figure 3). The rates for antipseudomonal carbapenem-nonsusceptible isolates were stable during the study (p = 0.66 by test for trend). For community-acquired, community-onset isolates, the proportion of nonsusceptible isolates increased (4.8% in 2003–2007 and 7.9% in 2008–2013, p = 0.03 by test for trend), but there was no significant trend for other categories (healthcare-associated, community-onset; p = 0.45 and hospital-onset; p = 0.40). Healthcare exposure was associated with higher rates of nonsusceptibility to antipseudomonal carbapenems (community-acquired, community-onset 6.6%; healthcare-associated, community-onset 10.0%; and hospital-onset 24.1%, p<0.01).

Healthcare exposure also increased nonsusceptibility to antipseudomonal extended-spectrum cephalosporins (community-acquired, community-onset 9.1%; healthcare-associated, community-onset 15.5%; hospital-onset 33.2%, p<0.01). Although 22.3% of all isolates were nonsusceptible, the proportion that were nonsusceptible decreased (24.8% in 2003–2007 and 20.0% in 2008–2013, p<0.01

by test for trend). Decreases were limited to healthcare-exposed patients (healthcare-associated, community-onset; p = 0.01 and hospital-onset; p = 0.04, but not community-acquired, community-onset; p = 0.16).

The overall rate for aminoglycoside nonsusceptible isolates was 20.6%, and nonsusceptible bacteria were more frequently isolated from patients with healthcare exposure (community-acquired, community-onset 10.7%; healthcare-associated, community-onset 16.4%; hospital-onset 28.0%, p<0.01). The overall trend was toward lower rates of nonsusceptible isolates (24.1% in 2003–2007 and 17.3% in 2008–2013, p<0.01 by test for trend). However, this trend of decreasing aminoglycoside nonsusceptibility rates was significant only for patients with healthcare exposure (community-acquired, community-onset, p = 0.14; healthcare-associated, community-onset, p<0.01; and hospital-onset, p<0.01).

The overall rate for antipseudomonal fluoroquinolone-nonsusceptible isolates was 29.6%; these isolates were obtained more frequently from patients who had healthcare-associated, community-onset (24.6%) and hospital-onset episodes (39.4%) than from patients who had community-acquired, community-onset episodes (15.4%, p<0.01). The proportion that was nonsusceptible decreased during the study (32.2% in 2003–2007 and 27.2% in 2008–2013, p<0.01 by test for trend), but this trend was significant only for patients with healthcare exposure (community-associated, p = 0.29; healthcare-associated, community-onset, p<0.01; and hospital-onset, p = 0.02).

Acinetobacter spp.

Antipseudomonal carbapenem nonsusceptibility results were reported for 9.8% of the isolates (Figure 4). The proportion of nonsusceptible isolates was higher for isolates from patients with healthcare exposure (community-acquired, community-onset 4.1%; healthcare-associated, community-onset 9.4%; and hospital-onset 32.6%, p<0.01). The nonsusceptible proportion for hospital-onset isolates increased significantly from 15.0% in 2003 to 56.6% in 2009, but decreased to 29.7% in 2013 (trend in 2003–2009, p<0.01; trend in 2010–2013, p<0.01). The nonsusceptibility proportion for community-acquired, community-onset isolates showed a significant trend (p =

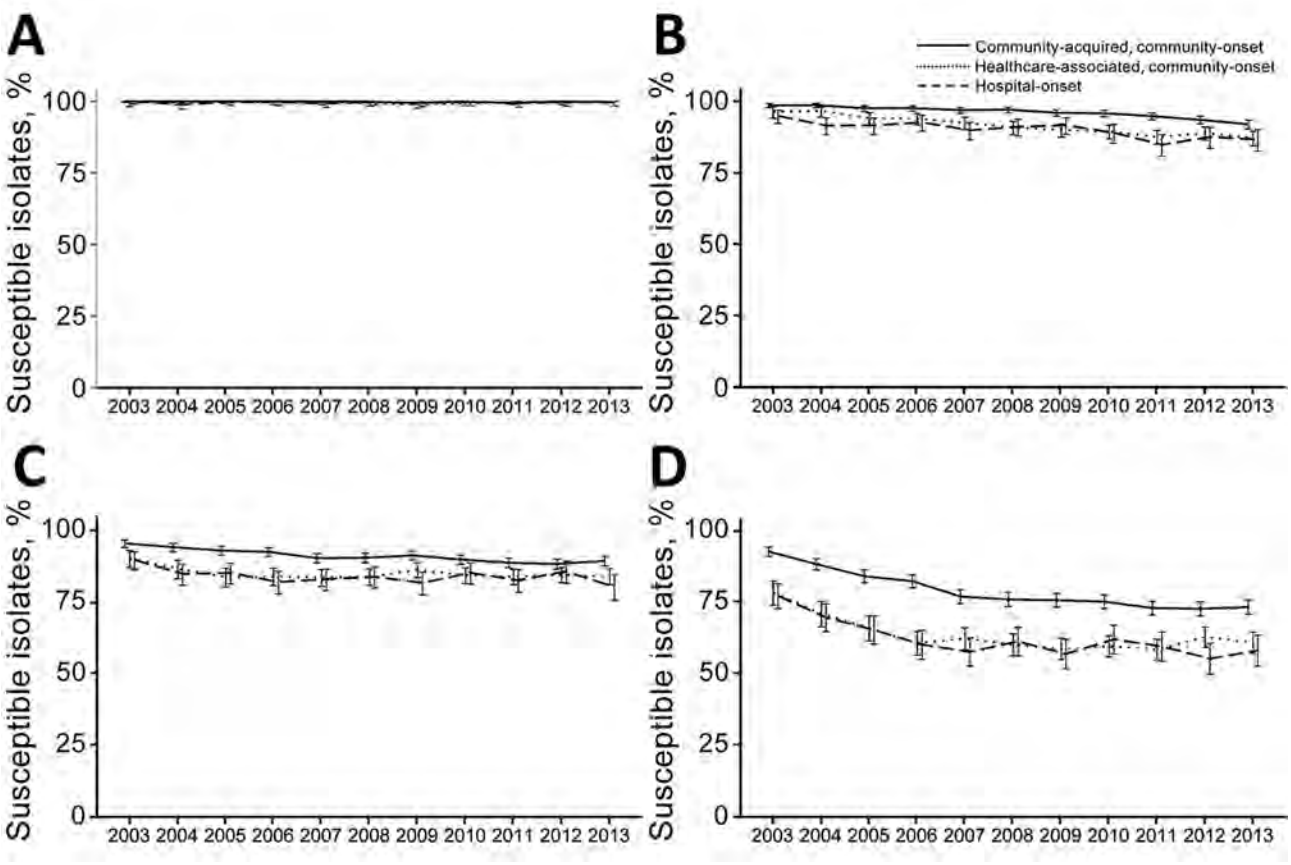

Figure 1. Trends of selected antimicrobial susceptibilities for *Escherichia coli* isolates from patients with bacteremia, Veterans Health Administration System, United States, 2003–2013. A) Carbapenems, B) Extended-spectrum cephalosporins, C) Aminoglycosides, D) Fluoroquinolones. Error bars indicate 95% CIs.

0.41), but this trend increased for healthcare-associated, community-onset isolates (5.4% in 2003–2007 and 12.1% in 2008–2013, p = 0.04).

Overall, 48.1% of isolates were nonsusceptible to extended-spectrum cephalosporins, and this proportion decreased during the study (54.3% in 2003–2007 and 42.5% in 2008–2013, p<0.01 by test for trend). This decrease was caused primarily by significant decreases in nonsusceptibility for hospital-onset isolates since 2009 (community-acquired, community-onset, p = 0.28; healthcare-associated, community-onset, p = 0.23; and hospital-onset, p<0.01). Extended-spectrum cephalosporin-nonsusceptible isolates were more frequent for patients with healthcare exposure (community-acquired, community-onset 19.6%; healthcare-associated, community-onset 30.1%; hospital-onset 70.4%, p<0.01).

Although the overall proportion of aminoglycoside-nonsusceptible isolates was 37.4%, the proportion considered nonsusceptible was higher as healthcare exposure increased (community-acquired, community-onset 9.6%; healthcare-associated, community-onset 19.9%; and hospital-onset 60.0%, p<0.01) The overall trend was toward decreasing rates of nonsusceptibility (45.7% in 2003–2007 and 29.9% in 2008–2013, p<0.01 by test for trend). The decrease was significant only for patients with healthcare exposure (community-acquired, community-onset, p = 0.08; healthcare-associated, community-onset, p = 0.04; and hospital-onset, p<0.01).

The overall proportion of isolates that were nonsusceptible to fluoroquinolones was 47.2%. Patients with healthcare exposure were more likely to be infected with nonsusceptible strains (community-acquired, community-onset 14.2%; healthcare-associated, community-onset 19.6%; hospital-onset 71.9%, p<0.01) There were significant decreases in nonsusceptibility to fluoroquinolones (54.3% in 2003–2007 and 41.0% in 2008–2013, p<0.01 by test for trend), which was observed for hospital-onset isolates only (community-acquired, community-onset, p = 0.07; healthcare-associated, community-onset, p = 0.73; hospital-onset, p< 0.01).

Discussion

We analyzed 47,746 cases of gram-negative bacteremia in the VHA system over an 11-year period and report 3 major findings. First, for *E. coli*, rates of nonsusceptibility to aminoglycosides, fluoroquinolones, and extended-spectrum cephalosporins increased across all care settings. Second, for *Klebsiella* spp., rates of carbapenem nonsusceptibility

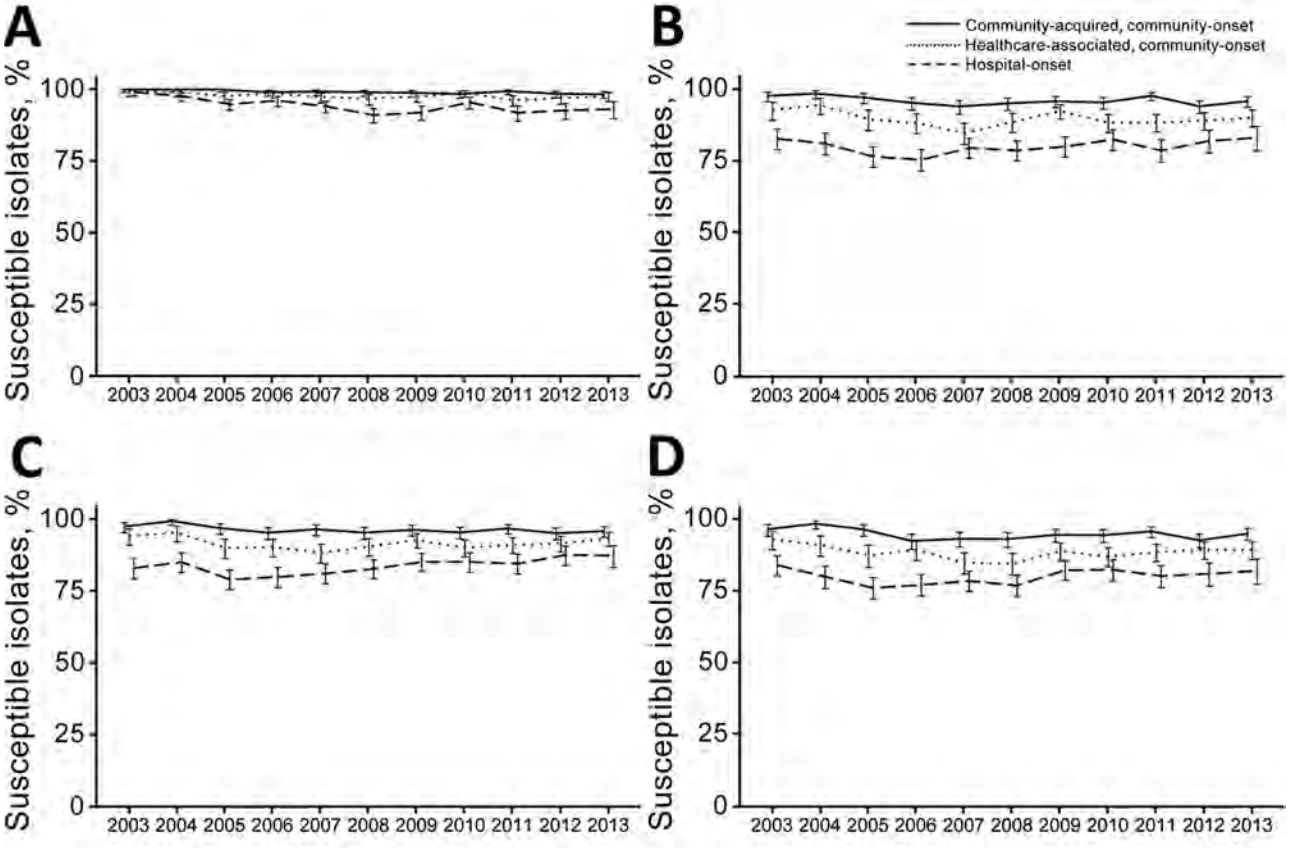

Figure 2. Trends of selected antimicrobial susceptibilities for *Klebsiella* spp. isolates from patients with bacteremia, Veterans Health Administration System, United States, 2003–2013. A) Carbapenems, B) Extended-spectrum cephalosporins, C) Aminoglycosides, D) Fluoroquinolones. Error bars indicate 95% CIs.

increased across all care settings, and nonsusceptibility to other antimicrobial classes increased for community-onset isolates and was stable or decreased for isolates from patients with healthcare exposure. Third, for *P. aeruginosa* and *Acinetobacter* spp., rates of nonsusceptibility to aminoglycosides, fluoroquinolones, and extended-spectrum cephalosporins mostly decreased, but only for isolates from patients with healthcare exposure.

Overall incidence rates for gram-negative bacteremia were comparable with those from previous population-based studies from the United States and Europe (*3*,*13*,*20*–*22*,*30*). We recently reported major changes in hospital-onset bacteremia, which was possibly caused by expansion of horizontal infection control programs in the VHA system during this time (*15*). An additional finding in our more recent study was the decreased proportion of nonsusceptible bloodstream isolates across nearly all antimicrobial classes for *Klebsiella* spp., *P. aeruginosa*, and *Acinetobacter* spp. in patients with healthcare exposure. However, trends for community-onset bloodstream isolates were stable or showed increased nonsusceptibility.

Our findings suggest that efforts to improve infection control practices within the VHA might have had effects on antimicrobial susceptibilities for isolates from patients with healthcare exposure, although the extent of these effects needs to be determined. With increased nonsusceptibility rates among community-acquired, community-onset isolates, we generally expect upward trends of nonsusceptibility rates for healthcare-associated and hospital-onset isolates caused by increased colonization pressure (*31*,*32*). However, our results showed improvements in nonsusceptibility rates for isolates of *Klebsiella* spp., *P. aeruginosa*, and *Acinetobacter* spp. in hospital-onset and healthcare-associated, community-onset infections, which might suggest successful interruption of transmission in inpatient settings. Infection control programs within the VHA have been expanded over the past 10 years, especially since introduction of the Methicillin-Resistant *Staphylococcus aureus* Prevention Initiative in 2007 (*33*). Our recent study showed a substantial decrease in nosocomial, gram-negative bacteremia after implementation of this initiative, which suggests a collateral benefit beyond the originally intended scope of this initiative (*15*). In addition, the VHA released a mandatory policy for hand hygiene practices in 2011 (*34*).

Antimicrobial stewardship efforts within the VHA, which have been evolving over the past 10 years, might

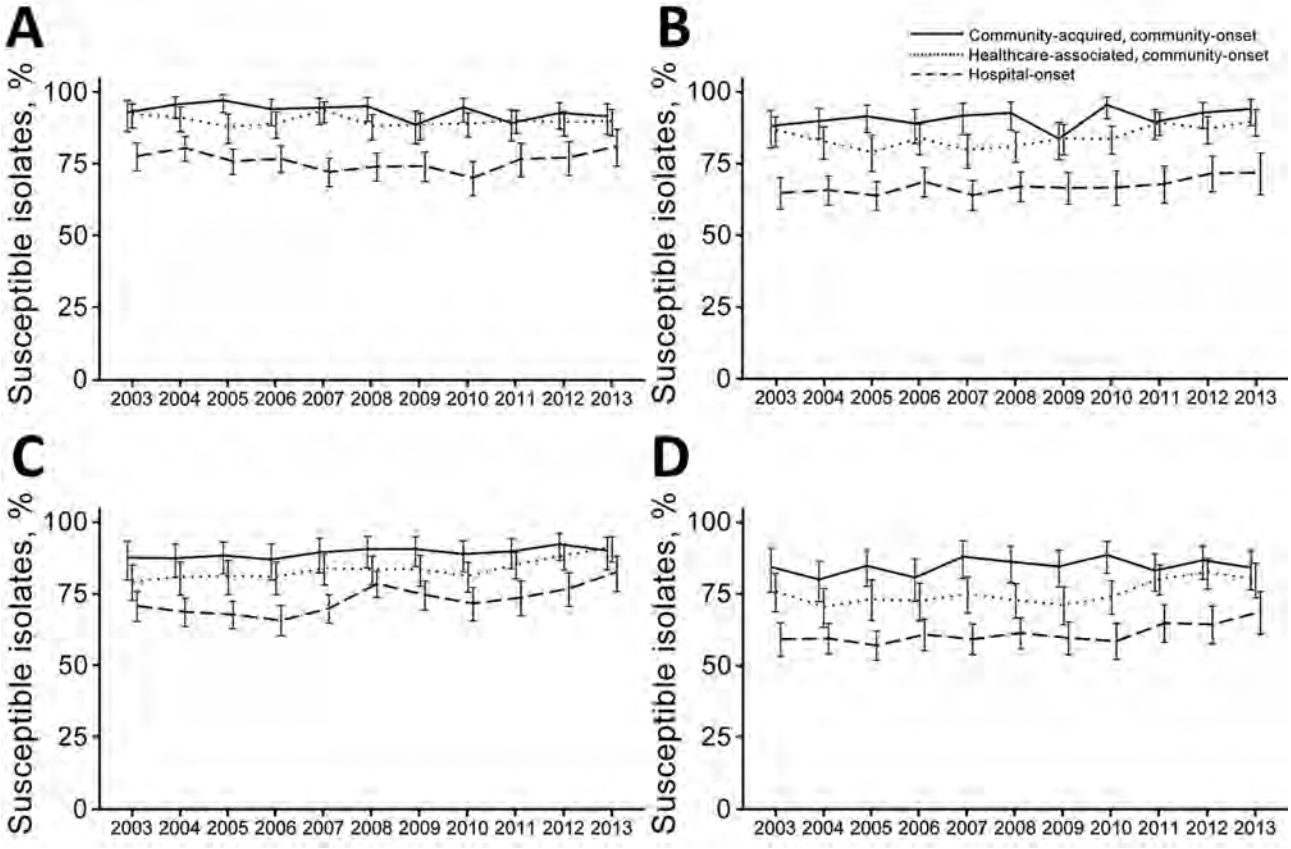

Figure 3. Trends of selected antimicrobial susceptibilities for *Pseudomonas aeruginosa* isolates from patients with bacteremia, Veterans Health Administration System, United States, 2003–2013. A) Antipseudomonal carbapenems, B) Antipseudomonal cephalosporins, C) Aminoglycosides, D) Antipseudomonal fluoroquinolones. Error bars indicate 95% CIs.

have contributed to increased antimicrobial susceptibilities. The National VA Antimicrobial Stewardship Task Force, established in 2011, has been leading systemwide educational efforts, and a mandate for inpatient stewardship was enacted in 2014. However, even before these broad interventions, facilities were actively performing stewardship. According to a survey of VHA facilities conducted by the VA Healthcare Analysis and Information Group, 86 (66.2%) of 130 VHA facilities had either a formal or informal antimicrobial stewardship policy in place in 2012 (*35*). At the time of the survey, 14 (16.3%) of these facilities had a stewardship policy in place for 2 years, 7 (8.1%) for 3 years, and 33 (38.4%) for ≥4 years.

We speculate that increased rates of antimicrobial susceptibility were not observed for *E. coli* healthcare-associated, community-onset and hospital-onset isolates because patients were probably colonized with *E. coli* before admission and these commensal strains might have developed or acquired resistance in the community setting. Antimicrobial drug resistance can develop in *E. coli* by its exposure to retail meat (*36*) and antimicrobial drug exposure outside VHA hospitals. Antimicrobial stewardship efforts within the VHA, like most healthcare systems, have typically not focused on outpatients. In addition, many non-VHA hospitals, which might have cared for patients in this cohort but were not captured by our analysis, did not have stewardship programs during this study (*37*,*38*).

A notable exception to these trends is increased rates of carbapenem nonsusceptibility for *Klebsiella* spp. in community and hospital settings. The increase in carbapenem-resistant *Klebsiella* spp. was also reported outside the VHA (*39*–*41*). This observation deserves attention because it might indicate a lapse of infection control efforts at the VHA. The VHA implemented internal guidelines for carbapenem-resistant *Enterobacteriaceae* to address this issue in 2015, and additional research is needed to determine whether this intervention has had any positive effect on this major threat (*42*).

The strength of our study was inclusion of all bacteremia episodes from the entire VHA system, which enabled us to perform population-based analysis, including diverse care settings, for wide geographic regions. Our study demonstrated the advantage of an integrated health informatics infrastructure with a clinical data warehouse within the VHA system. To accurately monitor the burden and trends

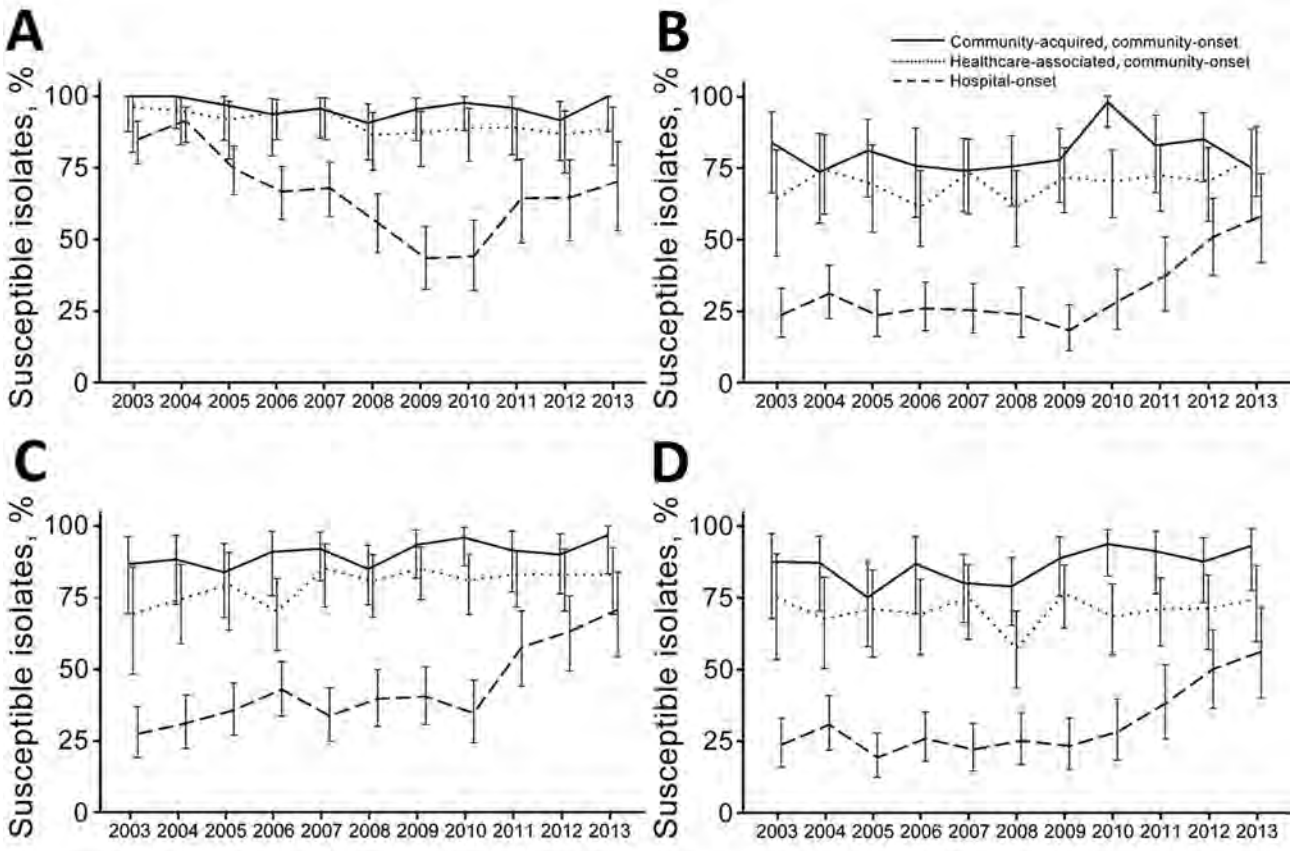

Figure 4. Trends of selected antimicrobial susceptibilities for *Acinetobacter* spp. isolates from patients with bacteremia, Veterans Health Administration System, United States, 2003–2013. A) Antipseudomonal carbapenems, B) Extended-spectrum cephalosporins, C) Aminoglycosides, D) Antipseudomonal fluoroquinolones. Error bars indicate 95% CIs.

of antimicrobial resistance, more investments in data integration informatics infrastructure need to be made.

This study had several limitations. First, most patients seen within the VHA system were male. Therefore, results might not be generalizable to the rest of the population. Second, because information was collected through patient medical records, detailed microbiological information, such as break points of antimicrobial susceptibility testing, molecular typing, or mechanisms of resistance, was often not available. Third, the Clinical and Laboratory Standards Institute (CLSI) revised its break point recommendations for extended-spectrum cephalosporins and carbapenems in 2010 (*43*), and antimicrobial susceptibility trends in the later part of the study might have been affected by this change. However, the FDA did not revise its recommendations to match those of the CLSI until 2013 (*44*). Because most microbiology laboratories in the VHA system use commercial systems that are regulated by the FDA for susceptibility testing, it is less likely that the change of CLSI break points substantially affected our observations. Fourth, this study focused on nationwide overall trends, and results might reflect local trends in each specific region. Previous studies showed geographic variabilities in incidence rates and antimicrobial susceptibilities for gram-negative bacteria (*45*–*47*). We also recently reported regional variations in fluoroquinolone nonsusceptibilities for *E. coli* isolates and plan to analyze geographic variabilities for other organisms and antimicrobial agents in future studies (*48*). Fifth, resistance can develop in gram-negative bacteria during patient therapy, and assessing susceptibilities for the primary isolate from each episode might underestimate the prevalence of nonsusceptibilities. Because there were diversities in reporting practices of susceptibilities for subsequent isolates across the system, it was not feasible to collect information for those isolates in a standardized manner.

In conclusion, we observed an increase in antimicrobial drug nonsusceptibility for *E. coli* in all healthcare exposure categories and stable or decreased rates of nonsusceptibility for *Klebsiella* spp., *P. aeruginosa*, and *Acinetobacter* spp. in patients with healthcare exposure. The extent to which these findings might reflect VHA infection control and antimicrobial stewardship activities remains to be determined. Despite encouraging decreases in nonsusceptibility for pathogenic gram-negative bacteria in acute-care hospital settings, increased attention to infection prevention and antimicrobial stewardship is needed for outpatient and nonacute inpatient settings.

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Mycoplasma genitalium Infection in Adults Reporting Sexual Contact with Infected Partners, Australia, 2008–2016

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Data on the likelihood of *Mycoplasma genitalium* infection in sexual contacts, particularly for men who have sex with men (MSM), are needed to form an evidence base for guidelines. We conducted a cross-sectional analysis of patients attending a sexual health clinic in Melbourne, Victoria, Australia, during 2008–2016. We calculated the proportion of contacts with *M. genitalium* infection and determined factors associated with infection. Among those patients reporting sexual contact with an *M. genitalium*–infected person, 48.2% of women, 31.0% of heterosexual men, and 41.7% of MSM were infected. Among heterosexual contacts, women were twice as likely to be infected; among MSM, rectal infection was more common than urethral infection; and among persons in heterosexual partnerships, concordance of infection was high. High positivity among female and MSM contacts and high concordance within heterosexual partnerships provide some justification for presumptive treatment; however, clinicians should consider antimicrobial drug resistance and toxicity of quinolones.

Mycoplasma genitalium is an established sexually transmitted pathogen that causes nongonococcal urethritis, and recent evidence indicates that it increases the risk for cervicitis, pelvic inflammatory disease, preterm delivery, and spontaneous abortion (*1*,*2*). The estimated prevalence of *M. genitalium* infection is 1%–3% in men and women, according to community-based studies from the United Kingdom, United States, Australia, and Scandinavia (*3*–*7*). Early diagnosis and effective treatment are therefore important in preventing sequelae and ongoing transmission, particularly the transmission of drug-resistant strains to sex partners.

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Published data are limited regarding the likelihood of transmission of *M. genitalium* and the proportion of persons who are likely to be infected after contact with an infected sex partner. Several small studies, with the number of participants ranging from 8 to 88, have examined the proportion of persons infected when their partner has a confirmed *M. genitalium* infection, with results indicating a range of 20.6%–66.7% (*8*–*14*). However, the CIs are broad, and greater precision would provide a more accurate evidence base for partner-notification guidelines and clinical practice. To our knowledge, no published estimates of the likelihood of *M. genitalium* infection in contacts of infected men who have sex with men (MSM) are available. Studies of *M. genitalium* in MSM attending clinics report rectal infection prevalence of 1%–5% in predominantly asymptomatic men, whereas a recent study of MSM in Australia with proctitis found 8% of HIV-negative MSM and 20% of HIV-positive MSM had rectal *M. genitalium* infection (*15*–*19*).

Treatment guidelines are inconsistent about the need for presumptive treatment of sexual contacts of *M. genitalium*–infected patients; guidelines in the United States and United Kingdom do not recommend presumptive treatment, whereas guidelines in Australia do (*20*–*22*). Potential disadvantages of presumptive treatment include cost, unnecessary use of antimicrobial drugs, and risk for adverse effects, particularly from fluoroquinolones used for macrolide-resistant *M. genitalium*. The potential advantages are that early treatment might prevent reinfection of the index patient or transmission to others and prevent sequelae. The higher the likelihood of infection in a contact of a person with confirmed infection, the stronger the argument for presumptive treatment. Presumptive treatment for chlamydial infection, a sexually transmitted infection with similar characteristics to *M. genitalium* infection, is recommended based on prevalence estimates of 36%–68% among contacts of sex partners with confirmed chlamydial infection (*23*–*26*).

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We performed a retrospective analysis of clinical records of patients attending a large urban sexual health service in Melbourne, Victoria, Australia, who reported sexual contact with a partner with diagnosed *M. genitalium* infection. We aimed to determine the proportion of cases with *M. genitalium* and the factors associated with infection in women, heterosexual men, and MSM.

Methods

We conducted our study at the Melbourne Sexual Health Centre, the largest public STI clinic in Victoria, Australia. Starting August 2008, the clinic began treating sexual contacts of *M. genitalium*–infected patients and recording these cases in the clinic database. We defined a contact as someone who reported anal or vaginal sex with or without condoms with a person reporting a recent diagnosis of *M. genitalium* infection. Persons reporting only oral sex did not meet our definition of a contact. Persons were included at first report of being a contact, and repeat presentations were excluded. MSM were defined as men reporting any sex with men within the preceding 12 months.

We tested contacts by using an in-house real-time PCR assay targeting the 16s rRNA gene (*27*). Men were predominantly tested by using a first-pass urine sample, rarely with a urethral swab, and with an anorectal swab if anal sex was reported. Women were tested using a high vaginal swab or cervical swab, but a first-pass urine sample was used if patients preferred, and an anorectal swab was taken if anal sex was reported. We did not test for pharyngeal *M. genitalium* in men or women because of the absence of published evidence for infection at this anatomic site (*15*,*28*).

We recorded all sexual contacts of the *M. genitalium*–infected patients who attended the clinic during August 2008–July 2016 in the clinic database. We extracted demographic, behavioral, laboratory, and clinical data from the clinic's electronic medical records, including number and sex of sex partners, sexual practices within the preceding 3 months, whether these partners were considered casual or regular partners, and consistency of condom use. Data were routinely obtained by clinicians and computer-assisted self-interview. Signs and symptoms among men reporting sexual contact with an infected person were urethral discharge, irritation, dysuria, rectal pain, and bleeding. Signs and symptoms among women reporting sexual contact with an infected person were abnormal vaginal discharge, dysuria, abnormal bleeding, and lower abdominal pain.

We performed statistical analyses by using Stata version 12 (StataCorp LLP, College Station, TX, USA). We calculated the proportion of contacts infected with *M. genitalium*, including 95% CIs, for 3 groups: women, heterosexual men, and MSM. We examined factors associated with infection for 2 groups: 1) heterosexual men and women, and 2) MSM. We conducted univariate logistic regression for each group by using demographic and behavioral characteristics as independent variables and detection of *M. genitalium* as the dependent variable. We treated age as a binary variable, with a cutoff at 27 years for all groups. We also treated the number of sex partners as a binary variable, with a cutoff at 1 for all groups. We used the χ^2 or Fisher exact test, where appropriate, to assess the statistical significance of these associations. We calculated crude odds ratios (ORs) with 95% CIs, entered variables with p values <0.10 in the univariate analysis in the multivariate analysis by using forward stepwise logistic regression, and calculated adjusted ORs (aORs) with 95% CIs. In multivariate analyses, we omitted the binary variable for number of partners because of collinearity with the variable indicating whether the notifying partner was a regular or casual partner. Because some MSM had urine tests, others had rectal swabs, and some had both, we entered each test, rather than each person, into a multivariate model examining risk factors for infection in MSM by using robust SEs to account for clustering around persons.

In a subset of contacts, we were able to identify the referring partner in the clinic's electronic medical record system. If this partner's *M. genitalium* infection was diagnosed at the clinic within 40 days of the contact's presentation, we included the contact in a further analysis of sexual partnerships (dyads).

Results

During the study period, a total of 441 presentations to the clinic were made by patients reporting sexual contact with a person with *M. genitalium* infection (Figure). We excluded repeat presentations by the same person (n = 25), those missing laboratory test results (n = 16), those missing >50% of the queried behavioral data (n = 1), and those not meeting our definition of a contact (n = 22). These exclusions left 377 (85.5%) persons (139 women, 126 heterosexual men, and 112 MSM) for analysis.

Baseline Characteristics of Study Population

We summarized baseline characteristics of the study population (Table 1). The median age of 139 female contacts was 26 years (interquartile range [IQR] 22–32 years). A total of 132 (95.0%) women were heterosexual, whereas 7 (5.0%) reported sex with men and women. The median age among 126 heterosexual male contacts of *M. genitalium*–infected patients was 28 years (IQR 24–35 years). The median age among 112 MSM contacts of *M. genitalium*–infected patients was 29 years (IQR 25–36 years). Most contacts in all 3 groups reported that their notifying partner was their regular partner, and most reported <100% condom use during the preceding 3 months.

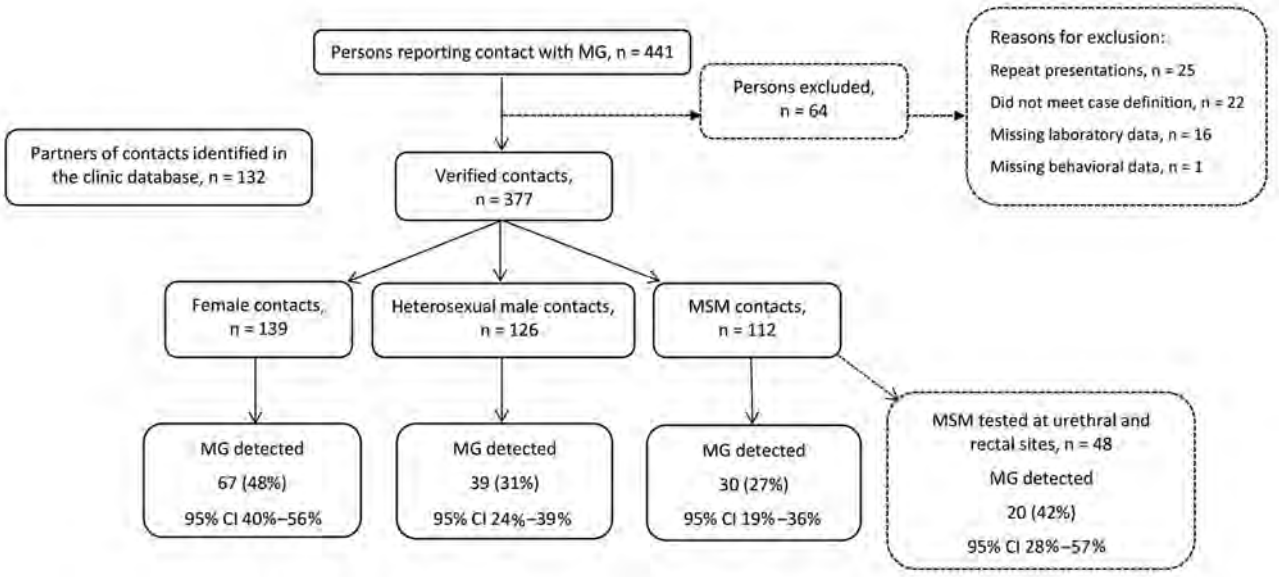

Figure. Flowchart for 441 persons examined at Melbourne Sexual Health Centre who reported sexual contact with a *Mycoplasma genitalium*–infected partner, Melbourne, Victoria, Australia, August 2008–July 2016. Dashed lines indicate persons excluded for analysis or subanalysis. MG, *Mycoplasma genitalium*; MSM, men who have sex with men.

M. genitalium Infection in Sexual Contacts

Heterosexual Women and Men

Because *M. genitalium* positivity did not significantly differ between cervical or high vaginal swabs or first-pass urine samples among women (50.0% vs. 46.1%; p = 0.643), we combined these samples for our analysis. The overall proportion of female contacts in whom *M. genitalium* was detected was 48.2% (95% CI 39.7%–56.8%). The proportion of heterosexual male contacts in whom urethral *M. genitalium* was detected was 31.0% (95% CI 23.0%–39.8%), which was significantly lower than the proportion of female contacts infected (p = 0.004).

MSM

The proportion of MSM contacts in whom *M. genitalium* was detected overall was 26.8% (95% CI 18.9%–36.0%). However, only 48 (42.9%) MSM were tested at both anatomic sites; 48 (42.9%) were tested only at the urethra, and 16 (14.3%) were tested only at the rectum. Of the 48 MSM contacts tested only at the urethra, 3 had *M. genitalium* detected (6.3%, 95% CI 1.3%–17.2%). In contrast, of the 16 MSM contacts tested only at the rectum, 7 had *M. genitalium* detected (43.8%, 95% CI 19.8%–70.1%). Of the 48 MSM contacts tested at both anatomic sites, 20 had *M. genitalium* detected (41.7%, 95% CI 27.6%–56.8%), with most (17/20) of these infections being rectal infections. Overall, 8 of 96 urethral sites tested for *M. genitalium* were positive (8.3%, 95% CI 4.3%–15.6%), compared with 24 of 59 rectal sites (40.7%, 95% CI 29.1%–53.4%).

Factors Associated with Having *M. genitalium* Infection

Heterosexual Female and Male Contacts

We examined potential predictors of *M. genitalium* infection among heterosexual women and men (Table 2). Factors associated with being infected with *M. genitalium* on univariate analysis included female sex (p = 0.004), having a regular partner as the notifying partner (p = 0.013), and having ≥2 sex partners in the preceding 3 months (p = 0.024). Factors that were significantly associated with being infected were included in a multivariate analysis; number of sex partners was not included because it was highly correlated with having a regular partner as the notifying partner, and condom use with the notifying partner was included given the protective effect of condoms against STI acquisition. Heterosexual contacts were more likely to be infected with *M. genitalium* if they were women (aOR 2.18, 95% CI 1.28–3.71) and the notifying partner was a regular sex partner (aOR 2.13, 95% CI 1.09–4.14). Contacts reporting <100% condom use with their notifying partner were 2.72 times more likely to have *M. genitalium* diagnosed, although this difference was not statistically significant (p = 0.066). The presence of any urethral discharge, irritation, or dysuria was associated with detection of *M. genitalium* in heterosexual men (OR 3.26, 95% CI 1.24–8.58). Symptoms were not associated with detection in women or in the combined (male and female) heterosexual model (Table 2).

MSM Contacts

Because most MSM were tested only at the urethra or the rectum, we based our analysis on the anatomic sites tested

Table 1. Baseline characteristics of 377 persons seen at Melbourne Sexual Health Centre who reported sexual contact with an *Mycoplasma genitalium*–infected partner, Melbourne, Victoria, Australia, August 2008–July 2016*

Characteristic	Women, n = 139	Heterosexual men, n = 126	MSM, n = 112
Age, y, median (IQR)	26 (22–32)	28 (24–35)	29 (25–36)
No. sex partners in preceding 3 mo†			
1	74 (53.6)	52 (41.9)	37 (35.6)
≥2	64 (46.4)	72 (58.1)	67 (64.4)
Condom use with all sex partners in preceding 3 mo			
100%	11 (8.0)	6 (4.9)	15 (14.4)
<100%	126 (92.0)	117 (95.1)	89 (85.6)
Nature of relationship with the notifying partner			
Casual	28 (20.9)	36 (29.0)	38 (36.2)
Regular	106 (79.1)	88 (71.0)	67 (63.8)
Condom use with the notifying partner in preceding 3 mo			
100%	15 (11.6)	6 (4.9)	15 (14.9)
<100%	114 (88.4)	117 (95.1)	86 (85.1)

*Values are no. (%) unless otherwise specified. IQR, interquartile range; MSM, men who have sex with men.
†Number of sex partners does not include female sex partners for female contacts or MSM contacts.

for *M. genitalium* rather than persons. By including each urethral (n = 96) and rectal (n = 59) test as individual observations within the dataset, we observed that 112 MSM contacts had 155 separate tests for *M. genitalium*. Factors that were significantly associated with infection on univariate analysis (or that were of borderline significance) included reporting having 1 sex partner in the preceding 3 months (p = 0.071), reporting <100% condom use with the notifying partner in the preceding 3 months (p = 0.061), and being tested at the rectal site (p<0.001) (Table 3). Including these 3 factors in a multivariate analysis, MSM contacts had an 8-fold increase in probability of *M. genitalium* infection if they were tested at the rectum instead of the urethra (aOR 8.39, 95% CI 3.14–22.42). In separate univariate analyses, restricted to persons tested at the relevant site, symptoms were not associated with detection of *M. genitalium* (Table 3).

M. genitalium Infection in Sexual Partnerships (Dyads)

Of 377 contacts, 132 (35%) reported having been notified by a partner who could be identified in the clinic's electronic medical record system. A total of 120 (91%) partnerships fulfilled the inclusion criteria for further analysis. In 86 heterosexual dyads, the median time between the contact and their partner being tested for *M. genitalium* was 8 days (IQR 6–16 days); in 34 MSM dyads, it was 7 days (IQR 4–11 days). Forty of 86 heterosexual dyads were concordant for *M. genitalium* infection (46.5%, 95% CI 36.4%–57.0%). Nine of 34 MSM dyads were concordant for infection (26.5%, 95% CI 14.6%–43.1%); however, few MSM dyads were tested for *M. genitalium* at both urethral and rectal sites. Of 34 MSM notifying partners that were identified, 29 (85.3%) had a history of urethral *M. genitalium* infection and 5 (14.7%) had a history of rectal *M. genitalium* infection.

Discussion

In this study, a high proportion of persons reporting contact with an *M. genitalium*–infected partner were infected, including 48% of women, 31% of heterosexual men, and 42% of MSM tested at both the rectum and urethra. The sample size for this study exceeds the combined total of sample sizes in previously published studies, adding precision to estimates of the probability of infection and transmissibility of *M. genitalium* between sex partners (*8*–*14*). These findings will inform guidelines for the management of sexual contacts of *M. genitalium*–infected patients and provide an evidence base for informed discussion between clinicians and their patients regarding the appropriateness of presumptive treatment for contacts of infected patients or recommending testing and return for treatment.

In this study, among heterosexual contacts, women were twice as likely as men to be infected with *M. genitalium*, after adjusting for condom use and nature of relationship. This finding could be attributable to the female genital tract's greater susceptibility to STIs, with the larger surface area of the cervico-vaginal mucosa compared with the urethral mucosa (*29*), and female sex hormones thought to enhance susceptibility to STIs (*30*). Heterosexual contacts notified by a regular partner were twice as likely to be infected, suggesting that multiple sexual acts or events of exposure might increase risk for acquisition of *M. genitalium.* Less than 100% condom use for penile-vaginal sex with a regular partner appeared to double the risk for *M. genitalium* infection among heterosexual contacts, and although this increased risk was not significant (p = 0.07), it does suggest that condoms provide protection against *M. genitalium* infection, as has been shown for other bacterial STIs. Concordance for *M. genitalium* infection in heterosexual dyads in which both partners were tested at our service was 47%, reflecting the high risk for concurrent infection in heterosexual partnerships. Overall, the prevalence of *M. genitalium* infection in heterosexual men and women was within the range reported for chlamydial infection in published studies (*23*–*26*).

The prevalence of *M. genitalium* that was observed among contacts in this study is substantially higher than the

Table 2. Potential predictors of *Mycoplasma genitalium* infection among heterosexual men and women seen at Melbourne Sexual Health Centre who reported sexual contact with an *M. genitalium*–infected partner, Melbourne, Victoria, Australia, August 2008–July 2016*

Characteristic	No.	Infected, no. (%)	Not infected, no. (%)	Unadjusted OR (95% CI)	p value	aOR† (95% CI)	p value
Total	265						
Age of sex partner, y‡							
≥27	141	53 (37.6)	88 (62.4)	1.0			
<27	124	53 (42.7)	71 (57.3)	0.81 (0.49–1.32)	0.393	–	–
Sex							
M	126	39 (31.0)	87 (69.0)	1.0		1.0	
F	139	67 (48.2)	72 (51.8)	**2.08 (1.25–3.43)**	**0.004**	**2.18 (1.28–3.71)**	**0.004**
No. of sex partners in preceding 3 mo							
1	126	59 (46.8)	67 (53.2)	1.0			
≥2	136	45 (33.1)	91 (66.9)	**0.56 (0.34–0.93)**	**0.024**	–	–
Nature of relationship with the notifying partner							
Casual	64	17 (26.6)	47 (73.4)	1.0		1.0	
Regular	194	86 (44.3)	108 (55.7)	**2.20 (1.18–4.10)**	**0.013**	**2.13 (1.09–4.14)**	**0.026**
Condom use with notifying partner in preceding 3 mo							
100%	21	5 (23.8)	16 (76.2)	1.0		1.0	
<100%	231	97 (42.0)	134 (58.0)	2.32 (0.13–1.27)	0.113	2.72 (0.93–7.91)	0.066
Symptoms in men§							
No	99	25 (25.3)	74 (74.7)	1.0			
Yes	21	11 (52.4)	10 (47.6)	**3.26 (1.24–8.58)**	**0.017**	–	–
Symptoms in women¶							
No	93	45 (48.4)	48 (51.6)	1.0		–	–
Yes	24	14 (58.3)	10 (41.7)	1.49 (0.60–3.70)	0.387		

*Bold text indicates a statistically significant association (p<0.05). Up to 7% of participants have missing data for some variables. aOR, adjusted odds ratio; MSM, men who have sex with men; OR, odds ratio.
†Adjusted for sex, nature of relationship with notifying partner, and reported condom use (100% vs. <100%).
‡The median age across all female, heterosexual male, and MSM contacts was 27 years.
§Discharge, dysuria, or urethral irritation; 6 men with urethral chlamydia excluded. In the combined heterosexual population symptoms were not significantly associated with infection and were not included in the multivariate model.
¶Vaginal discharge, dysuria, abnormal bleeding, or lower abdominal pain; 22 women with candidiasis, bacterial vaginosis, chlamydia, or urinary tract infections excluded.

prevalence reported in comparable study populations in Melbourne. Reported prevalence estimates from these studies were 2.4% (95% CI 1.5%–3.3%) in young women attending clinics, including the site of this study (*4*); 1.3% (95% CI 0.3%–3.7%) in urine samples from asymptomatic heterosexual men (*31*); and (2.1%; 95% CI 1.1%–3.6%) in rectal swabs and urine samples from asymptomatic MSM (*15*), all of which are much lower than the respective prevalence estimates reported in our study of 48.2% (95% CI 39.7%–56.8%), 31.0% (95% CI 23.0%–39.8%), and 41.7% (95% CI 27.6%–56.8%).

MSM contacts had a similar likelihood of being infected with *M. genitalium* as women when they were tested at both the urethra and the rectum. This study highlights the importance of rectal testing for *M. genitalium* in MSM. Urethral positivity was only 8% in MSM, compared with 31% in heterosexual men. However, overall rectal positivity was high at 38%, and when MSM were tested at both urethral and rectal sites, 42% were positive for *M. genitalium*, and most of these had rectal infections. The clinic records do not indicate why some men were not tested at both sites. The higher rate of rectal infection compared with urethral infection is consistent with studies of chlamydial infection among MSM but is also likely to be influenced by the notifying partner's reason for seeking care. When this factor was examined among MSM dyads, 29 of 34 MSM notifying partners sought care for urethral infections, suggesting that urethral infections might be more likely than rectal infections to cause symptoms.

Our study has several limitations. The study is retrospective and relies on self-report of exposure to infection without laboratory confirmation. As such, the data reflect the prevalence of infection only among those persons who seek care reporting exposure to *M. genitalium* rather than among all of those exposed. We have no information on contacts of infected patients who did not attend the clinic, and these persons are likely to be systematically different from those who did seek out testing and treatment. These findings might also not be generalizable to non–STI clinic populations or to other populations with a different background prevalence of *M. genitalium* infection. Although we considered the notifying partner the index patient for analytical purposes, we cannot ascertain the transmission direction between sex partners or whether transmission occurred through a third person. Sexual behavioral data were self-reported and hence subject to recall bias. The most notable limitation was the lack of dual-site testing for MSM contacts, which limited our ability to report precise estimates of infection among MSM and to examine concordance in MSM dyads.

Presumptive treatment of sexual contacts reduces the risk for reinfection and is recommended for STI syndromes

Table 3. Factors associated with detection of *Mycoplasma genitalium* infection among MSM examined at Melbourne Sexual Health Centre who reported sexual contact with an *M. genitalium*–infected partner, Melbourne, Victoria, Australia, August 2008–July 2016*

Characteristic	No.	Infected, no. (%)	Not infected, no. (%)	Unadjusted OR (95% CI)	p value	aOR† (95% CI)	p value
Total tests	155						
Age of sex partner, y‡							
≥27	51	11 (21.6)	40 (78.4)	1.0			
<27	104	21 (20.2)	83 (79.8)	0.92 (0.41–2.06)	0.840	–	–
No. of sex partners in preceding 3 mo							
1	51	15 (29.4)	36 (70.6)	1.0		1.0	
≥2	95	16 (16.8)	79 (83.2)	0.49 (0.22–1.06)	0.071	0.62 (0.25–1.54)	0.303
Nature of relationship with the notifying partner							
Casual	55	11 (20.0)	44 (80.0)	1.0			
Regular	90	21 (23.3)	69 (76.7)	1.22 (0.57–2.62)	0.615	–	–
Condom use with the notifying partner in preceding 3 mo							
100%	20	1 (5.0)	19 (95.0)	1.0		1.0	
<100%	120	31 (25.8)	89 (74.2)	6.62 (0.92–47.85)	0.061	5.41 (0.70–41.82)	0.105
Anatomic site tested							
Urethra	96	8 (8.3)	88 (91.7)	1.0		1.0	
Rectum	59	24 (40.7)	35 (59.3)	**7.54 (3.08–18.45)**	**<0.001**	**8.39 (3.14–22.42)**	**<0.001**
Urethral symptoms§							
No	78	6 (7.7)	72 (92.3)	1.0			
Yes	12	1 (8.3)	11 (92.2)	1.09 (0.12–9.94)	0.938	–	–
Rectal bleeding or pain¶							
No	49	19 (38.8)	30 (61.2)	1.0			
Yes	2	1 (50.0)	1 (50.0)	1.58 (0.09–26.78)	0.752	–	–

*Bold text indicates a statistically significant association (p<0.05). Up to 7% of participants have missing data for some variables. aOR, adjusted odds ratio; MSM, men who have sex with men; OR, odds ratio.
†Adjusted for number of partners in the previous 3 months, reported condom use (100% vs. <100%), and anatomic site tested.
‡The median age across all female, heterosexual male, and MSM contacts was 27 years.
§Discharge, dysuria, or urethral irritation. Analysis restricted to urine samples only after excluding coinfection with *Chlamydia trachomatis* (n = 3).
¶Analysis restricted to rectal swabs only after excluding coinfection with *Chlamydia trachomatis* (n = 2) and *Neisseria gonorrhoeae* (n = 6).

such as nongonococcal urethritis (*20*). In the contacts of *M. genitalium*–infected persons in this study, presumptive treatment would have treated 1 infection for every 2–3 treatments. However, the decision to recommend presumptive treatment must also take into account potential harms and benefits to the contact and their sex partners. Although heterosexual men had a slightly lower prevalence of positivity, presumptive treatment might be more important in reducing the risk for serious sequelae, such as pelvic inflammatory disease, in female partners.

The alternate approach of treating contacts only after confirmation of *M. genitalium* infection represents better stewardship of antimicrobial drugs but relies on access to sensitive testing practices and a high rate of return of patients to be effective. An important consideration before presumptively treating contacts for *M. genitalium* infection is the increasing prevalence of macrolide resistance, which is >40% in Europe, Japan, and the United States and >75% among MSM in Australia (*32*–*35*). Furthermore, macrolide resistance is selected in 12%–18% of seemingly susceptible infections after treatment with 1 g azithromycin and extended azithromycin regimens (*35*). Presumptive use of macrolides for *M. genitalium*–infected contacts might therefore not only be ineffective in those patients with detectable resistance but also contribute to development and spread of resistance, particularly in asymptomatic contacts who believe they have been effectively treated. The only recommended treatments for macrolide-resistant *M. genitalium* are fourth-generation fluoroquinolones, which are expensive and can cause tendinopathy, neuropathy, and adverse central nervous system effects, which are major considerations for determining their use in persons who do not have confirmed infection. Overall, a prudent approach entails managing sexual contacts according to the informed preferences of the person and, if known, the resistance status of the notifying partner. The results of our study provide an evidence base for informed discussions between clinicians and patients at risk for infection and can inform international treatment and partner-notification guidelines.

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Retrospective Observational Study of Atypical Winter Respiratory Illness Season Using Real-Time Syndromic Surveillance, England, 2014–15

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During winter 2014–15, England experienced severe strains on acute health services. We investigated whether syndromic surveillance could contribute to understanding of the unusually high level of healthcare needs. We compared trends for several respiratory syndromic indicators from that winter to historical baselines. Cumulative and mean incidence rates were compared by winter and age group. All-age influenza-like illness was at expected levels; however, severe asthma and pneumonia levels were above those expected. Across several respiratory indicators, cumulative incidence rates during 2014–15 were similar to those of previous years, but higher for older persons; we saw increased rates of acute respiratory disease, including influenza-like illness, severe asthma, and pneumonia, in the 65–74- and ≥75-year age groups. Age group–specific statistical algorithms may provide insights into the burden on health services and improve early warning in future winters.

Winter respiratory pathogens account for a large burden of community respiratory illness each year (*1*–*3*). Increased illness can result in pressures on hospitals, which can lead to local incidents where health services cannot meet patient demand, often leading to the closure of facilities (*4*). The elderly have been particularly implicated in driving hospital pressures due to increased admissions and increased lengths of stay (*5*). Although a wide range of viral and bacterial pathogens circulate each winter in the Northern Hemisphere, influenza viruses and respiratory syncytial virus (RSV) cause the greatest number of illnesses, especially among young children and the elderly. General practitioner (GP) consultations, emergency department (ED) visits, hospital admissions, and deaths all increase during periods of influenza and RSV activity: annual winter pressures on healthcare systems have been attributed in part to these pathogens (*6*–*10*). In the United Kingdom, seasonal influenza activity generally occurs during weeks 40–20 (October–May); the peak of activity is commonly December 25–January 1 or early January (*11*). RSV activity is typically more consistent, peaking during weeks 48–52 of each year, with evidence of a lag between the peaks among young children (week 48) and the elderly (week 52) (*12*,*13*). In the past, sentinel GP consultations for influenza-like illness (ILI) have been used as the standard for monitoring community-based influenza activity (*14*–*17*). These surveillance systems are now commonly interpreted as part of a suite of influenza-related monitoring systems.

Temporal analysis of GP ILI consultations in the United Kingdom has revealed a decreasing trend over the past 30 years, with particularly low to moderate activity recorded since winter 1999–2000 (*14*). There were 2 notable exceptions: the 2009–10 global pandemic, which saw substantial out-of-season summer and autumn activity (*18*); and winter 2010–11, when influenza activity reached levels higher than in any other winter since the millennium winter of 1999–2000 (*12*,*19*). To take such long-term trends into account, the United Kingdom, in common with many other countries in Europe, has implemented a Moving Epidemic Method to calculate the preepidemic threshold each season, with an approach that uses historical data to calculate the threshold (*20*). In more recent seasons, a spectrum of

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influenza surveillance systems, including virologic indicators, is used to determine levels of influenza activity in the community and health effects on the population, such as hospital admissions and excess deaths confirmed to be influenza related.

Public Health England (PHE) coordinates a surveillance program for influenza and other respiratory viruses including epidemiologic, virologic, and syndromic surveillance systems (*21*,*22*). Syndromic surveillance is the near real-time collection, analysis, interpretation, and dissemination of health-related data to enable the early identification of the impact (or absence of impact) of potential human or veterinary public health threats that require effective public health action (*23*). In England, PHE coordinates a national syndromic surveillance service that delivers daily real-time syndromic intelligence from several health data sources that have been described previously (*21*). Anonymized health data, including a range of symptoms and syndromes, are collected daily from several healthcare service providers across England. Counts of symptoms and syndromes are aggregated into several syndromic indicators (e.g., ILI, cough, vomiting, rash).

Moderate levels of influenza activity were seen in the community in the United Kingdom during 2014–15. Influenza A(H3N2) was the predominant virus circulating for most of the season, with influenza B circulating later in the season (*22*). The impact of A(H3N2) was predominantly seen in the elderly, with numerous outbreaks in residential care homes. Levels of all-cause excess deaths were also statistically much higher, at 5.4% excess deaths above the upper threshold (16,415 [95% CI 15,588–17,241] more excess deaths) than the last notable significant A(H3N2) season of 2008–09, which had 3.2% excess deaths above the upper threshold (10,438 [95% CI 9,977–10,964] more excess deaths) (*22*). However, in England there were reports of severe pressures within the National Health Service (NHS), particularly in emergency medicine, with more ED visits, longer waiting times in EDs, and a shortage of acute-care hospital beds (*4*). The aim of this study was to determine, retrospectively, whether syndromic surveillance could contribute intelligence toward identifying the underlying causes of these issues. We used national data collected routinely from a range of healthcare sources.

Methods

Study Period

PHE conducts enhanced surveillance of influenza and other respiratory viruses in the United Kingdom each winter from October (week 40) to May (week 20) (*22*). For this study, we used daily syndromic surveillance data extracted September 29, 2014–May 17, 2015. We extracted data from corresponding periods in 2 previous winters (2012–13, 2013–14) for comparison. To define a more specific period of intense winter activity during 2014–15, we selected a period of 5 weeks encompassing peak activity (*24*), corresponding to week 51 of 2014 through week 3 of 2015 (December 15, 2014–January 18, 2015).

Syndromic Surveillance Data

Syndromic surveillance data used in this study included general practitioner consultations "in hours," or during practitioners' regular office hours (GPIH), and "out of hours" (GPOOH) services; ED visits from a sentinel network that is part of England's Emergency Department Syndromic Surveillance System (EDSSS); and calls to the national NHS 111 telehealth service (*21*,*25*,*26*). We selected respiratory indicators from each data source, including ILI, upper and lower respiratory tract infection, acute respiratory infection, and pneumonia (Table 1). We captured, analyzed, and interpreted data contemporaneously with the study period by epidemiologic, statistical, and risk assessment processes (*27*,*28*).

Retrospective Descriptive Epidemiologic Analysis

We examined daily plots of syndromic surveillance data to establish trends over the winter period. We analyzed data according to the individual data source: incidence rates per 100,000 registered patient population (GPIH) or syndromic counts as a percentage of the total number of counts, where population coverage figures were unavailable (GPOOH, EDSSS, NHS 111). We stratified data by standard age groups (<1, 1–4, 5–14, 15–44, 45–64, 65–74, ≥75 years).

To compare respiratory activity during 2014–15 with previous winter seasons, we calculated baselines for each data source and syndromic indicator on the basis of available historic data for the data source. We also calculated weekly cumulative incidence plots for selected indicators and age groups for week 40 of 2014 through week 20 of 2015 and compared them to those for previous years.

Table 1. Syndromic surveillance data source and respiratory indicators used in study of an atypical winter respiratory illness season, England, 2014–15*

Source	Indicator
GPIH	Upper respiratory tract infection; lower respiratory tract infections; influenza-like illness; "severe asthma"; pneumonia
GPOOH	Acute respiratory infection; influenza-like illness; asthma/wheeze/difficulty breathing
EDSSS	Acute respiratory infection; influenza-like illness; bronchitis; asthma/wheeze/difficulty breathing; pneumonia
NHS 111	Cold/flu; cough; difficulty breathing;

*EDSSS, Emergency Department Syndromic Surveillance System; GP, general practitioner; GPIH, GP in hours; GPOOH, GP out of hours; NHS 111, National Health Service telehealth service.

Statistical Analysis

To establish whether there were any statistically significant differences in respiratory syndromic activity between 2014–15 and other years, we used a nonparametric Mann-Whitney test to compare mean incidence rates between winter 2014–15 and the combined winter periods 2012–13 and 2013–14. We calculated the mean daily incidence of each respiratory indicator by age group for the period weeks 51–3 for each data source: the rate for 2014–15 was compared with that for the same period during the 2 preceding winters. We performed all statistical analyses with Stata version 13.1 (*29*).

Results

Trends in Respiratory Indicators 2014–15

The analysis of daily trends of syndromic indicators by all ages during the 2014–15 season revealed an increase in activity beginning in November 2014, with activity generally peaking between week 52 of 2014 and week 1 of 2015. There were, however, differences in the timing of peak activity: the earliest peak occurred in the GPIH, where the 7-day moving average of upper respiratory tract infection (URTI) consultations peaked on December 22, 2014 (Figure 1, panel B); the latest peak was also in the GPIH, where ILI and pneumonia consultations both peaked on January 5, 2015 (Figure 1, panel A; Figure 2, panel D).

Compared with historical baseline data, the activity of GPIH ILI (for all ages) was below seasonally expected levels (as demonstrated by historical baselines; Figure 1, panel A), whereas GPIH URTI, EDSSS acute respiratory illness, and GPOOH asthma/wheeze/difficulty breathing activity was just over baseline levels (Figure 1, panels B, C, and D). However, the levels of GPIH lower respiratory tract infection (LRTI) and severe asthma and of EDSSS pneumonia were considerably higher than expected during 2014–15 (Figure 2, panels A–C).

For all-age incidence, GPIH and EDSSS pneumonia, GPIH LRTI, EDSSS ILI, and GPIH severe asthma were significantly higher ($p<0.05$) during week 51 of 2014 through week 3 of 2015 than during the same period in the comparator seasons (Table 2; full results in online Technical Appendix Table, https://wwwnc.cdc.gov/EID/article/23/11/16-1632-Techapp1.pdf). Calls to the NHS 111 line for cold/influenza were significantly higher ($p = 0.009$) in 2014–15, but due to the lack of historical data, we compared these data to those from 2013–14 only.

Cumulative Incidence of Respiratory Indicators

GPIH ILI all-age rates (Figure 3, panel A) during winter of 2012–13 and 2014–15 were similar with respect to the cumulative incidence slope, although the total cumulative incidence was greater for 2014–15 (307/100,000 population for 2012–13 and 339/100,000 population for 2014–15). Winter 2013–14 was demonstrably lower both in the slope of incidence and the final total cumulative incidence. GP ILI rates were noticeably higher in incidence for the 65–74- and ≥75-year age groups during 2014–15 (Figure 4, panel A; Figure 5, panel A); the cumulative rates for these age groups during 2014–15 started to diverge from the other winters during week 50 of 2014. Similarly, cumulative rates for GPIH severe asthma were comparable for all ages between 2014–15 and 2012–13 but were considerably higher for the 65–74- and ≥75-year age groups during 2014–15 (Figure 3, panel B; Figure 4, panel B; Figure 5, panel B); these rates were also higher for the 15–44- and 45–64-year age groups (data not shown).

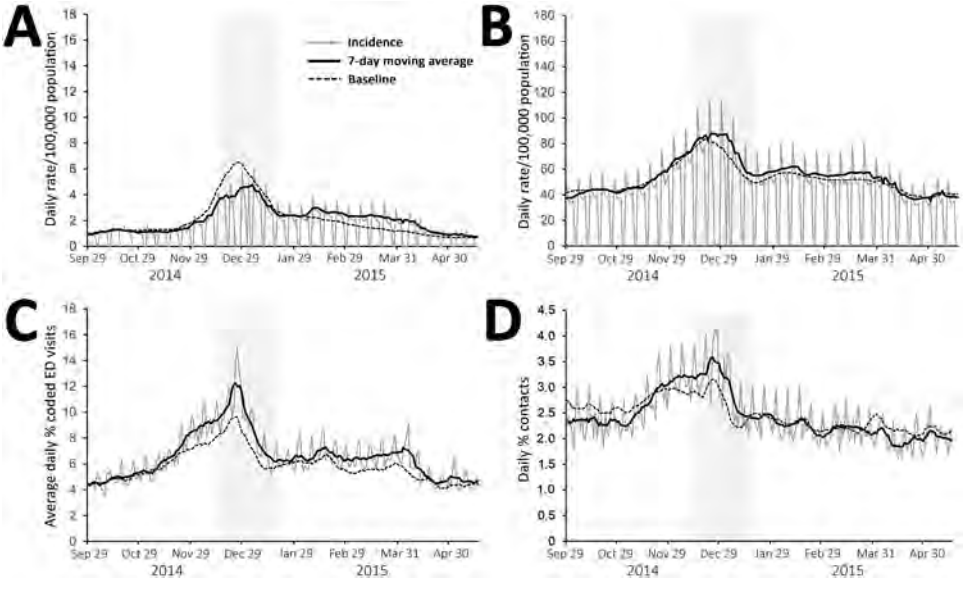

Figure 1. Daily incidence of acute respiratory indicators over winter 2014–15, England. A) General practitioner in hours (GPIH) influenza-like illness consultations; B) GPIH upper respiratory tract infection consultations; C) acute respiratory infection visits; D) general practitioner out of hours asthma/wheeze/difficulty breathing consultations. Vertical gray shaded area indicates period of peak winter activity (week 51 of 2014 through week 3 of 2015). ED, emergency department.

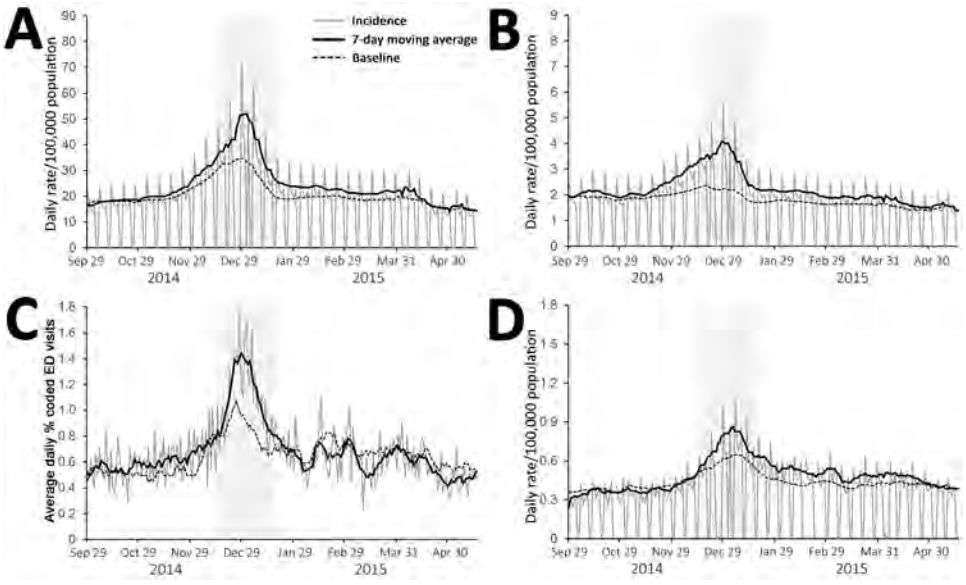

Figure 2. Daily incidence of severe respiratory indicators over winter 2014–15, England. A) General practitioner in hours (GPIH) lower respiratory tract infection consultations; B) GPIH severe asthma consultations; C) ED pneumonia visits; D) GPIH pneumonia consultations. Vertical gray shaded area indicates period of peak winter activity (week 51 of 2014 through week 3 of 2015). ED, emergency department.

The number of ED visits for pneumonia was similar between 2013–14 and 2014–15 across all ages and for the 65–74- and ≥75-year ages, with activity levels higher than observed in 2012–13 (Figure 3, panel C; Figure 4, panel C; Figure 5, panel C). GPOOH consultations for asthma/wheeze/difficulty breathing were similar across all age groups and years (Figure 3, panel D; Figure 4, panel D; Figure 5, panel D).

For several key indicators, the statistical analysis confirmed the significant increases in older age groups during 2014–15. Of those indicators without evidence for statistically significant differences in the all-age incidence over weeks 51–3 between 2014–15 and other years, GPIH URTI was significantly higher in the 65–74- ($p = 0.010$) and ≥75-year ($p = 0.002$) age groups. Similarly, both GPIH ILI (65–74 years, $p = 0.007$; ≥75-year, $p = 0.005$) and GPOOH asthma/wheeze/difficulty breathing (65–74 years, $p = 0.028$; ≥75-year, $p = 0.020$) were significantly higher for older age groups (Table 2; online Technical Appendix Table).

Discussion

We have undertaken a retrospective observational analysis of syndromic healthcare data that were monitored prospectively, on a daily basis, during winter 2014–15 as part of a national real-time syndromic surveillance service. Despite other indicators of influenza activity demonstrating moderate activity, although more severe in some instances (in

Table 2. Comparison of mean daily rate/percentage of selected respiratory indicators over weeks 51–3 between 2014–15 and previous 2 winters, England*

Syndromic surveillance		Age group, y							
Indicator	System	<1	1–4	5–14	15–44	45–64	65–74	≥75	All ages
URTI	GPIH	0.178	0.270	0.540	0.086	0.066	**0.010**	**0.002**	0.066
ARI	GPOOH	0.807	0.713	0.903	0.713	0.391	0.111	0.050	0.540
	EDSSS	0.178	0.624	0.624	0.142	**0.037**	0.111	**0.005**	0.142
ILI	GPIH	0.391	0.221	0.462	0.178	0.142	**0.007**	**0.005**	0.142
	GPOOH	0.327	0.327	0.540	0.624	0.713	0.221	**0.037**	0.462
	EDSSS	0.480	0.312	0.061	**0.010**	0.178	**0.009**	**0.019**	**0.007**
Cold/influenza	NHS 111	0.410	**0.016**	**0.009**	**0.009**	**0.009**	**0.009**	**0.016**	**0.009**
Fever	NHS 111	0.602	0.251	**0.009**	0.076	0.602	0.175	0.602	0.175
LRTI	GPIH	0.462	0.270	0.327	0.111	0.086	**0.020**	**0.005**	**0.028**
Pneumonia	GPIH	**0.037**	0.178	0.462	**0.002**	**0.003**	**0.007**	**0.002**	**0.002**
	EDSSS	0.156	0.713	0.266	0.178	0.111	0.066	**0.020**	**0.028**
Cough	NHS 111	0.917	0.347	0.117	**0.047**	**0.028**	**0.028**	**0.047**	0.175
DB	NHS 111	0.347	0.465	0.465	0.175	0.117	0.076	**0.028**	0.175
A/W/DB	EDSSS	0.221	0.066	0.624	0.391	0.086	0.807	0.391	0.540
	GPOOH	0.624	1.000	0.462	0.111	0.111	**0.028**	**0.020**	0.178
Asthma	GPIH	0.167	0.903	0.178	**0.028**	**0.003**	**0.005**	**0.003**	**0.005**

*Activity during winter 2014–15 is compared to combined mean activity during previous 2 winters and results of Mann-Whitney test are presented as p values. Statistically significant (at 95% confidence level) results are in **bold**. ARI, acute respiratory tract infection; A/W/DB, asthma/wheeze/difficulty breathing; DB, difficulty breathing; EDSSS, emergency department syndromic surveillance system; GP, general practitioner; GPIH, GP in hours; GPOOH, GP out of hours; ILI, influenza-like illness; LRTI, lower respiratory tract infection; URTI, upper respiratory tract infection.

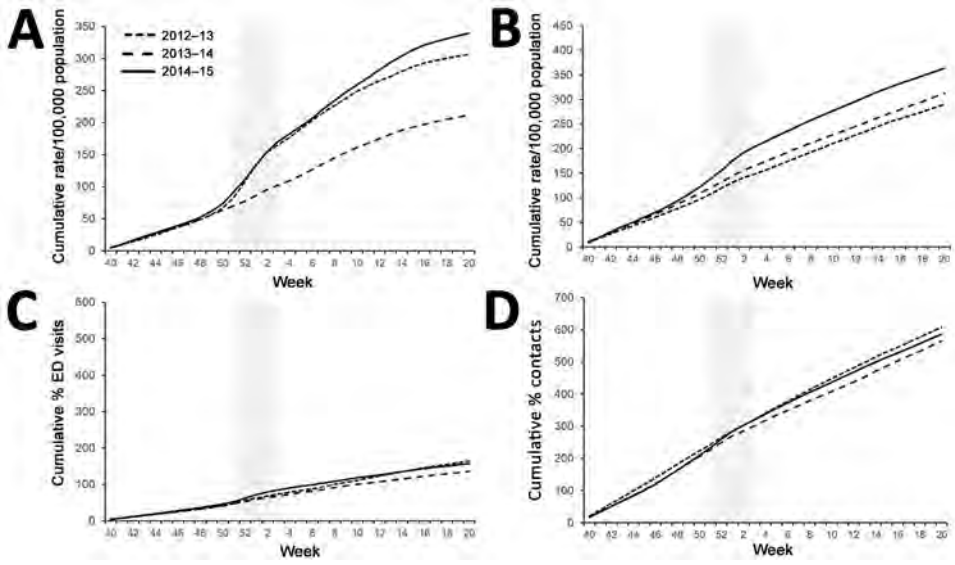

Figure 3. Weekly cumulative rates of selected respiratory indicators for all ages, England, winter 2014–15 compared with the previous 2 winters. A) General practitioner in hours (GPIH) influenza-like illness consultations; B) GPIH severe asthma consultations; C) ED pneumonia visits; D) general practitioner out of hours asthma/wheeze/difficulty breathing consultations. Vertical gray shaded area indicates period of peak winter activity (week 51 of 2014 through week 3 of 2015). ED, emergency department.

particular for excess all-cause deaths and respiratory outbreaks in residential care home settings) (*22*), the national incidence of syndromic GP ILI for all ages and other indicators of community-based respiratory activity (e.g., URTI and ARI) were within seasonally expected levels. However, unusual activity was seen for certain syndromic conditions, including GPIH severe asthma and LRTI, as reported contemporaneously during the winter (*30*). At the time, it was unclear what was driving these observations; however, when these syndromic surveillance data were further stratified by age group and compared between years, it was apparent that increases in respiratory activity in the 65–74- and ≥75-year age groups were significantly greater than those seen during the previous 2 years. Our results support the hypothesis that a particular feature of this winter was the effect of respiratory infections in the elderly. The elderly have a higher risk for severe respiratory disease and secondary complications, resulting in excess hospital admissions (*5*,*8*,*9*).

Another important factor to take into account is that the dominant circulating strain in 2014–15 was influenza A(H3N2), a strain known to cause more severe disease in the elderly compared with other influenza subtypes, resulting in higher rates of disease and more severe outcomes in the elderly during seasons of predominant A(H3N2) circulation (*10*,*31*,*32*). Although 2014–15 was characterized by circulation of antigenically and genetically drifted influenza A(H3N2) and B viruses, end-of-season vaccine effectiveness estimates ultimately demonstrated an overall effectiveness of 34%, highlighting that, although effectiveness was suboptimal, the vaccine still provided important levels of protection to vulnerable populations (*33*). This finding was in line with recently published vaccine effectiveness estimates for this generation of inactivated vaccines (*34*).

The timing of influenza activity can be a contributing factor to pressures on health services. In the United Kingdom, peak respiratory admissions for bronchitis usually occur during week 52 of 1 year through week 2 of the next, coinciding with circulation of RSV (*12*,*13*). During winters in which both influenza and RSV, and therefore ILI and bronchitis, circulate concurrently, a higher-than-expected number of hospital admissions occur as the number of patients with respiratory illness is condensed into a shorter, more intense period (*35*). During winter 2014–15, influenza activity breached threshold levels during week 50 of 2014 and peaked in weeks 1 and 2 of 2015, overlapping with the peak of RSV activity. Although RSV is mainly known as a cause of bronchiolitis in children, it can also be an important cause of illness the elderly, in whom it can cause pneumonia and other LRTI (*3*,*36*,*37*).

The peak in GP consultations for severe asthma recorded during the winter of 2014–15 was nearly twice the baseline level. The increase preceded the increase in GP consultations for influenza and affected persons ≥15 years of age. The association between influenza infections and exacerbations of asthma has been documented through increased asthma hospital admissions in the elderly (*38*). However, the high number of GP consultations for severe asthma compared with other respiratory indicators during an influenza epidemic is unusual, and further work on the underlying causes of these excess consultations may be warranted.

Our study has several limitations. Syndromic surveillance is limited to monitoring and reporting on initial symptoms and provisional diagnoses; therefore, the results of this work cannot be directly linked or attributed to individual respiratory pathogens. However, previous comparisons of syndromic surveillance data with laboratory

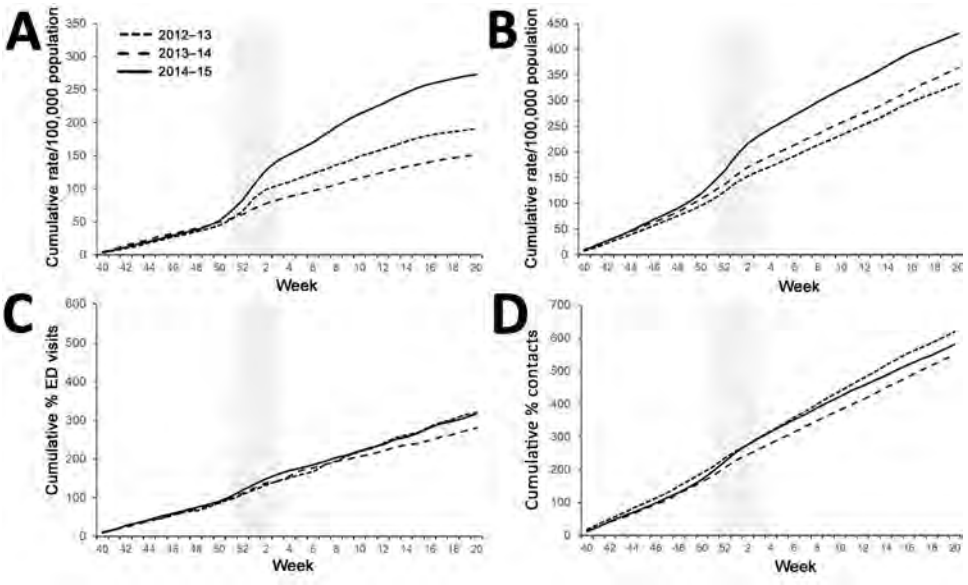

Figure 4. Weekly cumulative rates of selected respiratory indicators in the 65–74-year age group, England, winter 2014–15 compared with the previous 2 winters. A) General practitioner in hours (GPIH) influenza-like illness consultations; B) GPIH severe asthma consultations; C) ED pneumonia visits; D) general practitioner out of hours asthma/wheeze/difficulty breathing consultations. Vertical gray shaded area indicates period of peak winter activity (week 51 of 2014 through week 3 of 2015). ED, emergency department

reports has shown that respiratory syndromes are sensitive to individual pathogens and can provide early warning of seasonal respiratory activity such as influenza and RSV (*39–43*).

In addition, several of the national syndromic surveillance data sources have been established since 2012 and therefore had limited historical data available for use in this study, which limits the comparison with a larger number of winters (*21*,*26*). Comparable GP surveillance data with extensive historical data confirm that influenza activity over the last decade has been relatively low, and therefore the comparison years were not atypical (*14*).

The number of winters included in this analysis was limited by the availability of surveillance data. Interseasonal differences in influenza vaccine effectiveness may also be a limiting factor when making comparisons; we therefore recommend future research to update our findings, including more winters and possibly stratifying by vaccine effectiveness.

We undertook this retrospective analysis to determine the relative burden of respiratory illness during the winter. However, to translate this research into the active public health service, it is important to understand how to apply these results to prospective data analysis. A common limitation of prospective analysis is delayed reporting, which can limit the usefulness of these data. However, the PHE syndromic surveillance data sources are all obtained in near real-time (daily), and the data are recorded consistently and completed at the time of the patient event.

Our findings support clinicians, health service managers, and public health bodies not relying on a single indicator of influenza activity to anticipate winter pressures on healthcare systems. GP consultations for ILI have historically been used as a key indicator of influenza activity in the community. However, persons with influenza can experience a wide range of clinical signs and symptoms (*6*). Our work, and that of others, continues to show that several clinical indicators, including ILI, LRTI, and severe asthma stratified by age, should be monitored routinely to identify expected sources of pressure.

Our work also highlights the importance of ensuring high levels of vaccine uptake in groups at higher risk for severe disease after influenza infection and also for children. The pediatric influenza vaccination program in England aims to protect the vaccinated children themselves and also, by reducing their rates of infection, reduce transmission in the population and thus indirectly protect those at higher risk for severe disease, such as the elderly and those with underlying clinical disease.

In the elderly, influenza accounts for more hospital admissions than RSV (*10*,*36*,*44*), but for those admitted to hospital with RSV, length of stay, rates of use of intensive care, and mortality rates can be similar to those for influenza patients (*36*). A growing body of evidence indicates that the effect of RSV in older patients is underestimated because they are not representatively sampled in the community. Whereas the contribution of RSV to increased hospital admissions in the winter of 2014–15 is uncertain, it may have contributed to the total burden placed on hospitals over that period. Improved testing and recognition of the effects of RSV by clinicians, particularly those working in geriatric medicine, might improve further understanding of this burden.

Underpinning each of the PHE national syndromic surveillance data sources are statistical algorithms that contemporaneously compare current data to historical data to

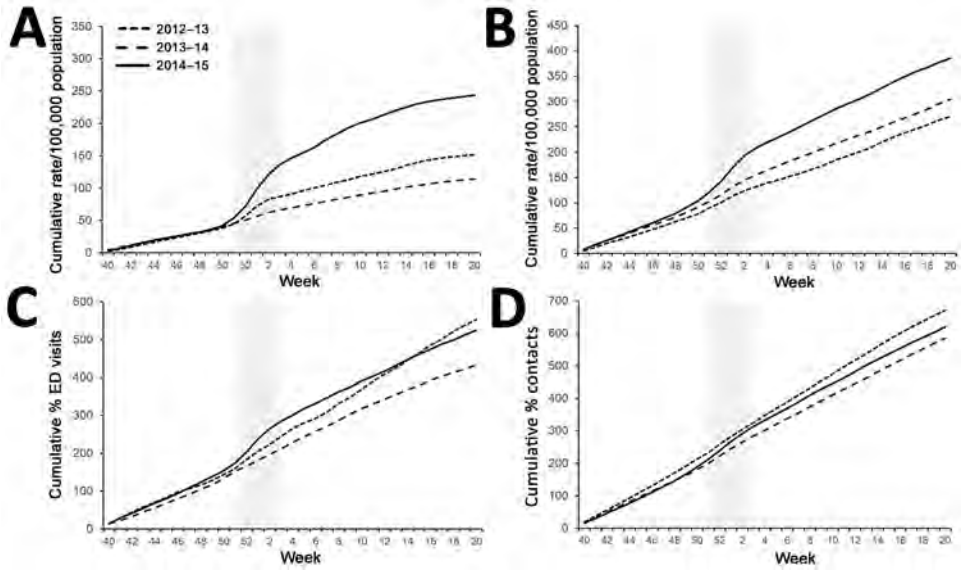

Figure 5. Weekly cumulative rates of selected respiratory indicators in the ≥75-year age group, England, winter 2014–15 compared with the previous 2 winters. A) General practitioner in hours (GPIH) influenza-like illness consultations; B) GPIH severe asthma consultations; C) ED pneumonia consultations; D) general practitioner out of hours asthma/wheeze/difficulty breathing consultations. Vertical gray shaded area indicates period of peak winter activity (week 51 of 2014 through week 3 of 2015) ED, emergency department.

determine whether activity is statistically higher and therefore requires public health action (*27*). The results of this study suggest that further development of these algorithms is needed to include age-specific statistics, that is, to monitor statistically significant activity in individual age groups rather than in all ages. This extended monitoring would offer a better means of providing early warning of increasing seasonal influenza severity and enable timelier public health action and interventions during future winters similar to 2014–15 (*45*). However, any increase in the daily workload required to monitor a large number of additional age-specific signals must be carefully considered. Using automated statistical algorithms to identify aberrations that require further epidemiologic interrogation can help minimize the impact on health systems while maintaining this extended monitoring (*27*).

The additional strain on the healthcare system during 2014–15 was experienced particularly in emergency medicine (*4*); the EDSSS was able to monitor trends at the national level, but the sentinel nature of this surveillance limited its usefulness for identifying and supporting local services. Future recruitment of additional EDs to the EDSSS across England, enabling expansion of this data source, would further facilitate use of local ED surveillance data.

Another area of potential future development is developing predictions and forecasting models to predict unusual activity in the elderly. Certain respiratory diagnoses, particularly in primary care and EDs, often peak in children with a lag before peaking in the elderly (*12*,*43*). It has also been shown that within primary care, elderly patients seek treatment later in the disease episode when symptoms have deteriorated (*46*). Using these observations, public health epidemiologists could monitor early incidence in children to model and predict unusual activity in the elderly.

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Weather-Dependent Risk for Legionnaires' Disease, United States

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Using the Nationwide Inpatient Sample and US weather data, we estimated the probability of community-acquired pneumonia (CAP) being diagnosed as Legionnaires' disease (LD). LD risk increases when weather is warm and humid. With warm weather, we found a dose-response relationship between relative humidity and the odds for LD. When the mean temperature was 60°–80°F with high humidity (>80.0%), the odds for CAP being diagnosed with LD were 3.1 times higher than with lower levels of humidity (<50.0%). Thus, in some regions (e.g., the Southwest), LD is rarely the cause of hospitalizations. In other regions and seasons (e.g., the Mid-Atlantic in summer), LD is much more common. Thus, suspicion for LD should increase when weather is warm and humid. However, when weather is cold, dry, or extremely hot, empirically treating all CAP patients for LD might contribute to excessive antimicrobial drug use at a population level.

Legionellosis is associated with a mild febrile illness, Pontiac fever, or Legionnaires' disease (LD) (*1*), a cause of severe, atypical, community-acquired pneumonia (CAP) (*2*). *Legionella* spp. are aerobic, gram-negative bacilli, common in the environment, that were identified as pathogenic after an outbreak of illness among attendees of a 1976 American Legion convention (*1*,*3*). Although there are several species of *Legionella* and different serotypes, *L. pneumophilia* causes most LD cases (*4*,*5*). The case-fatality rate for LD among community-dwelling persons is as high as 10% (*5*). Delayed initiation of appropriate antimicrobial drug therapy further increases death rates (*6*,*7*), and the severity of LD drives the rationale for covering atypical organisms in the guidelines for empiric treatment of CAP. In developed countries, *Legionella* causes 1%–4% of CAP cases (*4*,*8*,*9*). Thus, because the rate of LD is low, many persons with CAP may be unnecessarily treated for LD. In fact, a recent noninferiority study, which included aggressive diagnostic testing, showed similar outcomes when treating and not treating for atypical organisms (*10*).

A striking epidemiologic feature of *Legionella*-associated CAP is its seasonality; more cases are reported during the summer (*1*). In contrast, hospital-associated cases do not exhibit seasonality (*1*). Seasonality has been described in the Mid-Atlantic United States (*11*–*14*), England and Wales (*15*), and the Netherlands (*16*). Changes in use of cooling towers (*17*) or additional testing for pneumonia during the summer have been hypothesized as causes of this seasonality (*1*). However, strong evidence indicates that weather, particularly temperature and humidity, drive the summer spike in incidence (*11*,*12*,*15*,*16*). Although *Legionella* spp. are common in the environment, dry environments do not support them (*1*), and *Legionella* spp. are more sensitive than other pathogens to drying conditions (*18*). In contrast, warm and humid weather tends to support pathogen survival, growth, and the potential for aerosol exposures, increasing disease risk (*1*,*13*,*19*).

If the incidence of LD depends on local weather, the baseline rate of LD might be extremely low year-round in some locations and during specific seasons in other locations. Use of local weather data ultimately might provide information to help determine whether a specific CAP case is caused by *Legionella*. To establish the risk for LD across season, location, and weather conditions, we combined patient-level data on hospitalizations for pneumonia and LD from 26 US states with local weather data.

Methods

Data Source and Case Definition

We extracted individual-level inpatient-event data from the Agency for Healthcare Research and Quality's Healthcare Cost and Utilization Project (HCUP) Nationwide Inpatient Sample (NIS) for 1998–2011. The University of Iowa Institutional Review Board deems such studies as non–human subjects research. The NIS, a stratified 20% sample of discharges from nonfederal US hospitals, contains data from 47 states; after excluding the 21 NIS states that do not report the American Hospital Association identifier (AHA ID), patient race, or admission month, we used data from 26 states: Arizona, Arkansas, California, Colorado, Connecticut, Illinois, Iowa, Kentucky, Maryland, Massachusetts, Mississippi, Missouri, Montana, Nevada, New Hampshire, New Jersey, New York, North Carolina, Oregon, Pennsylvania, Rhode Island, Utah,

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Vermont, Virginia, Washington, and Wisconsin. Next, we mapped the hospitals in these 26 states to the AHA-reported addresses using the AHA ID; we then converted the addresses to geographic coordinates by using the US Census Bureau Geocoder (https://www.census.gov/geo/maps-data/data/geocoder.html) and Google Maps' Geocoding API (Google; Mountain View, CA, USA). We located 2,079 unique hospitals (Figure 1).

We identified LD cases as hospitalizations of persons with a primary diagnosis code of 482.84 (pneumonia due to Legionnaires' disease) from the International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM). The primary diagnosis in the NIS is the condition chiefly responsible for the hospital admission (*20*). The sensitivity and specificity of the 482.84 code for LD was previously evaluated in a New York, NY, hospital for 2003–2013 (*21*); the authors reported high sensitivity (83.5%) and specificity (99.9%), a positive predictive value of 88.0%, a negative predictive value of 99.8%, and agreement between the estimated cases observed in the NIS for 2012 and the Centers for Disease Control and Prevention (CDC) data (*21*).

As study controls, we used hospitalizations of persons with a primary diagnosis ICD-9-CM code of 481 and subcodes (pneumococcal pneumonia) and 482 and subcodes (other bacterial pneumonia) excluding 482.84 (pneumonia due to Legionnaires' disease). We refer to the combination of the codes 481 and 482 as bacterial pneumonia. Because these codes were assigned to patients as their primary diagnosis code for admission, we assumed that this collection of codes identified cases of community-acquired bacterial pneumonia. Finally, using CDC surveillance results (*22*), we computed the correlation between the national-level estimated number of LD cases in our data and the number reported by CDC (*23*).

We excluded records for persons <18 years of age and records that omitted any of our variables of interest: age, sex, payer, race, admission month and year, and hospital location. We also required the hospital to have ≥1 weather station within 100 km (62 miles).

Weather Definition

We obtained weather observations from the Integrated Surface Database (ISD) provided and maintained by the National Climatic Data Center of the National Oceanic and Atmospheric Administration. Because the NIS database provides only the month of admission, we aggregated the average temperature, relative humidity, and total rainfall by month for each weather station. We recorded each hospital's monthly weather data as the mean of these values observed at nearby (within 100 km [62 miles]) weather stations. Using only the states with hospital location reported, we considered different definitions of "nearby." Average temperatures computed using only the nearest station and stations within 10 or 25 miles were highly correlated with the average temperature using a 62-mile radius ($r>0.99$).

Modeling

Using logistic regression, we modeled whether a hospitalization for bacterial pneumonia had a diagnosis of LD on the basis of patient age, patient sex, payer, patient race, admission month, admission year, hospital latitude, total monthly rainfall, mean relative humidity, mean temperature, and an interaction between temperature and relative humidity. The interaction is required because relative humidity depends on temperature. We used mean temperature because it captures the nighttime and daytime temperature effects more accurately than does mean high temperature. However, the average high temperature and the average temperature for a month are highly correlated ($r = 0.988$). We used relative humidity rather than absolute humidity for 2 reasons. First, relative

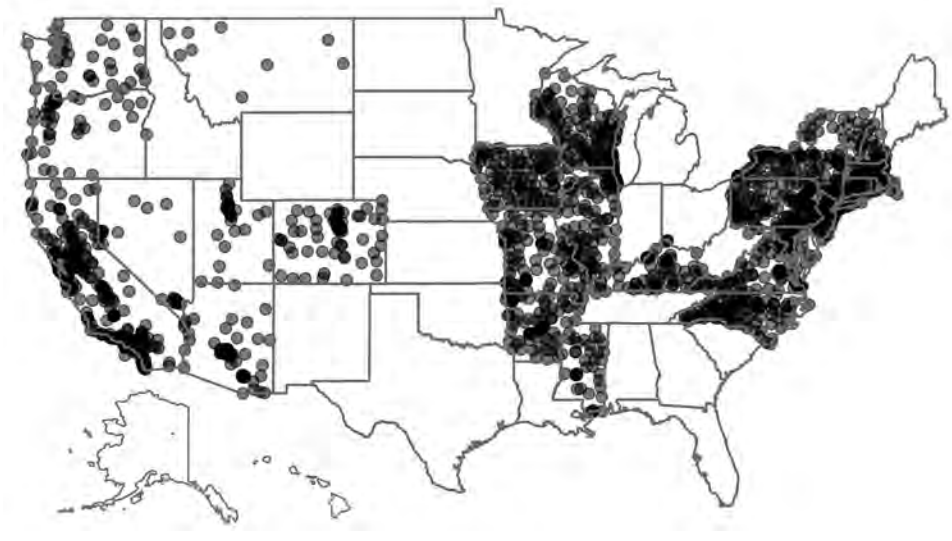

Figure 1. Locations of Healthcare Cost and Utilization Project (HCUP) hospitals used in the analysis of risk for Legionnaires' disease, 26 US states, 1998–2011. Because many hospitals are near each other, each hospital was plotted as a faint point. When multiple points overlap, the area becomes darker because of the stacking of the points. Thus, there are faint spots in more rural areas and dark clusters in more urban areas.

humidity ranges from 0% to 100% for any temperature, whereas absolute humidity ranges from 0 g/m^3 to some temperature-specific maximum, which introduces problems because often the lowest observed absolute humidity at high temperatures is impossible at lower temperatures. Second, the values of absolute humidity were extremely correlated with temperature (r = 0.85), whereas the correlation was much lower with relative humidity (r = –0.23). The correlation of temperature with relative humidity is negative because relative humidity is often much higher at cold temperatures. To make the results easier to interpret and because the expected responses are not linear, we converted humidity and temperature into bins: relative humidity <50.0%, 50.0%–55.0%, 55.1%–60.0%, 60.1%–65.0%, 65.1%–70.0%, 70.1%–75.0%, 75.1%–80.0%, and >80.0%; and mean temperature <60°F, 60.1–80°F, >80°F. Additionally, monthly rainfall was binned into dry (<18 mm, the lowest 25%), normal (18–85.85 mm, the middle 50%), and wet (>85.85 mm, the top 25%). Patient age was binned by decade, and we included hospital latitude and squared hospital latitude. The squared hospital latitude was included to enable the effect of latitude to be nonlinear.

To visualize the model and how LD risk varies with space and season, we computed the fitted values from this model using location and weather information and set the demographic variables to their individual modal values for cases of bacterial pneumonia observed in the NIS data. Because the weather data are nationally complete, unlike the NIS data, we can take a given demographic profile (e.g., white man, 68 years of age, on Medicare) and estimate the probability of an LD diagnosis for any location, given the weather data for each location and month.

Results

The NIS data provided a total of 5,172 LD cases from 447,132 hospitalizations for bacterial pneumonia (Table 1). After applying the discharge weights to produce a national-level estimate and before applying any exclusion rules other than reporting month and year, the NIS series compares favorably to CDC's reported monthly LD estimates (*23*), with a correlation of 0.74. After applying exclusion rules (age ≥18 years and provision of a complete set of predictor variables), we had data on 3,005 LD cases and 189,412 hospitalizations for pneumonia. The most common reasons for exclusion were lack of the AHA ID, admission month and year, and race, because some states elected not to report these variables.

Table 1. Sample sizes for Legionnaires' disease cases and other pneumonia controls in a study of weather-dependent risk for Legionnaires' disease, United States, 1998–2011*

Characteristics reported	No. (% of initial sample)	
	Cases	Controls
Total	5,172 (100.0)	447,132 (100.0)
Age ≥18 y	5,158 (99.7)	418,086 (93.5)
AHA ID	4,039 (78.1)	288,427 (64.5)
Sex	4,039 (78.1)	288,427 (64.5)
Payer	4,034 (78.0)	287,847 (64.4)
Admission month and year	3,542 (68.5)	253,725 (56.7)
Race	3,006 (58.1)	189,630 (42.4)
Weather station within 100 km (62 mi) of hospital	3,005 (58.1)	189,412 (42.4)

*The analysis comprised data from 26 states: Arizona, Arkansas, California, Colorado, Connecticut, Illinois, Iowa, Kentucky, Maryland, Massachusetts, Mississippi, Missouri, Montana, Nevada, New Hampshire, New Jersey, New York, North Carolina, Oregon, Pennsylvania, Rhode Island, Utah, Vermont, Virginia, Washington, Wisconsin. AHA ID, American Hospital Association identifier.

We compared several demographic and severity measures between the portion of the data dropped because of missing values and the proportion retained (Table 2). Among the cases, the only substantial difference was in the percentage of patients not insured; for 10.8% of dropped records, no insurance was reported, compared with 7.4% of those used in the model. Many statistically significant differences existed between the controls retained and those lost; however, the large sample size (retained sample n = 189,412) meant many non–clinically relevant differences would be statistically significant.

In general, hospitalized persons with LD were younger than those with other bacterial pneumonia and more likely to be male (Table 3). We found a large unadjusted

Table 2. Demographic and severity characteristics among dropped and retained records in a study of weather-dependent risk for Legionnaires' disease, United States, 1998–2011*

Characteristic	Cases			Controls		
	Dropped, n = 2,153	Retained, n = 3,005	p value	Dropped, n = 228,674	Retained, n = 189,412	p value
Mean age, y (± SD)	60.6 (15.7)	61.8 (15.6)	0.0078	68.2 (17.1)	68.8 (17.2)	<0.0001
Female, %	39.6	39.1	0.7138	48.1	48.2	0.4170
Privately insured, %	39.0	38.8	0.8814	19.6	17.0	<0.0001
Not insured, %	11.2	7.7	<0.0001	6.2	4.5	<0.0001
Mean no. diagnoses (± SD)	9.6 (4.1)	9.6 (4.3)	0.9213	8.2 (3.9)	9.4 (4.3)	<0.0001
Mean no. procedures (± SD)†	1.9 (2.5)	1.9 (2.8)	0.6027	1.0 (1.8)	1.4 (2.2)	<0.0001

*Many of the significant differences in the controls resulted from the large sample and might not be clinically significant. The analysis comprised data from 26 states: Arizona, Arkansas, California, Colorado, Connecticut, Illinois, Iowa, Kentucky, Maryland, Massachusetts, Mississippi, Missouri, Montana, Nevada, New Hampshire, New Jersey, New York, North Carolina, Oregon, Pennsylvania, Rhode Island, Utah, Vermont, Virginia, Washington, Wisconsin. †Any type of procedure recorded in the Agency for Healthcare Research and Quality's Healthcare Cost and Utilization Project; this was a measure of severity, and this variable was not included in the model.

Table 3. Key variables in the sample in a study of weather-dependent risk for Legionnaires' disease, United States, 1998–2011*

Variable	Cases, n = 3,005	Controls, n = 189,412
Age, y (± SD)	61.80 (15.61)	68.83 (17.15)
Sex, %		
F	39.13	48.18
M	60.87	51.82
Race/ethnicity, %		
White	76.64	79.60
Black	14.81	9.52
Hispanic	4.66	6.06
Other	3.89	4.83
Payer, %		
Medicare	45.82	69.22
Medicaid	7.69	9.27
Private	38.80	16.97
Uninsured	4.89	2.63
Other	2.80	1.92
Mean latitude, °N (± SD)	40.02 (2.75)	38.86 (3.30)
Mean monthly temperature, °F (± SD)	58.49 (14.96)	52.61 (15.32)
Mean monthly relative humidity, % (± SD)	70.03 (9.22)	67.34 (10.45)
Mean monthly total rainfall, mm (± SD)	80.39 (69.15)	61.68 (144.67)

*The analysis comprised data from 26 states: Arizona, Arkansas, California, Colorado, Connecticut, Illinois, Iowa, Kentucky, Maryland, Massachusetts, Mississippi, Missouri, Montana, Nevada, New Hampshire, New Jersey, New York, North Carolina, Oregon, Pennsylvania, Rhode Island, Utah, Vermont, Virginia, Washington, Wisconsin.

difference in the mean monthly environmental temperatures between cases (58.5°F) and controls (52.6°F), and monthly environmental relative humidity was higher on average for cases (70.0%) than for controls (67.3%). Additionally, there was nearly 20 mm more rain for cases (80.4 mm) than for controls (61.7 mm).

In the regression analysis, the primary variables of interest—mean temperature, mean relative humidity, and their interaction—were all significant (likelihood ratio test against model with only main effects, χ^2 test statistic = 350.42; p<0.0001) (Table 4, https://wwwnc.cdc.gov/EID/article/23/11/17-0137-T4.htm). Although total rainfall was independently a risk factor for LD, the effects of temperature and humidity were still significant.

The combination of the temperature and humidity main effects and interactions can make understanding the combined effect difficult. For this reason, we separately reported the estimated composite odds ratios for each combination (Table 5). Additionally, because odds ratios can be difficult to interpret, we provide the expected probabilities for a 61–70-year-old white man on Medicare admitted to a hospital at 42°N in April 2011 at the 3 different rainfall levels (Table 6). The relationship between temperature and relative humidity exhibits the Goldilocks principle: when it is too hot (>80°F) or too cold (<60°F), the odds of LD do not vary with humidity, but when the temperature is "just right" (60°–80°F), the odds have a dose-response pattern with humidity. The largest effect of this relationship between temperature and humidity was evident for warm and very humid months across all 3 rainfall levels.

Table 5. Odds ratios for Legionnaires' disease based on the interaction between average monthly temperature and average monthly relative humidity, United States, 1998–2011*

	Average monthly temperature, °F		
Relative humidity, %	<60	60–80	>80
0–50	1.00	0.55	1.57
50.1–55.0	0.85	0.77	0.00
55.1–60.0	0.58	0.49	0.00
60.1–65.0	0.66	0.78	1.28
65.1–70.0	0.79	0.98	0.77
70.1–75.0	0.80	1.29	0.48
75.1–80.0	0.76	1.37	1.09
80.1–100.0	0.65	1.70	0.00

*Estimated from the multivariable logit model shown in Table 4. The analysis comprised data from 26 states: Arizona, Arkansas, California, Colorado, Connecticut, Illinois, Iowa, Kentucky, Maryland, Massachusetts, Mississippi, Missouri, Montana, Nevada, New Hampshire, New Jersey, New York, North Carolina, Oregon, Pennsylvania, Rhode Island, Utah, Vermont, Virginia, Washington, Wisconsin.

We determined the monthly percentage of bacterial pneumonia discharges for which an LD diagnosis had been given within HCUP by US Census region (Figure 2). Percentages were relatively high in the Northeast and somewhat lower in the Midwest and South. The frequencies in the West were the lowest of all 4 regions and appeared not to be seasonal. The changes around 2002–2003 in all of the series are present in other data sources (*23*). The exact cause is unknown but is thought to be related to increased vigilance, testing, and reporting of atypical pneumonia after the outbreak of severe acute respiratory syndrome (*24*).

We also determined the probability of a case of bacterial pneumonia being diagnosed as LD in 2011 using the local weather data. We restricted this prediction to the states used to estimate the model (Figure 3). We set the nonweather, nonlocation covariates to their modal values for patients hospitalized with bacterial pneumonia (white 61–70-year-old man on Medicare) and used the weather station latitude and monthly average temperature, humidity,

Table 6. Estimated probability of Legionnaires' disease given bacterial pneumonia in a 61–70-year-old white man on Medicare located at 42°N and admitted to a hospital in April 2011, based on the interaction between average monthly temperature, rainfall, and relative humidity*

Relative humidity, %	Probability of Legionnaires' disease by rainfall level								
	<60°F			60°F–80°F			>80°F		
	Dry	Normal	Wet	Dry	Normal	Wet	Dry	Normal	Wet
0.0–50.0	1.46	1.98	2.13	0.81	1.10	1.18	2.28	3.08	3.31
50.1–55.0	1.24	1.68	1.81	1.13	1.53	1.64	0	0	0
55.1–60.0	0.86	1.16	1.25	0.72	0.98	1.06	0	0	0
60.1–65.0	0.97	1.32	1.42	1.15	1.56	1.68	1.86	2.52	2.71
65.1–70.0	1.16	1.57	1.69	1.44	1.95	2.10	1.13	1.53	1.64
70.1–75.0	1.17	1.59	1.72	1.88	2.54	2.74	0.71	0.96	1.03
75.1–80.0	1.12	1.52	1.63	1.99	2.69	2.90	1.59	2.16	2.32
80.1–100.0	0.95	1.29	1.39	2.46	3.32	3.57	0	0	0

*Because of the presence of month, latitude, and year in the model, these predicted probabilities are valid only on a line along 42°N in April 2011. The predicted values for other months (e.g., July or December) will differ, as will the predicted values for locations further north or south than 42°N. Dry, <18 mm; normal, 18–85.85 mm; wet, >85.85 mm.

and rainfall. The estimated probabilities of LD (Figure 3) are the fitted values from the model described by Table 4 and these covariate values. Since the ISD is national in scope, we extrapolated from the model estimated using HCUP data to the entire United States, including non-HCUP regions (Figure 4). The risk for LD varied considerably by location (low risk along the Gulf Coast and relatively low risk in the West) and calendar month (high–risk areas such as the Mid-Atlantic region are only actually at high risk during June–September and are at low risk during December–April) (Figure 4).

Discussion

Our results suggest that the incidence of LD varies considerably by season and local weather patterns. Specifically, LD is more likely to occur in warm (60°–80°F) and very humid (≥80.0%) months. For example, the odds of LD being diagnosed in a pneumonia patient during a month when the rainfall is <18 mm and the temperature is 60°–80°F was 3.1 (1.70/0.55) times higher when the relative humidity was >80.0% than when it was <50.0%. When rainfall amounts were greater, the risk also increased; however, regardless of rainfall, warm and humid weather was a major risk factor. Also, we found a dose-response relationship between relative humidity and the odds of an LD diagnosis during periods of warm weather. In contrast, hot, cool, or dry weather patterns produce no meaningful increase in LD.

Previous work has demonstrated seasonality and the effects of weather patterns on LD (*1*,*11*–*13*,*15*,*16*). However, much of this work was based on regional investigations where LD is common. Regional investigations are limited in their ability to more fully describe the relationship between weather patterns and LD incidence. For instance, in the Rocky Mountains or the US Southwest, community-associated LD is comparatively rare, and the rate for LD is much lower than what would be expected given patient factors. In contrast, the Mid-Atlantic has a higher-than-expected risk during certain months of the year.

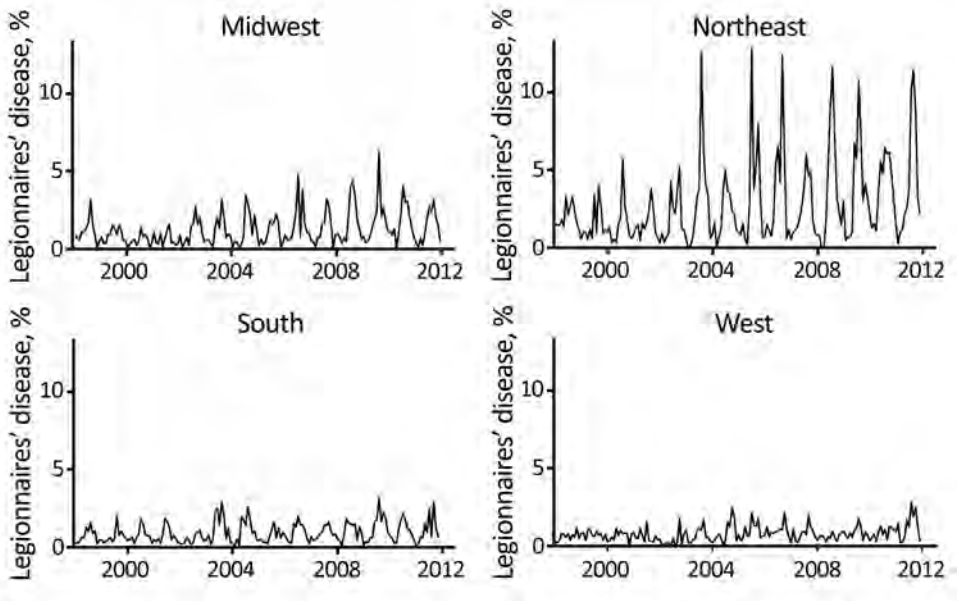

Figure 2. Time series of Legionnaires' disease as a percentage of bacterial pneumonia discharges in Healthcare Cost and Utilization Project hospitals, 26 US states, 1998–2011. The Legionnaires' disease series is highly seasonal in the Northeast, Midwest, and South. There are few cases and a lack of apparent seasonality in the West. The changes in the Legionnaires' disease series after 2002–2003 may result from increased vigilance, testing, and reporting of atypical pneumonias (*24*).

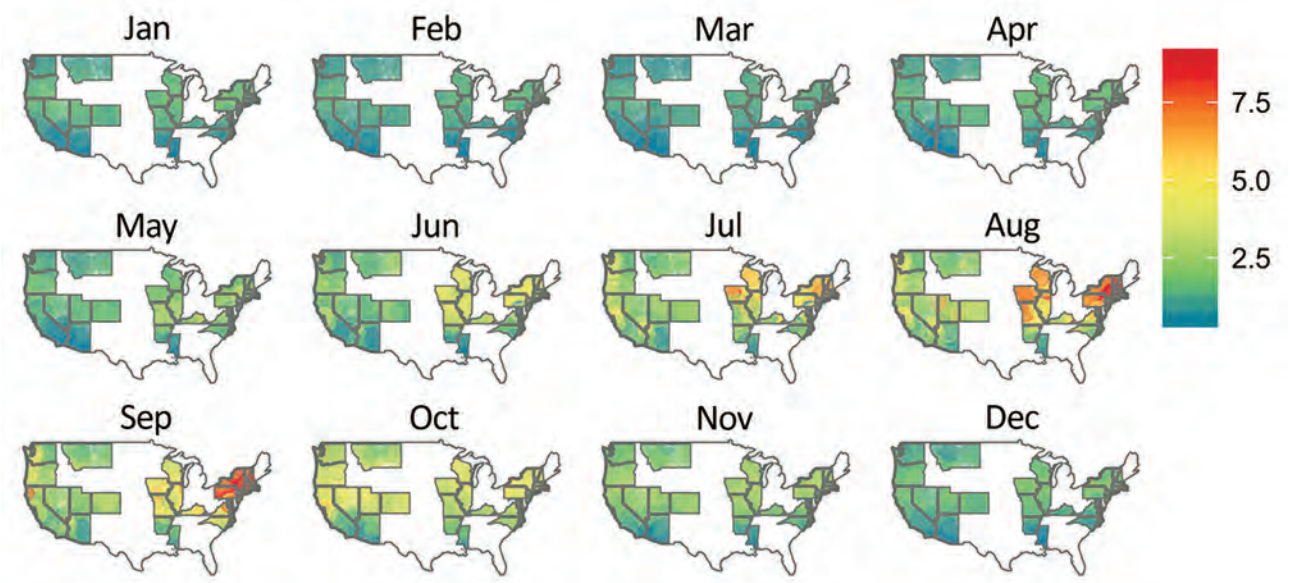

Figure 3. Predicted probability of an inpatient hospitalization for bacterial pneumonia being coded as Legionnaires' disease by location and month in 2011 for 26 US states. The predicted risk is for a 61–70-year-old white man on Medicare (the most common Legionnaires' disease patient in the data) by location in the United States for each month in 2011. These fixed covariates and actual monthly temperature, relative humidity, and latitude for each weather station in the Integrated Surface Database dataset were used to produce estimated probabilities using the model described in Table 4.

Using the national scope of our data, we estimated the differences that weather has on LD risk. The predicted probabilities of LD varied from 0% to 1% of all cases of bacterial pneumonia in the Southwest to nearly 8% during warm, humid, and rainy summer months in the Mid-Atlantic. Weather appears to drive these differences because our model had no geographic information other than latitude. This finding is consistent with prior regionally and seasonally limited studies (*11–13*,*15*,*16*). Areas previously studied (e.g., the Mid-Atlantic) vary considerably in risk depending on recent weather. Our results are biologically plausible because *L. pneumophilia* thrives in warm, wet environments (*25*), which support not only the pathogen's survival but also the existence of aerosolizations. In contrast, conditions are not as supportive for the pathogen in dry or excessively hot environments (*1*,*18*).

LD is difficult to diagnose on the basis of clinical manifestations alone (*26*). Furthermore, rapid diagnostic tests do not cover all strains (*27*), and some tests have relatively low sensitivity (*28*). More definitive culture results may take 3–5 days after therapeutic decisions are needed (*1*). Thus, incorporating local weather conditions into clinical decision-making ultimately might help increase or decrease clinical suspicion for LD, especially when combined with diagnostic testing. Current US CAP guidelines recommend empiric therapy routinely covering atypical pneumonias (*29*). Results of a recent noninferiority trial suggest that monotherapy with a β-lactam, aggressive diagnostic testing, and use of clinical judgment may safely avert the use of fluoroquinolones or dual therapy with a macrolide in patients with CAP (*10*). However, the same trial replicated elsewhere with a higher rate of LD might yield different results. Another study investigating the effect of a β-lactam alone versus a β-lactam with a macrolide found delays in clinical stability for persons treated with only 1 agent, but the authors failed to show that the β-lactam alone was not inferior (*30*). Our model suggests that warm, humid, and rainy summer months in the Mid-Atlantic may exhibit predicted probabilities of LD of nearly 8%. Accordingly, abandoning initial empiric coverage for LD might yield a differential effect on outcomes depending on season, region, and weather, and treating all CAP cases for atypical pneumonia in areas and seasons when LD is relatively uncommon may result in the excessive use of antimicrobial agents.

The antimicrobial drugs most commonly used to treat LD include either a fluoroquinolone or a macrolide (with a β-lactam), and resistance has increased for both (*10*,*31–34*). Thus, treating for LD only when and where risk is higher, along with increased diagnostic testing and good clinical judgement, may help reduce antimicrobial drug use, providing a new antimicrobial drug stewardship target. The temporal, climatologic, and geographic variations in LD risk emphasize the potential importance of regionally relevant guidelines. Basing treatment guidelines on estimates in high- or low-risk areas will lead to overuse or underuse of LD treatment for CAP. However, future work with more detailed clinical information

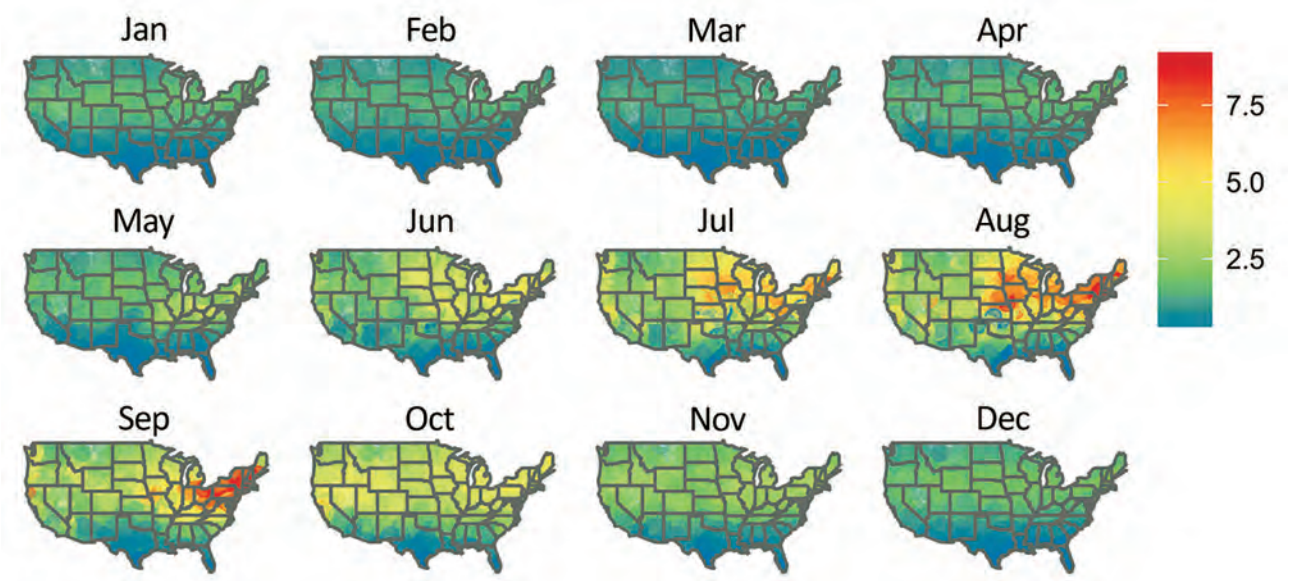

Figure 4. Predicted probability of an inpatient hospitalization for bacterial pneumonia being coded as Legionnaires' disease, all US states, 2011. The predicted risk is for a 61–70-year-old white man on Medicare (the most common patient in the pooled case–control sample) by location for each month in 2011. These fixed covariates and actual monthly temperature, relative humidity, and latitude for each weather station in the Integrated Surface Database dataset were used to produce estimated probabilities using the model described in Table 4.

is required to determine when and where antimicrobial drug use may be initially restricted and in which patients, without significantly impairing the quality of care. Specifically, future work will need to determine under which conditions and in which patients initial monotherapy with a β-lactam will not be inferior to combination therapy. Furthermore, future work will need to consider and incorporate diagnostic testing for LD and the patient's clinical status (e.g., intensive care unit admission).

Our work has several limitations. First, our study uses administrative data for inpatient hospitalizations. Some CAP and LD cases are treated on an outpatient basis. Furthermore, state participation in the NIS is voluntary, and some states omit key variables (e.g., AHA IDs). Although we found no meaningful differences between the parts of the sample we retained and parts we dropped, it is possible that participation/reporting varies nonrandomly. In addition, we do not have information about medications, test results, or microbiology data, so we cannot confirm an LD diagnosis or determine whether and what microbiological testing was performed. Thus, future work should incorporate alternative case-finding approaches, including more granular information about cases, specifically the CDC legionellosis database. Alternative data sources may also enable the generation of more granular geographic estimates and age-based estimates of disease risk. We also are unable to confirm without more clinical data the extent to which having a primary diagnosis of pneumonia correlates with CAP for the control patients in our analysis. Second, some of the geographic differences in the predicted probabilities may be due to differences in propensity to test for, and therefore diagnose, LD. Third, the NIS reports only the month of admission. Thus, we aggregated weather information by month. Future work needs to consider more granular (e.g., daily) data. Fourth, we consider the weather around a hospital, not the weather experienced by the patients admitted to the hospital. Fifth, meteorologic variables are interdependent: relative humidity depends on temperature because the maximum amount of water suspended in the air rises with the temperature, and our model may inadequately specify these relationships. Finally, our analysis did not contain possibly important geographic differences (e.g., use of monochloramine in municipal water).

The experience of warm and humid weather patterns common during summer resulting in substantial increases in LD might have driven the current view about the frequency of LD in the United States. Our results demonstrate the need to investigate the effects of incorporating recent weather patterns, particularly wet, warm, and humid weather, as an additional consideration in the clinical decision-making process for CAP. Our results suggest that the risk for LD is highly related to temperature and humidity regionally. We found locations where LD relatively rarely causes hospitalization for CAP, such as the Southwest and Rocky Mountains, but also the Mid-Atlantic region during the winter. Information about weather

exposures for patients also should help inform the design and interpretation of CAP-treatment trials. Future work examining more granular environmental data may ultimately enable clinicians to safely limit initial empiric antimicrobial drug selection for CAP to monotherapy with a β-lactam in specific seasons and regions.

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etymologia

Legionella pneumophila [le"jə-nel'ə noo"mo-fil'ə]

Ronnie Henry

In the summer of 1976, as the United States was celebrating the bicentennial of the Declaration of Independence, a mysterious acute respiratory illness developed in attendees at an American Legion convention in Philadelphia shortly after the attendees returned from the convention. In total, 182 Legionnaires became ill, and 29 died.

Researchers in the Leprosy and Rickettsia Branch at the Centers for Disease Control (CDC), headed by Charles C. Shepard, observed that guinea pigs became ill after being inoculated with lung tissues from patients who died. A few gram-negative bacilli were seen in guinea pig tissues, but these were believed to be normal flora or contaminants. The bacteria could not at first be isolated in embryonated eggs because the standard procedure for isolating rickettsiae at the time was to include penicillin and streptomycin to prevent contamination.

Figure: Left, Joseph McDade, CDC scientist who discovered the cause of Legionnaires' disease. Right, Lung cells with intra-alveolar exudate containing macrophages and polymorphonuclear leukocytes after infection with *Legionella pneumophila*, the causative agent of Legionnaires' disease. Photos: McDade, R.E. Bates/CDC; photomicrograph, F.W. Chandler/CDC.

Returning to work after Christmas 1976, CDC microbiologist Joseph McDade was bothered by these unexplained findings. He again attempted to grow the bacteria in embryonated eggs, this time without antibiotics, and successfully isolated a large inoculum of pure culture that could be grown on agar. These bacteria were determined to be the etiologic organism of Legionnaires' disease and were eventually named *Legionella* (for the Legionnaires) *pneumophila* (Greek *pneumon* [lung] + *philos* [loving]).

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Increased Detection of Emergent Recombinant Norovirus GII.P16-GII.2 Strains in Young Adults, Hong Kong, China, 2016–2017

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A new recombinant norovirus GII.P16-GII.2 outnumbered pandemic GII.4 as the predominant GII genotype in the winter of 2016–2017 in Hong Kong, China. Half of hospitalized case-patients were older children and adults, including 13 young adults. This emergent norovirus targets a wider age population compared with circulating pandemic GII.4 strains.

Noroviruses are leading causes of acute gastroenteritis (*1*). In the winter of 2016–2017, increased circulation of an uncommon recombinant norovirus genotype called GII.P16-GII.2 was reported in parts of Asia, including China (*2*) and Japan (*3*). Concurrently, winter norovirus cases peaked at an abnormally high level in Germany (*4*) and France (*5*) because of this emergent genotype. We report an increased detection of norovirus GII.P16-GII.2 infections in hospitalized case-patients beginning in August 2016 in Hong Kong, China. We also provide early evidence that this emergent GII.2 variant might target a wider age population than that targeted by circulating pandemic GII.4 strains.

The Study

Since August 2012, we have conducted an ongoing molecular surveillance of norovirus genotype distribution in all hospitalized gastroenteritis patients in our teaching hospital in Hong Kong (*6*). We admitted patients on the basis of clinical severity at presentation and routinely tested for norovirus on the basis of clinical suspicion of viral gastroenteritis. We collected stool samples and tested them for norovirus by using a 1-step quantitative reverse transcription PCR assay (*6*). We then subjected norovirus RNA–positive samples to genotyping that targeted the 5′ end (region C) of the viral protein 1 (VP1) gene as previously described (*6*). After Sanger-sequencing amplicons, we assigned norovirus genotypes by using the RIVM online norovirus genotyping tool (http://www.rivm.nl/mpf/norovirus/typingtool).

During July 2016–February 2017, we collected 399 norovirus RNA–positive stool samples from 393 patients. The median patient age was 2 years (interquartile range [IQR] 1–15 years). The female-to-male ratio was 1.04:1. We successfully genotyped 357 (90.8%) samples. The top 3 circulating VP1 genotypes during the study period were GII.4 (n = 214 [54.5%]), GII.2 (n = 86 [21.9%]), and GII.3 (n = 16 [4.1%]). Before this season, GII.2 had been a rare genotype, accounting for <1% of total strains circulating locally (*6*) and <1.5% of those circulating globally (*7*). However, we observed a rapid increase in the number of GII.2 cases starting in August 2016 (Figure 1, panel A). The number and proportion of GII.2 cases increased from 1 (2.7%) in August 2016 to 35 (64.8%) in February 2017. In contrast, the percentage of GII.4 cases decreased from 71.4% in July 2016 to 5.6% in February 2017. By January 2017, GII.2 had outnumbered GII.4 as the most predominant GII genotype detected in our surveillance.

We determined partial GII.2 VP1 gene sequences (1,322 nt in length) from the samples of 20 case-patients (GenBank accession nos. KY421044, KY677828–KY677833, and KY817742–KY817754) as previously described (*8*). We performed neighbor-joining phylogenetic inference by using MEGA 6.0 (http://www.megasoftware.net) (Figure 2, panel A). Tree topology showed that the surge of GII.2 infections in the winter of 2016–2017 in Hong Kong coincided with the emergence of a genetically distinct cluster that was different from other strains detected in Japan and Europe before 2016. Although we did not have epidemiologic data for our case-patients, formation of different subclusters in the neighbor-joining tree indicated a high genetic diversity, suggesting that emergence of GII.2 was unlikely to be an outcome of a point source outbreak. Instead, stepwise topology indicated frequent person-to-person transmission events.

The GII.2 strains we identified clustered most closely with the recombinant GII.P16-GII.2 strains from Germany

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during the same period (Figure 2). Subsequent genotyping of the RNA-dependent RNA polymerase gene in 28 (33%) of the case-patients indicated that all of them were infected with strains belonging to the GII.P16 genotype.

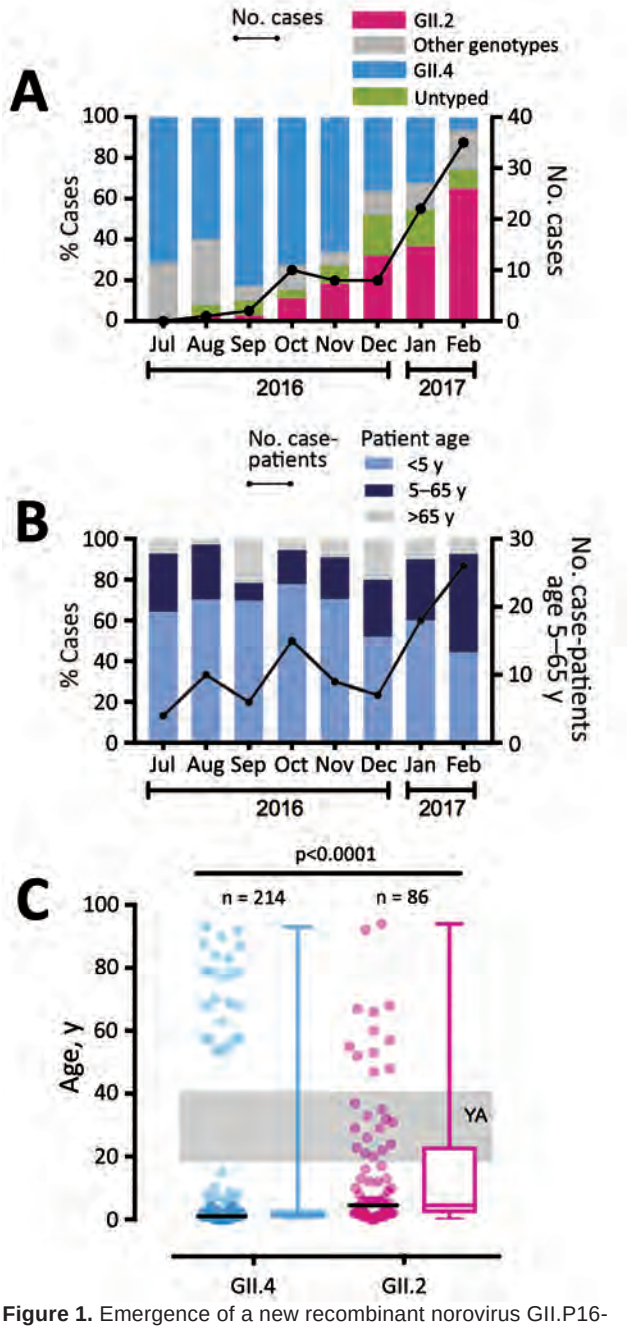

Figure 1. Emergence of a new recombinant norovirus GII.P16-GII.2, Hong Kong, China, winter 2016–2017. A) Distribution of norovirus genotypes, July 2016–February 2017. B) Proportion of norovirus case-patients among 3 stratified age groups. C) Age distribution of hospitalized case-patients with GII.4 and GII.2 infections. A total of 214 GII.4 and 86 GII.2 case-patients are shown. Black horizontal lines represent medians. Gray shading denotes young adults (YA; 18–40 years of age). p value calculated by using Mann-Whitney *U*-test.

We dual-genotyped specimens collected from an additional 8 GII.2-infected case-patients during January–February 2017 by using single amplicons covering partial RNA-dependent RNA polymerase and VP1 gene regions (GenBank accession nos. KY817734–KY817741). Phylogenetic analysis confirmed that our strains belonged to the recombinant GII.P16-GII.2 variant and were not an artifact of co-infections with 2 different norovirus genotypes (Figure 2, panel B).

Epidemiologic studies have shown that norovirus GII.4 and GII.2 infections more commonly occur in young children (*9*,*10*). However, this new GII.P16-GII.2 variant might target wider age groups. We observed an increasing trend of hospitalized older children and adults (i.e., persons 5–65 years of age); 30% of all January 2017 cases and 48% of all February 2017 cases of GII.2 infection occurred in patients from this age group (Figure 1, panel B). The median age of GII.2 case-patients was significantly higher than that of GII.4 case-patients (5 years [IQR 2–23 years] vs. 1 year [IQR 1–3 years]; p<0.0001 by Mann-Whitney *U*-test) (Figure 1, panel C). The proportion of older children and adults 5–65 years of age, an age group that previously had been less commonly seen with severe norovirus infections, was significantly higher among GII.2 case-patients than among in GII.4 case-patients (44% vs. 9%; p<0.0001 by Fisher exact test). More important, we observed 13 cases of GII.2 infections in young adults 18–40 years of age but no GII.4 infections in this age group (Figure 1, panel C). Among young adults 20–39 years of age, GII.2 incidence was higher than GII.4 incidence (4 vs. 0 cases/100,000 population) (online Technical Appendix Figure, https://wwwnc.cdc.gov/EID/article/23/11/17-0561-Techapp1.pdf).

We noted a gradual narrowing in the age distribution of GII.4 infections to young children <5 years of age in the past 5 seasons (Table), presumably a result of herd immunity development, given that the current GII.4 Sydney 2012 variant has been circulating for >4 years. Part of the observed differential age distribution between GII.2 and GII.4 case-patients might be attributed to the changing epidemiology of GII.4.

Conclusions

We report the emergence of a recombinant norovirus GII.P16-GII.2 variant that surpassed the previously predominant genotype GII.4 in hospitalized acute gastroenteritis case-patients in the winter of 2016–2017 in Hong Kong. However, unlike the recently emerged epidemic GII.17 Kawasaki variant that predominated only in part of Asia (China and Japan) during 2014–2016 (*12*,*13*), this new recombinant GII.P16-GII.2 variant also caused a steep rise in gastroenteritis cases in Asia (*2*,*3*) and Europe (*4*,*5*), indicating that it was geographically widespread across continents. We observed an increase in proportion, number, and

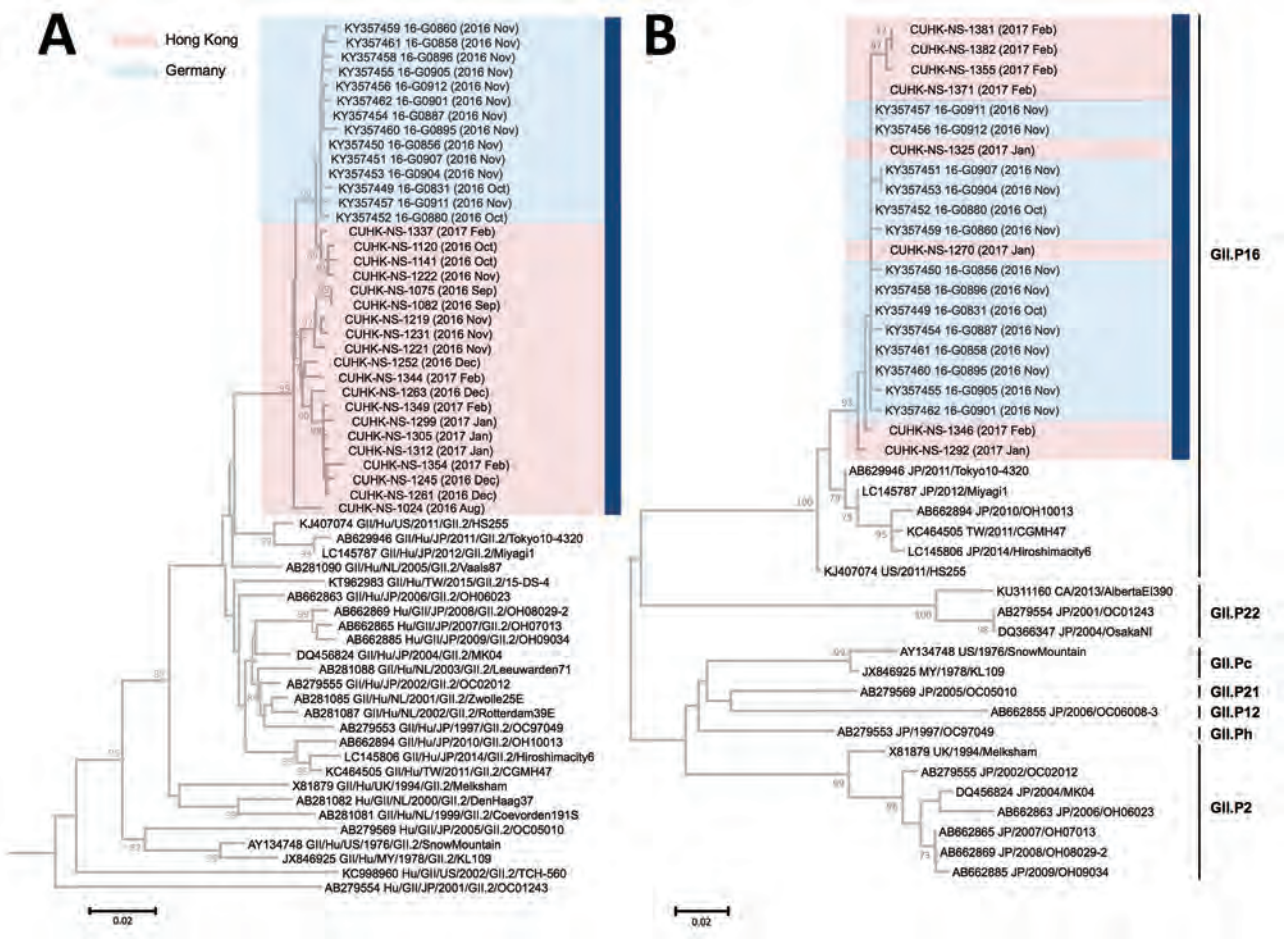

Figure 2. Neighbor-joining phylogenetic analysis of partial A) VP1 (1,322 nt) and B) RdRp (235 nt) gene sequences of norovirus genogroup II genotype 2 (GII.2) detected by molecular surveillance at a teaching hospital, Hong Kong, China, July 2016–February 2017. The trees were constructed by using Kimura-2-parameter distance method with 1,000 bootstrap replicates. Bootstrap values ≥70 (percentage) are shown at nodes. Blue bar indicates winter of 2016–2017. Pink shading denotes sequences obtained in this study. Blue shading denotes sequences from Germany during the same period. Year and month of strain collection are shown in parentheses. The VP1 tree is rooted to a genotype GII.5 strain (GII/Hu/GF/1978/GII.5/C15) (not shown), and the RdRp tree is mid-point rooted. Sequences shown in the RdRp tree were obtained from dual-typing of 8 single amplicons. All RdRp genotypes known to recombine with GII.2 VP1 are included in the RdRp tree. Scale bars are drawn to scale and indicate numbers of nucleotide substitutions per site. RdRp, RNA dependent RNA polymerase; VP1, viral protein 1.

incidence of hospitalized GII.2 case-patients in the 5–65-year age group. A similar shift in age distribution was also reported during the emergence of norovirus GII.17 Kawasaki variant in Hong Kong and Shanghai in the winter of 2014–2015 (*8*,*14*). This shift suggests a lack of preexisting herd immunity that provided a specific immunologically naive ecologic niche for this emergent norovirus to become an epidemic or even pandemic variant. A recent phylogenetic study showed that the evolution of GII.2 VP1 gene was relatively static in the past 40 years and suggested that, other than antigenicity, genetic changes in nonstructural proteins might play a role in the emergence of this recombinant GII.2 (*15*).

Table. Age distribution of hospitalized patients with norovirus GII.4 infections, by season, Hong Kong, China, 2012–2017

Seasons	Median age, y (IQR)	References
2012–13	3 (1–74)	(*11*)
2012–13, 2013–14	2 (1–60)	(*6*)
2014–15	1 (1–8)	(*8*)
2015–16	2 (1–4)	Unpublished†
2016–17	1 (1–3)	This study

*IQR, interquartile range.
†Chan MC. Molecular surveillance of norovirus in Hong Kong. Unpublished raw data; 2017.

Our study is limited by a small sample size, short study period, single-site setting, and lack of clinical severity evaluation. However, the infections in our hospitalized case-patients represented the severe end of the spectrum of disease, and to frequently encounter norovirus gastroenteritis in young adults in such a setting is unusual. We provide early evidence that this emergent

norovirus targets an age population wider than circulating pandemic GII.4 strains do and can cause severe infections (i.e., resulting in hospitalization) apart from causing outbreaks. Collectively, these findings might have important implications for norovirus vaccine formulation and vaccination strategy. Close monitoring of the global spread of GII.P16-GII.2 is warranted.

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P.K.S.C. and M.C.W.C. conceived the study. M.C.W.C. designed and coordinated the study. S.N., N.L., S.J., E.A.S.N., T.F.L., and R.W.M.L. coordinated sample collection; K.K., T-N.H., and L.-Y.C. performed experiments. K.K. and M.C.W.C. analyzed data and drafted the manuscript. All authors critically reviewed and commented on the manuscript prior to submission.

Ms. Kwok is currently an MPhil student in the Department of Microbiology at the Chinese University of Hong Kong. Her research interests include viral genomic epidemiology, pathogenesis, and surveillance.

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Emergence of *Bordetella holmesii* as a Causative Agent of Whooping Cough, Barcelona, Spain

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We describe the detection of *Bordetella holmesii* as a cause of whooping cough in Spain. Prevalence was 3.9% in 2015, doubling to 8.8% in 2016. This emergence raises concern regarding the contribution of *B. holmesii* to the reemergence of whooping cough and the effectiveness of the pertussis vaccine.

Whooping cough is a highly contagious respiratory disease, primarily caused by *Bordetella pertussis* (*1*). Other species, such as *B. parapertussis* and *B. holmesii*, have been recognized as causes of a syndrome that clinically resembles that of whooping cough (*1*,*2*). Pertussis is the term used for the disease specifically caused by *B. pertussis*, whereas pertussis-like illness or syndrome is more appropriately used when referring to the other etiologic agents. *B. holmesii*, a poorly studied pathogen, was originally identified in 1995 as a rare cause of bacteremia (*3*). Since then, it has been related to other invasive diseases, especially in asplenic and immunosuppressed patients and in healthy people with pertussis-like symptoms (*4*).

Microbiologic diagnosis of whooping cough by molecular tests provides a higher sensitivity and promptness than culture techniques, with PCR being the method most commonly used in clinical laboratories (*5*). Most molecular diagnostic kits used to detect *B. pertussis* target insertion sequence IS*481*, which is present in high copy numbers in the *B. pertussis* genome (*6*). However, IS*481* is not a specific target of *B. pertussis* because it is also found in other *Bordetella* species, including *B. holmesii*, leading to underestimation of this pathogen in this clinical scenario (*6*).

To date, several cases of *B. holmesii* associated with pertussis-like illness have been reported in North and South America, Asia, Africa, and Europe (*4*). Additionally, 2 important outbreaks of *B. holmesii* infection associated with pertussis-like illness were detected in France and Ohio (*7*,*8*). Recent reports of the detection of positive cases of *B. holmesii* infection in the Netherlands (*9*), which previous analysis had failed to identify (*10*), reinforce the emergence of this pathogen. To our knowledge, the presence of this microorganism in Spain has not been documented. We report the emergence of *B. holmesii* as a causative agent of whooping cough in the metropolitan area of Barcelona, Spain.

The Study

We evaluated 391 nasopharyngeal samples from patients from the metropolitan area of Barcelona who had a clinical and laboratory-confirmed diagnosis of whooping cough during January 2013–December 2016 at the Hospital Vall d'Hebron. All the samples were positive by the IS*481*-based SmartBp/Bpp (Cepheid, Sunnyvale, CA, USA) real-time PCR and thus were considered positive for *B. pertussis*.

We reevaluated all the samples by using species-specific multiplex real-time PCR (*10*). This method detects the promoter of the pertussis toxin operon (*ptxAPr*), which is specific for *B. pertussis*, and the *recA* gene (*Bh-RecA*), specific for *B. holmesii*. To corroborate the identification of *B. holmesii*, we further analyzed all the *Bh-RecA* RT-PCR–positive samples by sequencing an internal fragment of the housekeeping gene encoding the ribonucleoside-diphosphate reductase α chain (*nrdA*), which is useful for discriminating among the different species of *Bordetella* (*11*), and the *Bh-RecA* gene. The study was approved by the Clinical Research Ethics Committee of the hospital.

Among the 391 nasopharyngeal samples analyzed, 380 (97.2%) were confirmed positive for *B. pertussis* and 16 (4.1%) for *B. holmesii*. Among the *B. holmesii*–positive samples, 5 were positive for *B. pertussis* and *B. holmesii* and 1 for *B. parapertussis*, *B. holmesii*, and *Streptococcus pyogenes* (Figure).

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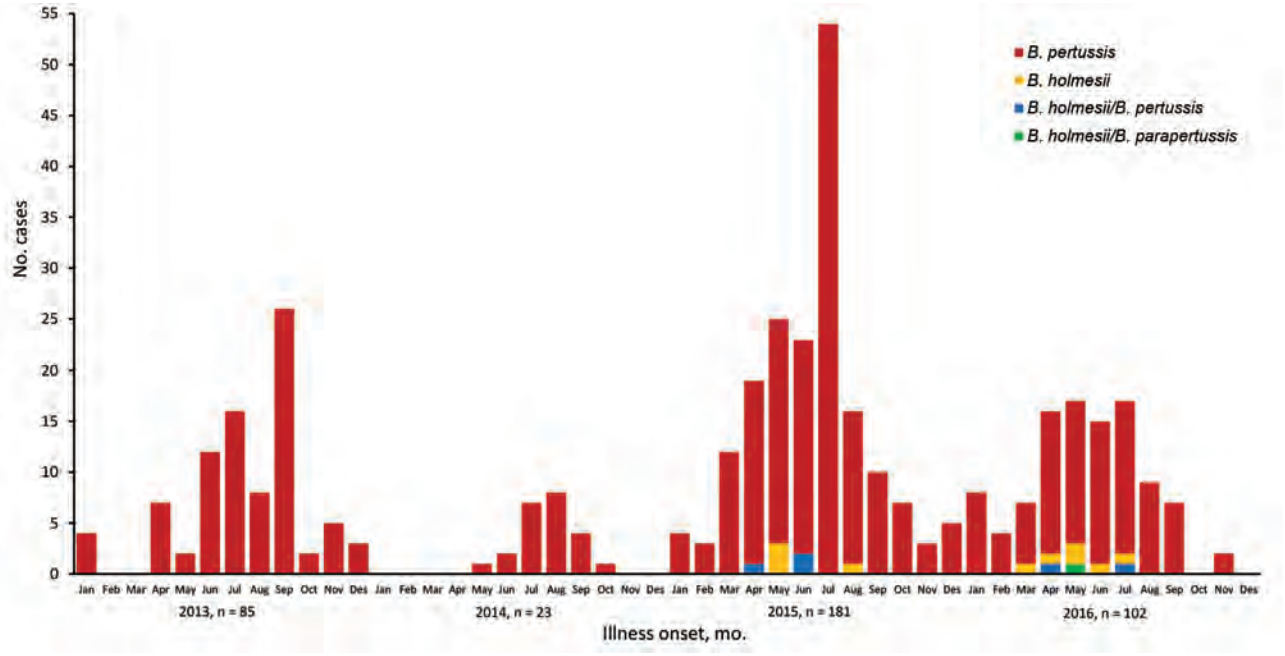

Figure. Timeline distribution of the 391 whooping cough cases diagnosed at the Hospital Vall d'Hebron, Barcelona, Spain, 2013–2016, showing *Bordetella* species detected.

None of the *B. holmesii*–positive cases was detected during 2013–2014. In total, 7 cases were reported in 2015, corresponding to 3.9% of whooping cough cases diagnosed in 2015, and the remaining 9 cases were reported in 2016, accounting for 8.8% of the cases diagnosed during that year (Figure).

Ten (62.5%) of the 16 *B. holmesii*–positive patients were female; the median age was 9 years (range 1–40 years), and 87.5% were pediatric patients (<14 years). Fourteen cases were detected in the context of a school-related (85.7%) or family (35.7%) outbreak; 3 of these cases were detected in both kinds of outbreaks.

Vaccination status was available for 14 of the 16 patients. Of these, all cases occurred in children 14 months to 14 years of age who had received a median of 5 doses of pertussis vaccine (range 2–5 doses) according to the current vaccination program (5 doses, administered at 2, 4, and 6 months and at 1.5 and 6 years of age). The median time since the last vaccination was 4.5 years (range 0.7–14.1 years) (Table 1). No cases of complications or malignant pertussis-like disease occurred. Information about antimicrobial therapy received was available for 15 patients, all of whom had been treated with

Table 1. Demographic, clinical, and epidemiologic characteristics of 16 patients with diagnosed whooping cough associated with *Bordetella holmesii* infection, Hospital Vall d'Hebron, Barcelona, Spain, 2015–2016*

Patient no.	Age, y/sex	No. vaccine doses received	Date last vaccine dose received	Diagnosis date	Treatment	Co-infections	Outbreak relatedness	Site of exposure
1	10/F	5	2010 Mar 16	2015 Apr 17	AZM	*B. pertussis*	Yes	School
2	12/F	5	2008 Jul 18	2015 May 5	AZM	ND	Yes	School†
3	9/F	5	2011 Dec 12	2015 May 13	AZM	ND	Yes	School†
4	13/F	5	2007 Oct 23	2015 May 25	AZM	ND	Yes	School†
5	12/F	5	2008 Sep 6	2015 Apr 6	AZM	*B. pertussis*	Yes	School
6	28/F	UNK	UNK	2015 Apr 6	AZM	*B. pertussis*	Yes	Home
7	4/F	4	2012 Mar 8	2015 Aug 14	AZM	ND	Yes	School and home
8	9/M	5	2012 Oct 18	2016 Sep 3	UNK	ND	Yes	School
9	1/M	2	2015 Jun 8	2016 April 13	AZM	*B. pertussis*	Yes	Home
10	8/M	5	2012 Jun 26	2016 Apr 21	AZM	ND	Yes	School and home
11	6/M	4	2011 Aug 8	2016 Mar 5	AZM	*B. parapertussis/ S. pyogenes*	Yes	School
12	40/F	UNK	UNK	2016 Sep 5	AZM	ND	UNK	UNK
13	14/F	3	2002 Aug 5	2016 May 24	AZM	ND	No	–
14	5/F	4	2012 Mar 2	2016 Sep 6	AZM	ND	Yes	School and home
15	9/M	5	2013 Sep 10	2016 Nov 7	AZM	ND	Yes	School
16	6/M	4	2011 Feb 16	2016 Jul 28	AZM	*B. pertussis*	Yes	School

*AZM, azithromycin; ND, not detected; UNK, unknown.
†These 3 patients' illnesses were related to the same school outbreak.

Table 2. Comparison of demographic, vaccination-related, and clinical characteristics between patients with *Bordetella pertussis* and *B. holmesii* infection, Hospital Vall d'Hebron, Barcelona, Spain, 2015–2016*

Characteristic	*B. pertussis*, n = 40	*B. holmesii*, n = 10	p value
Median age (range), y	5.5 (0.08–74)	9 (4–40)	0.07
Median pertussis vaccine doses received (range)	4 (0–5)	5 (3–5)	0.21
Median time from last pertussis vaccine dose received to date of diagnosis (range), y	1.92 (0.08–11.70)	3.82 (1.03–14.05)	0.1
Fever, no. (%)	5 (12.5)	1 (10)	1
Whoop, no. (%)	9 (22.5)	1 (10)	0.66
Paroxysms, no. (%)	4 (10)	1 (10)	1
Cough ≥14 d, no. (%)	12 (30)	4 (40)	0.7
Hospitalized, no. (%)	4 (10)	0	0.57

*Differences were assessed for significance using the chi-squared exact test (in comparison with independent qualitative variables) and the Mann-Whitney *U*-test (for quantitative variables; no normality was observed in data distribution). We selected a randomized sample of confirmed *B. pertussis* cases with a 4:1 relation with *B. holmesii*–infected patients as a comparison group. p values <0.05 were considered statistically significant at the 95% CI level.

azithromycin, and no patient experienced therapeutic failure or relapse.

No statistical differences were observed between age, clinical features, and vaccination status among the case-patients with *B. holmesii* and *B. pertussis* infections (Table 2). However, *B. holmesii* infections tended to be more prevalent in older children (median age 9 vs. 5.5 years; p = 0.07) compared with *B. pertussis* infections.

Conclusions

B. holmesii is an underdiagnosed emerging respiratory pathogen that triggers clinical manifestations similar to those caused by *B. pertussis* (*1*). In this retrospective study, we detected 10 cases in which *B. holmesii* was found to be the only putative agent of a pertussis-like infection and 6 cases in which *B. holmesii* was co-detected with another causative agent of whooping cough. We observed no differences in the demographics, clinical features, and vaccination status among patients infected by *B. holmesii* and *B. pertussis*, but a trend toward higher involvement of *B. holmesii* infections was observed in older children, as reported previously (*7*,*8*).

We found that 4.1% of the respiratory samples from patients with laboratory-confirmed whooping cough during 2013–2016 were positive for *B. holmesii*, for which detection was reported from April 2015 onward. The number of positive cases of *B. holmesii* infection doubled from 3.9% in 2015 to 8.8% in 2016. Of note, 2015 was considered the year with the highest incidence of whooping cough since the introduction of the acellular vaccine in Spain. In the autonomous community of Catalonia, incidence (cases/100,000 inhabitants) was 13.3 for 2013, 14.8 for 2014, 48.9 for 2015, and 24.6 for 2016 (http://canalsalut.gencat.cat/ca/actualitat/llista_butlletins/salut_publica/butlleti_epidemiologic_de_catalunya).

Even in the absence of clear recommendations to treat pertussis-like respiratory infections caused by *B. holmesii*, several studies have reported controversial results about a possible lower activity of macrolides compared with other antimicrobial agents (*4*,*12*). Unfortunately, because we could not recover the bacterial isolates, we were unable to perform antimicrobial drug susceptibility testing. However, no evidence of complications or relapses was observed in any patient after treatment with azithromycin.

B. holmesii lacks most of the antigens present in the pertussis acellular vaccine or the proteins produced differ phenotypically (*4*). This situation, together with the lack of protection against replication observed in immunized mice (*13*), suggests the absence of cross-protection against *B. holmesii* infections. In our study, most of the patients had received the complete immunization schedule of 5 doses (Table 1). Thus, the increasing trend of whooping cough might be attributed not only to *B. pertussis* adaptation to the introduction of the acellular pertussis vaccine, decreased vaccine efficacy, or waning immunity, as previously reported (*14*,*15*), but also to the emergence of secondary pathogens, such as *B. holmesii*, which the pertussis vaccine might not prevent.

Our study describes the emergence of *B. holmesii* as a causative agent of whooping cough in Spain. Accurate diagnosis of the causative agent of this disease is crucial to determine the real incidence and prevalence of the microbial species involved, to assess its contribution to the epidemiology of whooping cough, to evaluate whether specific antimicrobial drug treatments should be implemented and, in terms of public health, to assess the efficacy of the pertussis vaccine.

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Highly Pathogenic Avian Influenza A(H7N9) Virus, Tennessee, USA, March 2017

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In March 2017, highly pathogenic avian influenza A(H7N9) was detected at 2 poultry farms in Tennessee, USA. Surveillance data and genetic analyses indicated multiple introductions of low pathogenicity avian influenza virus before mutation to high pathogenicity and interfarm transmission. Poultry surveillance should continue because low pathogenicity viruses circulate and spill over into commercial poultry.

In early March 2017, concurrent outbreaks of highly pathogenic avian influenza (HPAI) and low pathogenicity avian influenza (LPAI) A(H7N9) were occurring at poultry farms in Tennessee, USA. The first report of high loss due to death was received from a commercial broiler breeder facility in Lincoln County, Tennessee. The facility contained 74,000 chickens in 6 houses, but only 1 house was affected. Signs of disease included respiratory distress and increased death. Two days after disease onset, the number of dead birds increased from 50 to 500 within 24 hours, and oropharyngeal swab samples tested positive by real-time reverse transcription PCR (rRT-PCR) for the matrix and H7 genes at the C. E. Kord Animal Health Diagnostic Laboratory (Nashville, Tennessee). Samples were forwarded to the National Veterinary Services Laboratories of the US Department of Agriculture (Ames, Iowa, USA) for confirmation, and depopulation of the flock was initiated. The next day, the virus was confirmed as H7N9 HPAI virus by sequencing. A second HPAI-positive broiler breeder flock of 55,000 birds was identified in the control zone <10 days after identification of the first site; this site also had only 1 house affected. During the time between the 2 HPAI virus detections, H7N9 LPAI was confirmed in a different broiler breeder flock in Giles, a neighboring Tennessee county (online Technical Appendix Figure 1, https://wwwnc.cdc.gov/EID/article/23/11/17-1013-Techapp1.pdf). Subsequently, additional commercial and backyard flocks in Alabama, Kentucky, and Georgia were identified to have H7N9 LPAI through zone and routine surveillance. To trace the origin and understand their genetic relationship, we performed whole-genome sequencing and comparative phylogenetic analysis of the available H7N9 viruses identified in Tennessee and Alabama.

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The Study

We sequenced the complete genomes of 12 HPAI viruses (7 from the first and 5 from the second site), 3 LPAI viruses from Giles County, and 7 LPAI viruses from Alabama by next-generation sequencing (online Technical Appendix Table). We generated maximum-likelihood phylogenies by using RAxML (*1*) and Bayesian relaxed clock phylogenies by using BEAST version 1.8.3 (*2*). After removing the insertion sequences at the hemagglutinin (HA) cleavage sites from HPAI viruses, we concatenated all available whole-genome sequences and analyzed them by using the median-joining method implemented by NETWORK version 5.0 (online Technical Appendix) (*3*).

H7N9 HPAI and LPAI viruses from North America were genetically distinct from those from China, which have been infecting poultry and humans since 2013 (online Technical Appendix Figure 2). All H7N9 viruses shared high levels of nucleotide identity (>99.2%–99.7%) across all 8 gene segments except for the insertion at the HA cleavage site. Insertion sequences, thought to be critical for the HPAI phenotype, elongate the proteolytic cleavage site, making HA more susceptible to cleavage by ubiquitous proteases. The insertion sequence at the HA cleavage site (PENPKTDRKSRHRRIR/G, insertion sequence is underlined) in H7 viruses had 100% sequence homology to chicken 28S rRNA (GenBank accession no. AC147447.3), suggesting the mutation occurred during virus replication in chickens. Similar events of chicken rRNA insertion have been reported in the North America H7N3 virus lineage in poultry (*4*). All previously reported H7 HPAI viruses had insertions near the HA cleavage site ranging from 6 to 54 nt (*5*).

An H7N9 LPAI closely related to the Tennessee and Alabama H7N9 viruses was previously detected on September 7, 2016, in a Wildlife Services wild bird surveillance pooled oropharyngeal and cloacal swab sample collected from a blue-winged teal in Goshen County, Wyoming, geographically located within the North American Central Migratory Flyway. The LPAI A/blue-winged teal/Wyoming/AH0099021/2016(H7N9) virus (BWT/WY/2016) shared

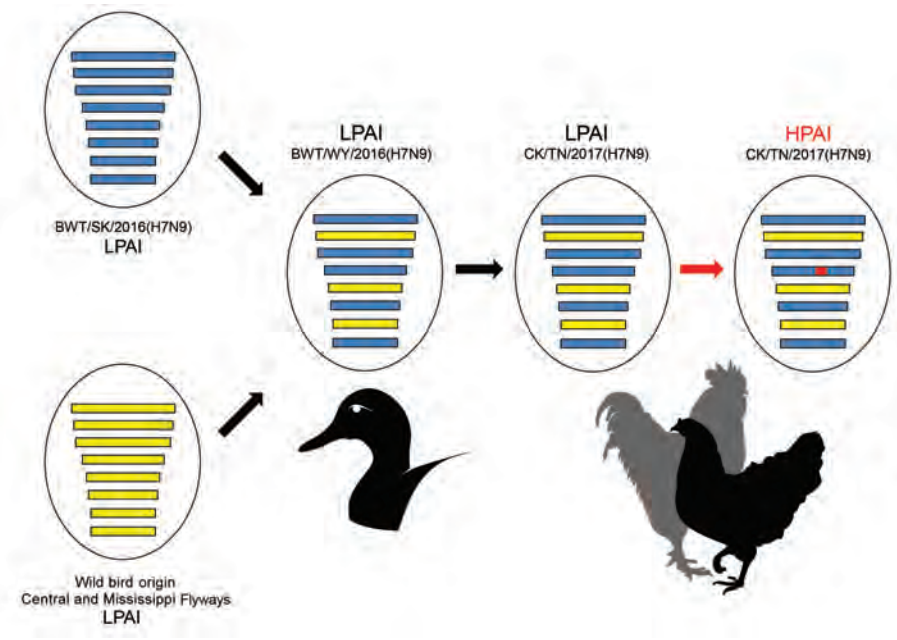

Figure 1. Genome constellation of influenza A(H7N9) viruses. Viruses are represented by ovals containing horizontal bars that represent the 8 influenza gene segments (from top to bottom: polymerase basic 2, polymerase basic 1, polymerase acidic, hemagglutinin, nucleoprotein, neuraminidase, matrix, and nonstructural). A genome reassortment event between the H7N9 virus from Saskatchewan, Canada (blue segments) and viruses from wild birds of the US Central and Mississippi Migratory Flyways (yellow segments) led to the genome assortment present in the Wyoming LPAI virus (BWT/WY/2016). The genome constellation of BWT/WY/2016 is the same as those of the Tennessee H7N9 viruses. A red bar in the hemagglutinin gene of the HPAI virus indicates the insertion at the hemagglutinin cleavage site. HPAI, highly pathogenic avian influenza; LPAI, low pathogenicity avian influenza.

a high level of nucleotide identity (>99.1%–99.8%) with the H7N9 outbreak strain across all 8 gene segments and appeared to be a precursor of the H7N9 HPAI virus that caused the outbreak among poultry (Figure 1; online Technical Appendix Figures 3, 4). Surveillance in Canada led to the identification of a similar virus, A/blue-winged teal/Saskatchewan/109–701/2016(H7N9) (BWT/SK/2016), on August 12, 2016. In phylogenetic analyses, 5 genes (polymerase basic 2, polymerase acidic, HA, neuraminidase, nonstructural) from BWT/WY/2016, BWT/SK/2016, and the H7N9 outbreak isolates clustered together (online Technical Appendix Figure 2, panels A–C, E, H). The polymerase basic 1, nucleoprotein, and matrix genes of BWT/WY/2016 and H7N9 outbreak isolates were derived from a variety of different North America lineage LPAI viruses circulating in the Central and Mississippi Migratory Flyways during 2016 (online Technical Appendix Figure 2, panels D, F, G).

A previous report suggests that the genomes of North America wild bird lineage influenza A viruses are evolving through a remarkably high rate of genome reassortment, forming transient genome constellations that rapidly change with no apparent pattern of gene segment association (*6*). In contrast, a high level of nucleotide identity among all segments of the BWT/WY/2016 and H7N9 outbreak isolates (online Technical Appendix Figure 4) suggests that this genome constellation might have been maintained in the wild bird population since its emergence in 2016, with subsequent dissemination to poultry in the southeastern states in 2017.

The median-joining phylogenetic network analysis suggests separate introductions occurred from a common source into Tennessee and Alabama (Figure 2). The estimated time to most recent common ancestor of the HA genes was October 5, 2016 (95% Bayesian credible interval August 8, 2016–December 10, 2016) (Table), corresponding with the waterfowl fall migration season and time of BWT/WY/2016 sample collection. The LPAI viruses identified at Alabama broiler breeder farms were probably introduced separately. The network analysis also suggested that the HPAI and LPAI viruses identified at chicken farms in Tennessee probably originated from a common LPAI virus, and a single mutational event led to the HPAI virus that was detected at the first farm and

Table. Times to most recent common ancestors of H7N9 viruses, Tennessee and Alabama, USA, March 2017*

Virus clusters (no. taxa)†	tMRCA‡	BCI 95%	Posterior probability
Tennessee and Alabama LPAI and HPAI viruses (22)	2016 Oct 5	2016 Aug 8–2016 Dec 10	1.00
Tennessee LPAI and HPAI viruses (15)	2016 Dec 21	2016 Nov 7–2017 Feb 3	1.00
Tennessee LPAI virus (3)	2017 Jan 23	2016 Dec 3–2017 Feb 25	0.98
Tennessee HPAI virus (12)	2017 Jan 30	2017 Jan 1–2017 Feb 25	1.00
Alabama LPAI virus from backyard guinea fowl (3)	2017 Jan 8	2016 Nov 18–2017 Feb 21	1.00
Alabama LPAI virus from commercial chicken (3)	2017 Jan 26	2016 Dec 10–2017 Mar 8	1.00

*BCI, Bayesian credible interval; HPAI, highly pathogenic avian influenza; LPAI, low pathogenicity avian influenza; tMRCA, time to most recent common ancestor.
†See online Technical Appendix Table (https://wwwnc.cdc.gov/EID/article/23/11/17-1013-Techapp1.pdf) for specific virus strains used in analysis.
‡Times to most recent common ancestors of hemagglutinin segment.

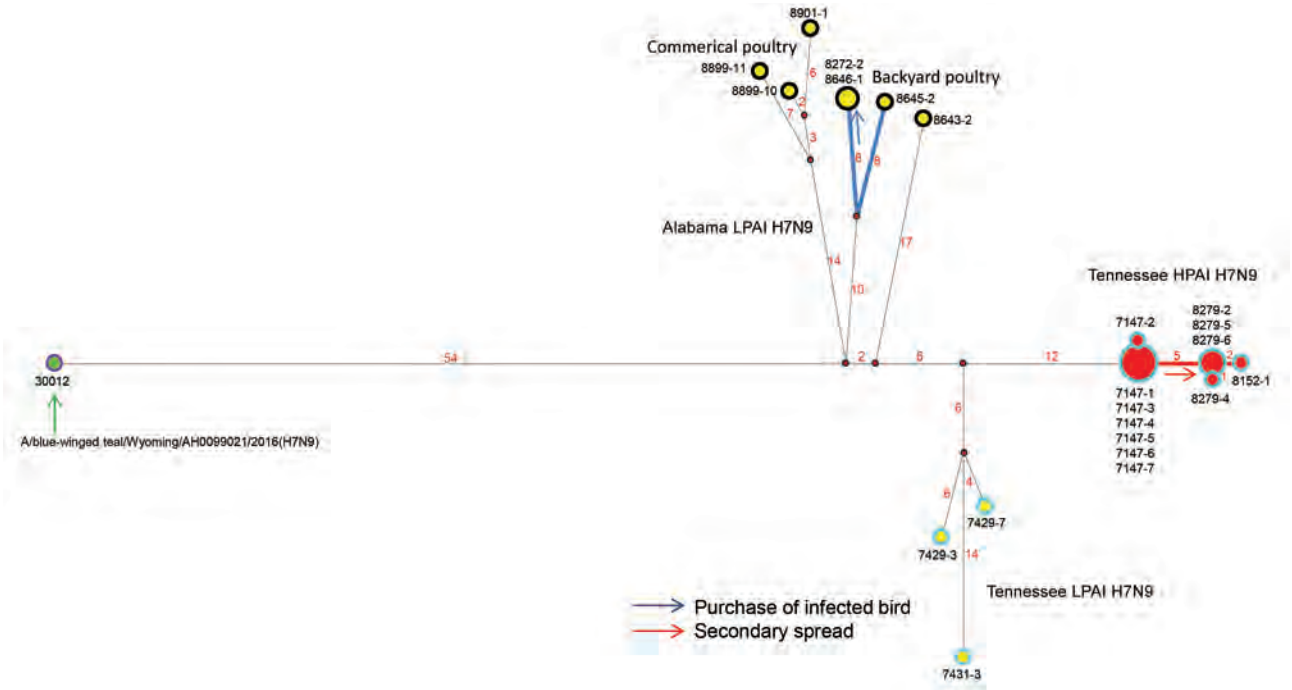

Figure 2. Median-joining phylogenetic network of influenza A(H7N9) viruses, United States, 2017. The median-joining network was constructed from concatenated H7N9 virus genomes containing all 8 segments. This network includes all the most parsimonious trees linking the sequences. Each unique sequence is represented by a circle sized relative to its frequency in the dataset. Isolates are colored according to the sample: red inner circle represents HPAI in poultry, yellow inner circle represents LPAI in poultry, green inner circle represents LPAI in a wild bird, purple outer circle represents isolates from Wyoming, black outer circle represents isolates from Alabama, and sky-blue outer circle represents isolates from Tennessee. Bold lines indicate farm-to-farm transmission verified by epidemiologic investigations. Red numbers indicate number of nucleotide changes between isolates. Black numbers are abbreviated isolate names. HPAI, highly pathogenic avian influenza; LPAI, low pathogenicity avian influenza.

subsequently spread to the second (Figure 2). An epidemiologic investigation revealed that delayed carcass disposal at the first site with HPAI virus might have facilitated transmission to the second and that the purchase of an infected guinea fowl caused transmission of an LPAI virus between 2 sites in Alabama. The 8-gene network analysis suggested the potential for ≥3 separate virus introductions: 1) LPAI in Alabama commercial poultry, 2) LPAI in backyard Alabama poultry, and 3) LPAI and HPAI in Tennessee commercial poultry. Bayesian phylogenetic analyses indicated high posterior probabilities (>0.98) for each of the clusters of viruses in these introductions; the time to most recent common ancestor of each of these 3 clusters was December 2016–January 2017, corresponding to the wintering season for wild birds in this area (Table). The distribution of cases across 4 states and discovery of antibody-positive flocks with low or no viral RNA identified during routine and zone surveillance during March 2017 together with the phylogenetic analysis indicating a theoretical poultry precursor and variability in viruses from the same farm suggest that the H7N9 virus circulated in the area undetected in poultry before the initial HPAI virus detection.

Conclusions

Whole-genome sequencing and comparative genetic analyses of all available sequences of the North America wild bird H7N9 lineage suggest that the virus in the Wyoming blue-winged teal represents a precursor to the poultry viruses in the southeastern United States and that the mutation from an LPAI virus to an HPAI virus occurred in poultry. Our data suggest that the virus circulated in the region undetected in poultry before the initial HPAI virus detection and that ≥3 separate virus introductions occurred. These findings highlight the need for routine and frequent testing of poultry for avian influenza virus because reportable LPAI viruses might circulate without causing any clinical signs.

Dr. Lee is a postdoctoral researcher at the Southeast Poultry Research Laboratory in Athens, Georgia, USA. His research interests include molecular epidemiology and host–pathogen interactions of avian influenza viruses.

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Mycobacterium lepromatosis Lepromatous Leprosy in US Citizen Who Traveled to Disease-Endemic Areas

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We report *Mycobacterium lepromatosis* infection in a US-born person with an extensive international travel history. Clinical symptoms, histopathology, and management are similar to those of infections caused by *M. leprae*. Clinicians should consider this pathogen in the diagnosis of patients with symptoms of leprosy who have traveled to endemic areas.

A 59-year-old white man born in the United States came to the Travel and Tropical Medicine Clinic at Mayo Clinic (Rochester, Minnesota, USA) in March 2017 reporting 12 months of progressive skin lesions and prior onset of peripheral neuropathy and arthritis. The lesions started on his forearms, then progressed to nearly diffuse involvement of his face, neck, ears, and trunk. The lesions were not pruritic, painful, or ulcerative. Two years earlier, arthralgia of his hips, knees, and hands led to a diagnosis of rheumatoid arthritis on the basis of positive results for rheumatoid factor, negative cyclic citrullinated peptide antibody, and antinuclear antibodies. He received prednisone for several weeks, then methotrexate (20 mg/wk). He also had an electromyography-confirmed sensorimotor peripheral neuropathy that began 1 year before this presentation. After an unsuccessful empiric course of topical corticosteroid therapy, skin biopsies were collected from his neck and forearm; results showed sheets of histiocytes and scattered well-formed granulomas with perineural involvement. Fite stain was positive for numerous intracellular acid-fast bacilli, leading to a diagnosis of lepromatous leprosy; the patient was referred to the Division of Infectious Diseases at Mayo Clinic for further evaluation.

The patient was an administrator who worked indoors and did not have substantive outdoor exposure. He was born and raised in the United States but had an extensive travel history as an adult to many countries over several decades, including 2 trips to the Pacific coast of Mexico (7 days each in Puerto Vallarta, Jalisco, in April 2005, and Acapulco in March 2007), a region to which *Mycobacterium lepromatosis* leprosy is endemic (*1*). He had no known exposure to a person with leprosy or to armadillos, which are known vectors for leprosy.

On physical examination, we found nearly diffuse erythema and induration of the patient's face, ears, neck (Figure 1), and chest, as well as his upper extremities, more focally involving the dorsal aspects of the forearms. He had partial loss of eyebrows bilaterally. The right auricle had a small, crusted ulcer. His nasal mucosa was thickened and nasal passages almost blocked. Ulnar and superficial cervical nerves were not easily palpated. He showed no clinical signs of active synovitis.

Initial laboratory test results were within reference ranges or negative, including complete blood count, erythrocyte sedimentation rate, C-reactive protein, creatinine, QuantiFERON-TB Gold In-Tube Test (Quest Diagnostics, Madison, NJ, USA), HIV serologies, rhumatoid factor (despite positive test 2 years prior), and cyclic citrullinated peptide antibodies. Chest radiograph results were unremarkable.

Skin biopsies of the neck, chin, and forearm showed patchy or diffuse granulomatous dermal inflammation, with foamy and epithelioid histiocytes. We found numerous acid-fast bacilli within histiocytes and invading nerves (Figure 2), highlighted by Fite, Gomori methenamine silver, and Gram stains; Ziehl-Neelsen stain highlighted only a few organisms. The combination of clinical, histopathologic, and histochemical staining features was diagnostic for multibacillary lepromatous leprosy.

We achieved a diagnosis by using the broad-range 16S ribosomal RNA gene PCR assay on the formalin-fixed, paraffin-embedded block of the chin biopsy, as follows: specimens were lysed with proteinase K, then incubated with 0.1-mm silica beads in a thermomixer at 100°C with rapid mixing. DNA was extracted from the lysate with the Genomic DNA Clean & Concentrator 10 kit (Zymo Research, Irvine, CA, USA). We used PCR with 5 µL of the DNA extract and previously described primers (*2*) on a Roche LightCycler 480 (Roche Molecular Systems Inc., Branchburg, NJ, USA) with SYBR Green stain. The PCR target is an ≈400-base-pair portion, including the V3-V4 region, of the 16S ribosomal RNA gene. The

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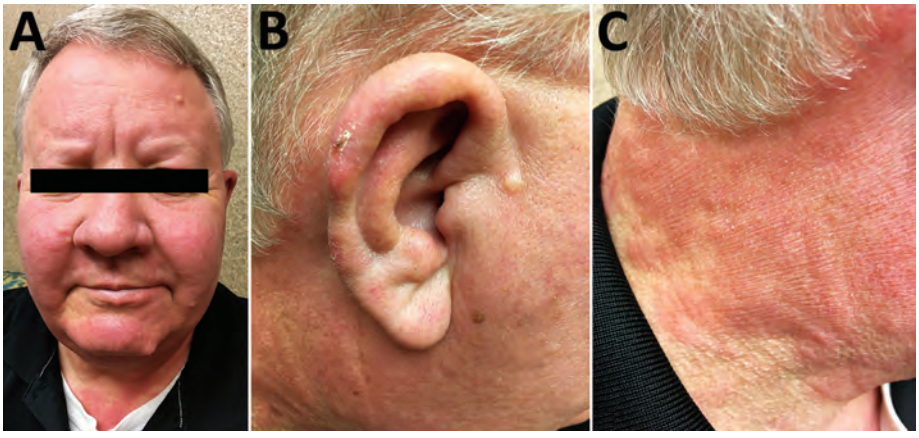

Figure 1. Signs of *Mycobacterium lepromatosis* infection in 59-year-old white male US citizen, 2017. A) *Leonine facies* with partial loss of eyebrows and nodular lesion of chin. B) Right ear nodularity with focal crusted ulceration. C) Confluent erythema from face to neck.

amplified product, which we sequenced by using Sanger sequencing, showed identical nucleotides to *M. lepromatosis* strain FJ924 (positions 368–765, GenBank accession no. EU203590) and a 3-nt difference from *Mycobacterium leprae* Br4923 (GenBank accession no. FM211192).

The presence of *M. lepromatosis* was also confirmed by using *M. lepromatosis*–specific PCR at the National Hansen's Disease Program (Baton Rouge, LA, USA). In consultation with this program, we prescribed clarithromycin, rifampin, and dapsone in April 2017. Within 3 months of treatment, the patient had decreased skin induration, nasal obstruction, and pinna thickening but minimal improvement in arthralgias or peripheral neuropathy symptoms. No immune reactions occurred during treatment.

Until the advent of molecular methods, all leprosy worldwide was assumed to have been caused by *M. leprae*. In 2008, a novel *Mycobacterium* species, *M. lepromatosis*, was identified by multigene analysis on tissue obtained from 2 immigrants to the United States from Mexico who died from diffuse lepromatous leprosy (*3*), which is endemic to Mexico and Costa Rica and is rarely reported from other geographic locations (*4*).

Comparative genomics show that *M. leprae* and *M. lepromatosis* are closely related and derived from a common ancestor (*4*) with ≈9.1% genetic difference between them, suggesting species-level divergence of ≈10 million (*5*) to 13.9 million (*4*) years ago. A phylogeographic survey suggests that *M. lepromatosis* is not widespread (*4*). Since 2008, PCR testing of patients identified *M. lepromatosis* from Mexico (*1*,*6*), Singapore (*7*), Canada (*8*), and Myanmar and Brazil (*9*).

Unlike *M. leprae* (*10*), *M. lepromatosis* has not been found in armadillos. Red squirrels are infected with *M. lepromatosis* in the United Kingdom (*11*), but the geographic distribution, zoonotic transmission risks, and other animal reservoirs are unknown.

Excluding the patient we report, *M. lepromatosis* lepromatous leprosy has been diagnosed in 10 persons (including the 2 index case-patients) in the United States: all were immigrants (8 from Mexico, 2 from Costa Rica) (*3*,*6*,*12*). Our report emphasizes that US citizens can acquire *M. lepromatosis* when traveling to Mexico or other locations as tourists. Our patient's earliest symptoms of arthritis and neuropathy were in December 2014, suggesting acquisition in Puerto Vallarta (2005) or Acapulco (2007), consistent with the leprosy incubation period of 7–8 years (*6*). His signs and symptoms were similar to those of patients from Mexico (*1*,*6*,*12*,*13*) and a patient from Canada in whom peripheral neuropathy developed 1–2 years before onset of skin lesions. Nasal mucosal

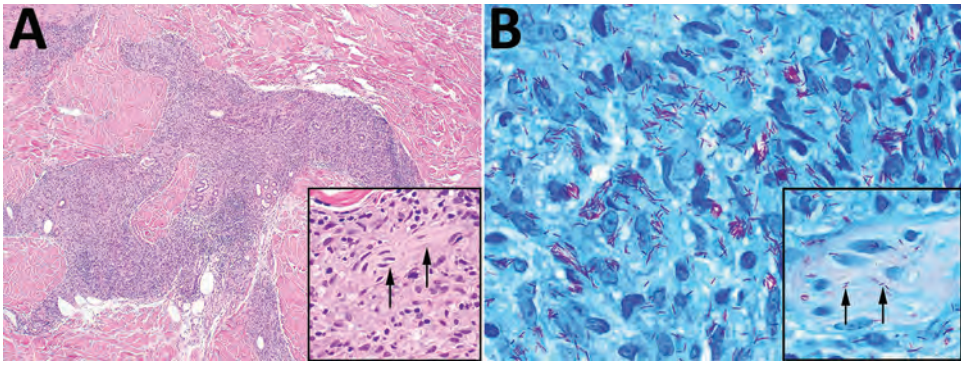

Figure 2. Skin biopsies of 59-year-old white male US citizen showing *Mycobacterium lepromatosis* infection, 2017. A) Hematoxylin and eosin–stained section of a specimen from the chin showing granulomatous dermal inflammation (original magnification ×100); inset shows nerve involvement (arrows) that is diagnostic for leprosy (original magnification ×400). B) Fite-stained section of a specimen from the chin highlights numerous acid-fast bacilli within histiocytes (original magnification ×1,000); inset shows peripheral nerve involvement (arrows) that is diagnostic for leprosy (original magnification ×1,000).

involvement was prominent in both. Loss of eyebrows, eyelashes, or both is common in *M. lepromatosis* infection (*6*,*8*,*13*) and might be an early sign of infection. Future reports may help determine if this feature is specific for *M. lepromatosis*. In the case reported by Han et al. (*13*), cure was associated with nearly complete regrowth of eyebrows and eyelashes.

The full spectrum of manifestations, outcomes, and global burden of *M. lepromatosis* infection remains unknown. In a study of persons who had leprosy, specifically diffuse lepromatous leprosy in Mexico, *M. lepromatosis* was identified more often (63.2%) than *M. leprae* and caused dual infections in 16.1% (*1*).

Leprosy can resemble autoimmune disorders, including systemic lupus erythematosus or rheumatoid arthritis. Chronic polyarthritis is described in up to 75% of leprosy patients, possibly secondary to an immune response to mycobacterial heat-shock proteins (*14*,*15*). Overlapping occurrences of rheumatoid and leprous arthritis make it difficult to differentiate these disorders. The arthritis in the patient we report could be leprous.

Reported patients who have *M. lepromatosis* and *M. leprae* lepromatous leprosy have been treated similarly (*12*). Because of the multibacillary load and higher risk for immune reactions and pigmentation, especially with clofazimine or minocycline, we prescribed clarithromycin, rifampin, and dapsone for this patient. He was maintained, at our advice, on methotrexate to modulate these reactions.

In summary, *M. lepromatosis* lepromatous leprosy is a travel-related hazard for travelers to Mexico or other disease-endemic areas. Specific exposure risks for acquisition of *M. lepromatosis* are unknown. The presence of leprosy-like skin lesions should prompt detailed evaluation, including skin biopsy for histopathology, histochemical stains for mycobacterial organisms, and 16S ribosomal RNA gene PCR to identify the causative agent.

A.V. is an inventor for Travel Health and Wellness, LLC; R.P. reports grants from CD Diagnostics, BioFire, Curetis, Merck, Hutchison Biofilm Medical Solutions, Accelerate Diagnostics, Allergan, and The Medicines Company. R.P. is a consultant to Curetis; monies are paid to Mayo Clinic. In addition, R.P. has a patent on *Bordetella pertussis*/*parapertussis* PCR issued; a patent on a device/method for sonication with royalties paid by Samsung to Mayo Clinic; and a patent on an anti-biofilm substance issued. R.P. serves on an Actelion data monitoring board; receives travel reimbursement from Roche, ASM, and IDSA; an editor's stipend from ASM and IDSA; and honoraria from the NBME, Up-to-Date, and the Infectious Diseases Board Review Course.

Dr. Virk has been a consultant in the Division of Infectious Diseases at the Mayo Clinic in Rochester, Minnesota, since 1997. She is the chair of the Enterprise Antimicrobial Stewardship Program at the Mayo Clinic and previously was the director of the Travel and Tropical Medicine Clinic.

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Lineage-Specific Real-Time Reverse Transcription PCR for Yellow Fever Virus Outbreak Surveillance, Brazil

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The current yellow fever outbreak in Brazil prompted widespread yellow fever virus (YFV) vaccination campaigns, imposing a responsibility to distinguish between vaccine- and wild-type YFV-associated disease. We developed novel multiplex real-time reverse transcription PCRs that differentiate between vaccine and American wild-type YFV. We validated these highly specific and sensitive assays in an outbreak setting.

Yellow fever virus (YFV) is a mosquitoborne member of the genus *Flavivirus* within the family *Flaviviridae* (online Technical Appendix Figure 1, panel A, https://wwwnc.cdc.gov/EID/article/23/11/17-1131-Techapp1.pdf) that is endemic to Africa and South America (*1*). Within YFV, 2 American and at least 3 African genotypes can be differentiated (*2*). The American YFV genotypes evolved from ancestral African viruses several hundred years ago and now are found only in South America (*3*).

In December 2016, Brazil reported the country's largest yellow fever (YF) outbreak in decades. Through May 31, 2017, a total of 3,240 suspected cases were reported, including 435 deaths (*4*). The geographically widespread outbreak was caused by the American genotype 1 (online Technical Appendix Figure 1, panel B) (*5*). In response to the outbreak, authorities launched large-scale vaccination campaigns aimed at distributing >20 million doses of YFV vaccine (*6*). Two different live-attenuated vaccines are being deployed. Most contain the vaccine strain 17DD, produced in Brazil (*7*). International authorities are deploying another 3.5 million doses of the standard vaccine strain, 17D (*6*). Both vaccine strains originate from the same parental strain, Asibi, and represent the West African genotype 2 (online Technical Appendix Figure 1, panel B).

While YFV vaccines are considered safe, rare vaccine-associated adverse events (YF-VAAE) can occur (*8*). Viscerotropic YF-VAAE symptoms can overlap those of YF disease (*9*). In YFV-endemic regions, it is essential to distinguish between YF-VAAE and wild-type YFV infection (*10*). Routine diagnostic procedures can take several days, usually requiring 2 separate steps, detection and strain characterization by nucleotide sequencing. Here, we present 2 highly sensitive real-time reverse transcription PCRs (RT-PCRs) designed to detect and discriminate between YFV vaccine and American wild-type (hereafter referred to as wild-type) strains within 1 hour.

The Study

We followed 2 rationales for real-time RT-PCR design. First, a small number of oligonucleotides per assay can be beneficial in resource-limited settings. Therefore, we designed 5 different single-target assays using primers capable of simultaneously amplifying vaccine and wild-type YFV strains. However, these criteria restricted the ability to design optimal oligonucleotides. Therefore, we designed 2 additional dual-target assays that target 2 separate genomic regions in which vaccine and wild-type strains differ sufficiently from one another (Figure 1, panel A). Vaccine and wild-type strains were generally discriminated by lineage-specific hydrolysis probes within a single tube reaction, incapable of detecting the heterologous lineage due to high numbers of nucleotide mismatches under oligonucleotide binding sites (Figure 1, panel B) (*12*).

We selected the 2 most sensitive single- and dual-target assays on the basis of preliminary experiments using full viral RNA of wild-type and vaccine strains (Table; online Technical Appendix Figure 2). For assay validation

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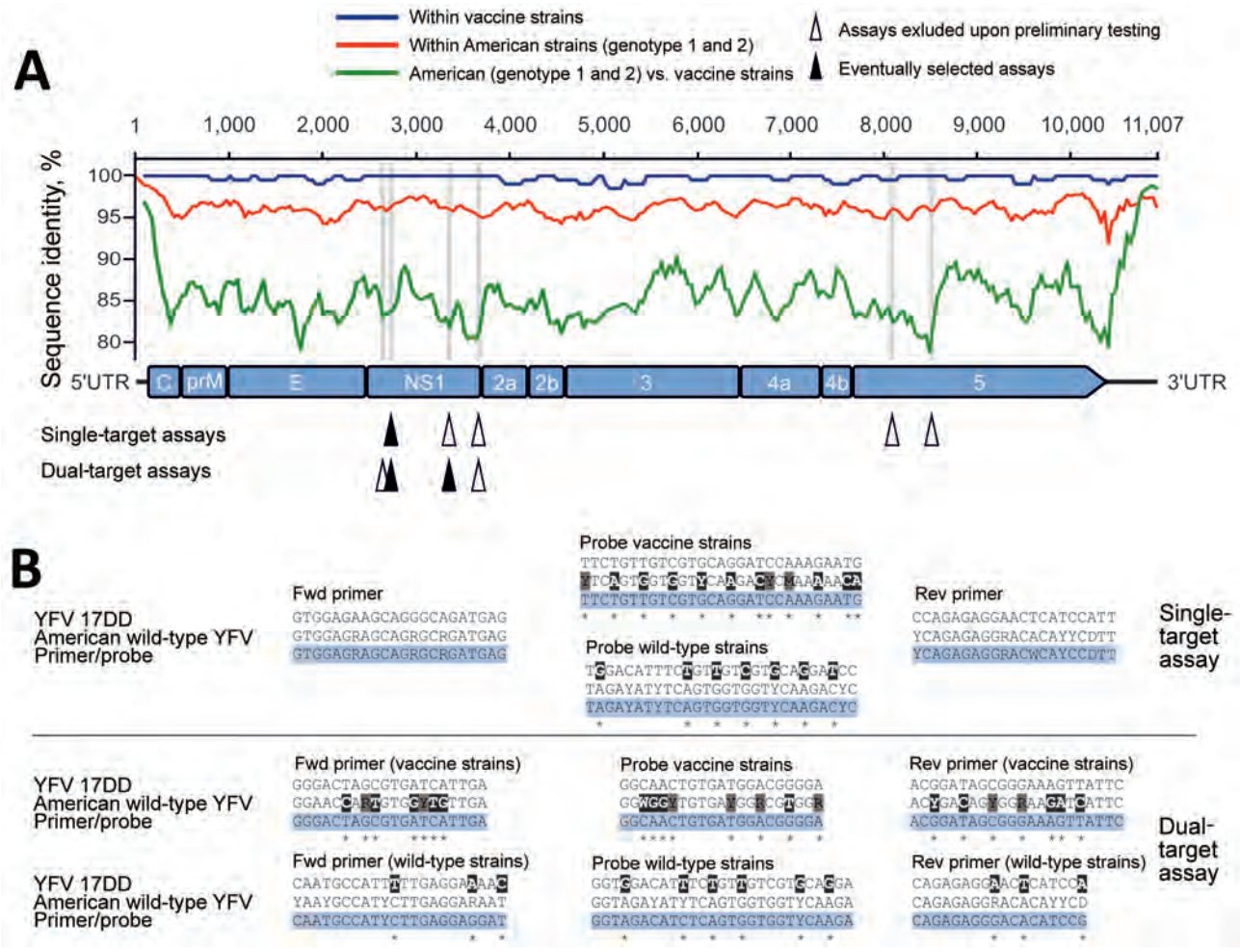

Figure 1. Design of new real-time RT-PCRs for differentiation between vaccine and wild-type YFV. A) YFV genomic representation (GenBank accession no. DQ100292) with real-time RT-PCR target sites, indicated by arrowheads, and identity plot of all complete YFV sequences available in GenBank as of May 24, 2017. Plots were done in SSE version 1.2 (*11*) using a sliding window of 200 and a step size of 40 nt. Target sites of the eventually selected assays are indicated by filled arrowheads; all other designed assays excluded after preliminary testing by open arrowheads. Of the real-time RT-PCR assays developed in this study, 1 assay targets only 1 genomic region, whereas the other assay targets 2 different genomic regions of vaccine and wild-type YFV strains. Both PCRs are duplex assays in which vaccine and wild-type YFV RNA are detected by lineage-specific probes. We called the assay targeting only 1 genomic region a single-target assay and the assay targeting 2 separate genomic regions a dual-target assay, even though the term dual-target commonly refers to detection of 2 different genes of a single pathogen, which is not the case in this study. B) Alignment of real-time RT-PCR oligonucleotide binding sites with YFV 17DD and American wild-type strains. The 100% consensus sequences were generated in Geneious (Biomatters Ltd., Auckland, New Zealand) and mapped to respective PCR primers and probes. Potential nucleotide mismatches are indicated by asterisks. D = A/G/T, M = A/C, R = A/G, W = A/T, Y = C/T. Black indicates a mismatch with all American wild-type strains, gray a mismatch with some American wild-type strains, based on the complete genetic information of American YFV strains and YFV vaccine strains available in GenBank as of March 24, 2017. C, capsid; E, envelope; Fwd, Forward; NS, nonstructural protein; prM, precursor membrane; Rev, reverse; RT-PCR, reverse transcription PCR; UTR, untranslated region; YFV, yellow fever virus.

and quantification, we designed 2 in vitro transcripts (IVTs) based on the vaccine strain 17DD and an outbreak strain from Brazil (*5*), as described previously (*12*).

The 95% lower limit of detection of the single- and dual-target assays ranged from 4.0 to 8.8 RNA copies/reaction for vaccine and wild-type YFV strains (online Technical Appendix Figure 3). Discrimination between vaccine and wild-type strains was reliable even at high concentrations of IVTs and full viral RNA in the range of 10^6 copies/reaction. Assay specificity was assessed using a set of 39 high-titer flavivirus cell culture isolates (online Technical Appendix Figure 1, panel A), all of which tested negative in the novel assays.

Hypothetically, near-simultaneous infection with wild-type YFV and vaccination may occur in the outbreak setting in Brazil. In the case of co-occurrence of vaccine and wild-type YFV within a single sample, 1 target may

Table. Oligonucleotides for new yellow fever virus real-time RT-PCRs*

Oligonucleotide name	Primer/probe†	Sequence, 5′ → 3′‡	Target genomic domain, no. bases	Orientation
Single-target assay				
YFVsingle-fwd	Primer	GTGGAGRAGCAGRGCRGATGAG	2,653–2,674	+
YFVsingle-rv	Primer	AAHGGRTGWGTYCCTCTCTGR	2,743–2,763	-
YFVsingleP-vac	Probe (FAM)	TTCTGTTGTCGTGCAGGATCCAAAGAATG	2,710–2,738	+
YFVsingleP-wt	Probe (YAK)	TAGAYATYTCAGTGGTGGTYCAAGACYC	2,703–2,730	+
Dual-target assay				
YFVdual-fwd-vac	Primer	GGGACTAGCGTGATCATTGA	3,296–3,315	+
YFVdual-rv-vac	Primer	GAATAACTTTCCCGCTATCCGT	3,356–3,377	-
YFVdualP-vac	Probe (FAM)	TCCCCGTCCATCACAGTTGCC	3,317–3,337	-
YFVdual-fwd-wt	Primer	CAATGCCATYCTTGAGGAGAAT	2,677–2,698	+
YFVdual-rv-wt	Primer	CGGATGTGTCCCTCTCTG	2,744–2,761	-
YFVdualP-wt	Probe (YAK)	TCTTGRACCACCACTGAGATGTCTACC	2,701–2,727	-

*25 µL real-time RT-PCR reactions were performed using the Superscript III one-step RT-PCR system with Platinum Taq polymerase (Thermo Fisher Scientific, Darmstadt, Germany). Target genomic domain positions according to GenBank reference genome NC_002031. Reactions were set up with 5 µL of RNA; 12.5 µL of 2× reaction buffer; 0.4 µL of a 50 mM magnesium sulfate solution (Superscript III one-step RT–PCR system with Platinum Taq polymerase kit, Thermo Fisher Scientific); 1 µg of nonacetylated bovine serum albumin; and 1 µL enzyme. Single-target assay reactions contained 400 nM forward primer, 600 nM reverse primer, and 280 nM of each probe. Dual-target assay reactions contained 400 nM of each primer and 220 nM of each probe. RT-PCR, reverse transcription PCR.
†Probes are labeled with either fluorescein amidite (FAM) or Yakima Yellow (YAK) at the 5′-end and a Black Hole Quencher (TIB MOLBIOL Syntheselabor GmbH, Berlin, Germany) at the 3′-end. Primer concentrations were optimized using the YFV vaccine strain IVT and the wild-type YFV IVT. Amplification involved 50°C for 15 min, followed by 95°C for 3 min and 45 cycles of 95°C for 15 s and 58°C for 30 s with fluorescence read at the 58° annealing/extension step on a LightCycler 480 thermocycler (Roche, Basel, Switzerland).
‡H = A/C/T, M = A/C, R = A/G, W = A/T, Y = C/T.

occur in relatively higher concentrations than, and thus outcompete amplification of, the other target, resulting in an incomplete test result. We observed no target competition with the dual-target assay even in the presence of high concentrations of the heterologous RNA (Figure 2, panel A). In contrast, the single-target assay showed decreased sensitivity at ≤1,000 copies/reaction upon the presence of 100–500-fold higher concentrations of the heterologous target.

Commonly used clinical specimens for YFV diagnostics may contain substances that can interfere with PCR (*13*). To assess our assays' performance in different clinical matrices, we used human plasma and urine previously tested negative for YFV and spiked them with 10^1–10^6 copies/mL of either vaccine or wild-type YFV. Three replicates of each spiked specimen were purified individually and tested by using our PCRs and a YFV reference assay (*13*). We detected samples containing ≥1,000 copies/mL in all replicates irrespective of the clinical matrix (Figure 2, panel B). Detection of samples containing ≤100 copies/mL was unreliable in all 3 assays. As exemplified before for Zika virus, final RNA copy numbers in eluates used for RT-PCR will depend on the RNA extraction protocol, illustrating that even assays with analytical sensitivity in the single-copy range may not correctly detect weakly positive clinical specimens (*12*).

Clinical specimens may differ from spiked materials, and assay performance needs to be assessed in an outbreak context. Therefore, we compared the new assays to the reference assay (*13*) in a Brazilian flavivirus reference laboratory, using different clinical specimens obtained from 11 YF cases previously confirmed by nucleotide sequencing as wild-type YFV infections. The sensitivity of the dual-target assay was identical to that of the reference assay, whereas the single-target assay was slightly less sensitive (Figure 2, panel C). Identification of wild-type YFV was reliable in all cases, consistent with specific detection of lineages even in highly positive clinical specimens.

Conclusions

The new PCRs we describe enable YFV detection with diagnostic sensitivity. The dual-target assay was superior to the single-target assay in sensitivity and robustness to target competition. However, the single-target assay may be advantageous in resource-limited settings and may be more convenient for multiplex usage in combination with assays targeting co-circulating arboviruses, such as chikungunya, Zika, and dengue viruses. Beyond rapid test results, the real-time RT-PCR–based protocols provide considerably higher sensitivity than protocols aiming at generating longer PCR amplicons necessary for strain discrimination by nucleotide sequencing, enabling conclusive results even when virus concentrations in specimens are low or when these materials are available only in limited quantity. Of note, PCR-based YFV detection is most reliable 5–7 days after symptom onset, during the viremic phase. Reliable YFV surveillance should thus include serologic methods. However, serologic tests used for virologic diagnostics cannot discriminate between vaccination and wild-type YFV infection.

Of note, our novel assays are limited to vaccine and American YFV wild-type strains. West African wild-type stains would be detected by our YFV vaccine assays due to the close genetic relatedness between these strains, but our assays are not suitable to detect the genetically diverse Eastern and Central African wild-type strains. If needed, one

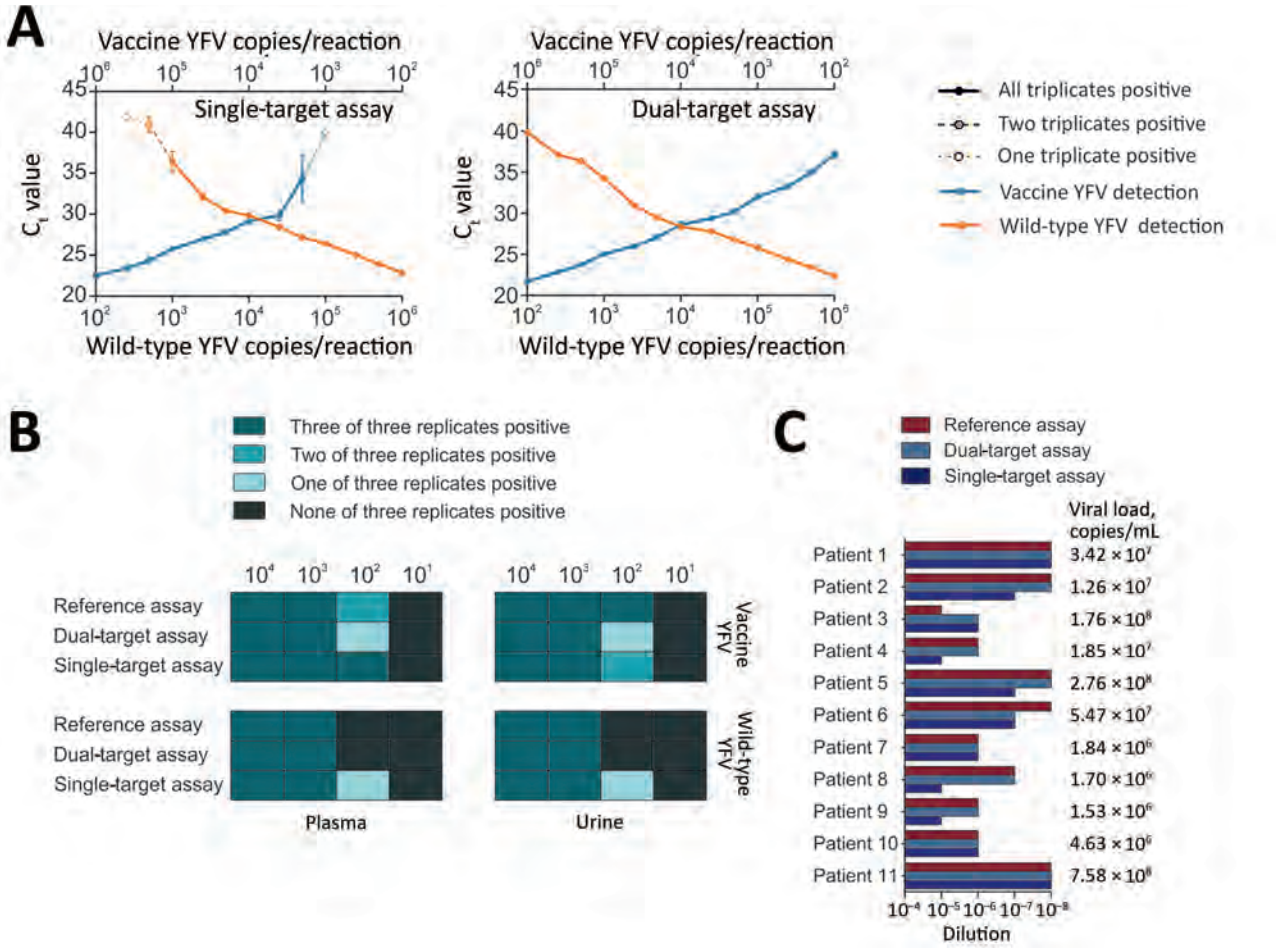

Figure 2. Validation of new real-time RT-PCRs for differentiation between vaccine and wild-type YFV. A) Effects of target competition on YFV real-time RT-PCRs. Mean cycle threshold (C_t) values are plotted against IVT concentrations. Triplicates were tested for each datum point. B) Validation of the assays with clinical matrices. Spiked viruses were vaccine strain 17D and the American genotype 2 wild-type strain BOL88/1999. RNA purification was performed using the MagNA Pure 96 Viral NA Small Volume Kit (Roche, Basel, Switzerland) according to the manufacturer's instructions. C) Clinical validation. Clinical specimens (serum, liver, whole blood, and plasma) from 11 YFV-infected patients were tested. RNA was extracted using the MagMAX Pathogen RNA/DNA Kit (Thermo Fisher, São Paulo, Brazil) and serial dilutions of the RNA were tested using the new assays and a YFV reference assay (*12*). Viral loads were determined for clinical specimens using a commercially available quantitative real-time RT-PCR (Bio Gene Research Yellow Fever PCR kit; Bioclin, Minas Gerais, Brazil), following the manufacturer´s instructions. Standard curves and sample copies per millileter were calculated using an in-house IVT standard. IVT, in vitro transcript; RT-PCR, reverse transcription PCR; YFV, yellow fever virus.

could extend our assays by an additional primer/probe combination targeting the Eastern and Central African genotypes.

Recently, the first attenuated live dengue virus vaccine was approved in several countries, including Brazil (*14*). Inactivated Japanese encephalitis virus vaccines are currently replaced by attenuated live vaccines in Asia (*15*), and an attenuated live West Nile virus vaccine has completed a phase II clinical trial (*16*). Large-scale deployment of these vaccines will raise the need to discriminate between potential vaccination-associated events and wild-type virus infection in symptomatic patients. Our work with YFV may provide a diagnostic blueprint for establishing and validating suitable methods for differentiating between vaccine and wild-type viruses for these other viruses as well.

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Phylogenetic Analysis of *Klebsiella pneumoniae* from Hospitalized Children, Pakistan

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Klebsiella pneumoniae shows increasing emergence of multidrug-resistant lineages, including strains resistant to all available antimicrobial drugs. We conducted whole-genome sequencing of 178 highly drug-resistant isolates from a tertiary hospital in Lahore, Pakistan. Phylogenetic analyses to place these isolates into global context demonstrate the expansion of multiple independent lineages, including *K. quasipneumoniae*.

Klebsiella spp. are gram-negative bacteria that are widely distributed in the environment, and *K. pneumoniae* is a common cause of infection in humans (*1*). Increasingly, *K. pneumoniae* is reported as a cause of invasive bloodborne infections, particularly in healthcare settings and in immunocompromised patients (*2*). Of concern is that infection-associated *K. pneumoniae* is often multidrug resistant (MDR) and can harbor resistance determinants against most, if not all, commonly used antimicrobial drugs, posing a major threat to public health. The World Health Organization recently highlighted finding new treatments against MDR *Enterobacteriaceae* (including *Klebsiella*) as priority 1 (critical) (http://www.who.int/mediacentre/news/releases/2017/bacteria-antibiotics-needed/en/).

K. pneumoniae is a major pathogen in economically developed settings, and multiple outbreaks in different countries have been reported. Less is known about its prevalence in economically challenged areas, including lower and middle income countries (LMIC). Reports are now appearing about *Klebsiella*-associated infections in Nepal (*3*) and in Indonesia, Laos, and Vietnam (*1*). *Klebsiella* can spread rapidly in hospital environments, and the increasing prevalence of MDR strains has raised concern among major health organizations (*4*,*5*). Thus, high-resolution insight into the diversity of *Klebsiella* spp. isolated in LMICs will provide vital data for improving epidemiologic management of infections and for better understanding of the mechanisms of spread between LMICs and more developed countries.

The Study

Clinical samples were collected during a 22-month period (May 2010–February 2012) from The Children's Hospital & The Institute of Child Health, Lahore (Lahore, Pakistan), the largest tertiary care hospital in the region (Figure 1, panel A). The hospital had a capacity of 650 beds during the study period but is under pressure to handle up to 2,000 inpatients at any given time. The primary catchment area is Lahore (population ≈10 million); the hospital also receives patients from the greater area of Punjab province (population ≈100 million) (Figure 1, panel A). The Ethical Committee of The Children's Hospital & Institute of Child Health, Lahore, approved the study.

A total of 44,260 samples were collected in the course of routine sampling from children; 5,475 (12.4%) resulted in laboratory-positive cultures. Of these, 710 (13.0%) samples were positively identified as *K. pneumoniae*, the third most dominant isolate after *Escherichia coli* (1,336 [24.4%]) and coagulase-negative staphylococci (724 [13.2%]) (Figure 1, panel B). We screened all *K. pneumoniae* isolates for resistance to ceftazidime (30 μg disc, zone of inhibition ≤17 mm) or cefotaxime (30 μg disc, zone of inhibition ≤22 mm). We further tested *K. pneumoniae* isolates that were resistant to any of these indicator drugs using the Clinical and Laboratory Standards Institute combined-disc confirmatory test (*6*); extended-spectrum β-lactamase (ESBL) production was confirmed when the zone of inhibition by either cephalosporin drug increased by ≥5 mm in the presence of clavulanate. A total of 214 of *K. pneumoniae* isolates were ESBL-positive (Figure 1, panel C); most were isolated from children with bloodstream infections (Figure 1, panel D). The outcomes were severe, especially among neonatal patients (Figure 1, panel D); 56 died, 31 were taken home against medical advice, and 127 were discharged (Figure 1, panel D). Almost all patients infected

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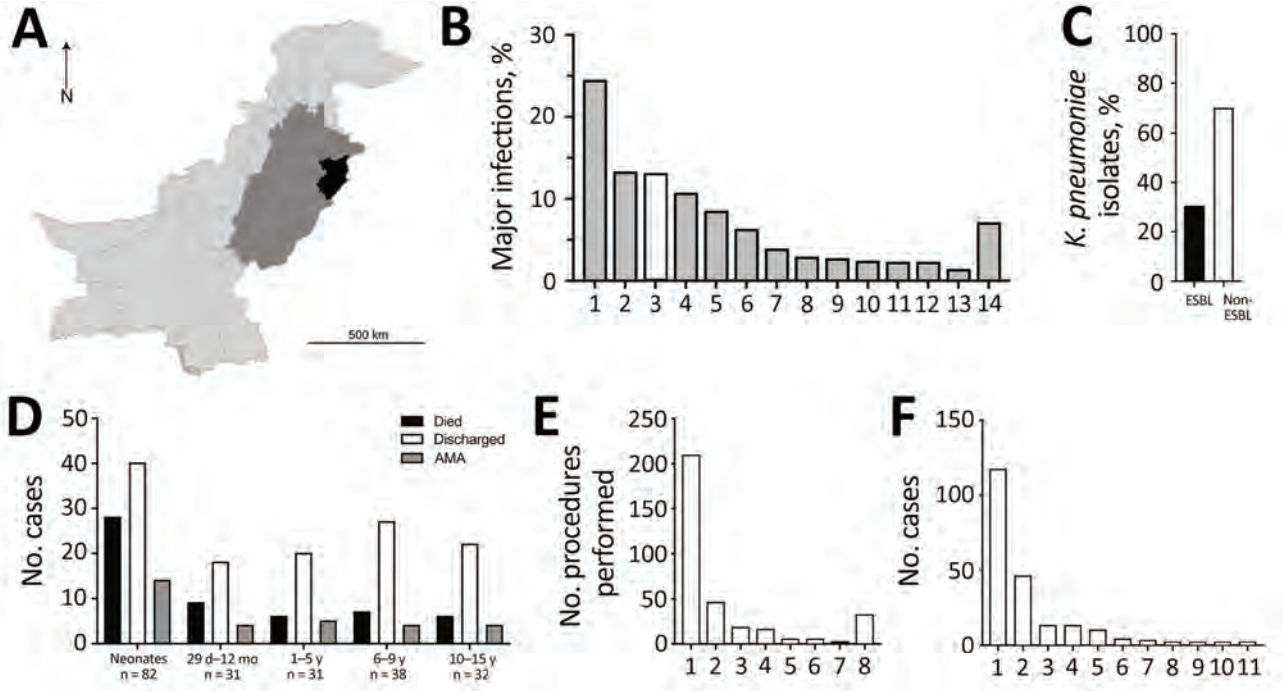

Figure 1. Statistical overview of bacterial isolates from clinical samples collected during May 2010–February 2012 from The Children's Hospital & The Institute of Child Health, Lahore, Pakistan. A) Map of Pakistan highlighting the main catchment area of Lahore (black, population ≈10 million) and the wider area of Punjab (medium gray, population ≈100 million). B) A total of 5,475 samples collected from children resulted in laboratory-positive cultures; the 5 most frequently occurring bacterial species accounted for ≈70% of total bacterial infections, and *Klebsiella pneumoniae* (white bar) was the third most dominant (710 isolates). 1, *Escherichia coli*; 2, coagulase-negative *Staphylococcus*; 3, *K. pneumoniae*; 4, *Pseudomonas aeruginosa*; 5, *K. oxytoca*; 6, *Staphylococcus aureus*; 7, *Acinetobacter* spp.; 8, *Enterococcus faecalis*; 9, *Citrobacter* spp.; 10, *Streptococcus pyogenes*; 11, *Burkhoderia cepacia*; 12, *Enterobacter clocae*; 13, *Salmonella enterica* var. Typhi; 14, others (>100 species). C) The proportion of ESBL-producing *K. pneumoniae* (214 isolates) among all *K. pneumoniae* isolates demonstrated high prevalence of antimicrobial resistance. D) A total of 38.3% of ESBL-producing *K. pneumoniae* infections occurred in neonates (<29 d), an age group that also showed the highest fatality rate (34.1%). Patients who were removed from the hospital against medical advice (AMA) typically were critically ill and were taken home by the family to avoid dying in the hospital. E) The apparent hierarchy shown in panel E closely correlated with interventions given. IV line (97.7%), urinary catheter (27.5%), and ETT (8.4%) were the 3 most commonly administered procedures among sampled patients, although no temporal relationship between procedure and sample collection could be established. 1, IV line; 2, urinary catheter; 3, ETT; 4, PD catheter; 5, surgery; 6, NG tube; 7, CVP; 8, others. F) A total of 54.6% of ESBL-producing *K. pneumoniae* isolates were from patient blood samples, followed by urine (21.5%), CSF (6%), and ETT (6%). 1, Blood; 2, urine; 3, CSF; 4, ETT; 5, PD catheter; 6, tracheal secretions; 7, pus; 8, CVP tip; 9, ear swab; 10, pleural fluid; 11, wound swab. CSF, cerebrospinal fluid; CVP, central venous catheter tip; ESBL, extended-spectrum β-lactamase; ETT, endotracheal tube; IV, intravenous; NG, nasogastric; PD, peritoneal dialysis catheter. The regional map was derived from the Global Administrative Areas online resource (http://www.gadm.org/).

with ESBL *Klebsiella* had received an intravenous line (209 [97.7%]) (Figure 1, panels E,F), and a high number received a urinary catheter (46 [21.5%]).

We performed whole-genome sequencing on 178 isolates (online Technical Appendix Table 1, https://wwwnc.cdc.gov/EID/article/23/11/17-0833-Techapp1.pdf). We prepared Illumina sequencing libraries (Illumina, San Diego, CA, USA) with a 450-bp insert size according to the manufacturer's protocols and sequenced them on an Illumina HiSeq2000 with 100-bp–long paired-end reads before assembly using an open-source high-throughput assembly and improvement pipeline as described (*7*) (https://github.com/sanger-pathogens/) and annotated using prokka (*8*). Initial clustering using mash (*9*) enabled aligning of these isolates to published reference sequences (online Technical Appendix Figure 1, panel A). The clustering indicated a strong structure for the isolates that fell within the species *K. pneumoniae* (online Technical Appendix Figure 1, panel B). However, the analysis also revealed a large group of sequences most similar to *K. quasipneumoniae*; closer inspection focusing on this species showed strongest similarity to subspecies *similipneumoniae* (online Technical Appendix Figure 1, panel C) (*10*). We combined several independent datasets: a large global collection (*1*); 2 hospital outbreaks obtained in a comparable time frame, 1 of which was based in Nepal in 2012 (*3*); and a hospital study from Spain that also focused on diversity within ESBL-producing strains (*11*) (online Technical Appendix Table 2). We applied the pan-genome pipeline Roary version 3.7.0 (*12*) with a blastp (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins)

percentage identity of 90% and a core definition of 99%, resulting in a core gene alignment comprising 1,793 genes for all studies (Figure 2) and 3,486 genes for the strains of this study (online Technical Appendix Figure 2). We first extracted single-nucleotide polymorphisms using snp-sites version 2.3.2 (*13*), then calculated a maximum-likelihood tree using RAxML version 8.2.8 (*14*) with the general time-reversible model and 100 bootstrap repeats. The core gene phylogeny (Figure 2) shows a wide distribution of the isolates from Pakistan across different lineages rather than 1 clonal lineage. The diversity of our strain collection is further emphasized through the diversity of multilocus sequence types (STs). No single ST dominates (Figure 2 outer ring; online Technical Appendix Figure 2); however, a large group of isolates belongs to ST15, which is known to be problematic. The presence of *K. quasipneumoniae* isolates agrees with an overall lower percentage of reads mapped against *K. pneumoniae* (online Technical Appendix Table 1) and with recent descriptions of virulent *K. quasipneumoniae* strains (*1*,*9*,*15*). Assessing the metadata in phylogenetic context highlights the association of the *K. quasipneumoniae* lineage with patients in the neonatal ward, suggestive of its nosocomial residency (online Technical Appendix Figure 2). However, other main lineages (e.g., ST15, ST48) show a dynamic spread across wards and age groups, indicating against ≥1 resident lineages but instead a frequent movement of *K. pneumoniae* through the hospital, general population, or both.

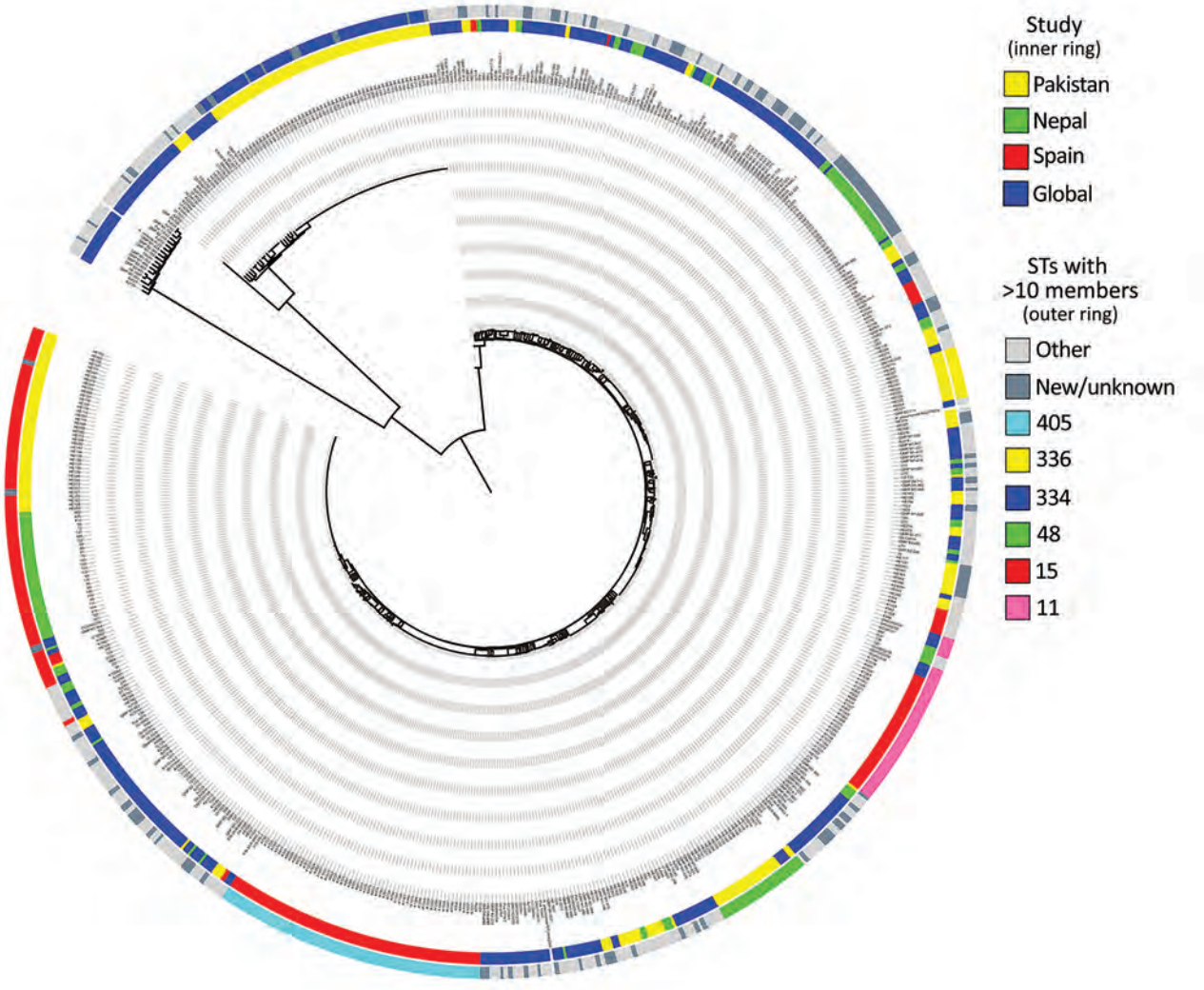

Figure 2. Phylogenetic analysis demonstrating the diversity of *Klebsiella pneumoniae* isolates from clinical samples collected during May 2010–February 2012 from The Children's Hospital & The Institute of Child Health, Lahore, Pakistan, in a global context. The core gene tree based on the alignment derived from Roary (*12*) was calculated using RAxML (*14*) and shows the wide diversity of samples analyzed in this study (inner ring, yellow) in context with a large-scale global analysis (inner ring, blue [*4*]) and 2 hospital outbreaks, which show a more clonal pattern (inner ring: red, outbreak in Spain [*11*]; green, outbreak in Nepal [*3*]). The sequence types observed (outer ring) also reflect the diversity; most sequence types have <10 members even in this combined collection. STs, sequence types.

The high number of *K. quasipneumoniae* isolates, even if potentially restricted to most sequences derived from a lineage potentially resident in a specific ward, highlights the importance of a diverse set of sampling sites to be studied. It also highlights the need for continued monitoring of new emerging strains and that our knowledge of the diversity of potentially problematic lineages is far from exhaustive.

Conclusions

The *Klebsiella* isolates in this study represented the *Klebsiella* isolates routinely present in infections over a protracted period. Our findings highlight a consistent problem with ESBL-encoding strains belonging to a multitude of lineages. We observed sporadic single-isolate lineages, as well as smaller, related clusters of 5–10 strains per lineage, in addition to 2 larger clusters of strains. More studies are needed to better delineate the distinguishing features for successful spread and persistence of lineages such as the ST15 cluster. Also, the large spread of *K. quasipneumoniae* is unusual. Further intense monitoring of LMIC hospital environments is urgently needed to prevent the persistence of resident lineages with very high base-level drug resistance, which, through the inevitable acquisition of a few more genes, would lead to untreatable infections.

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Bartonella quintana and Typhus Group Rickettsiae Exposure among Homeless Persons, Bogotá, Colombia

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In 2015, we investigated *Bartonella quintana* and typhus group rickettsiae in body lice from homeless persons in Bogotá, Colombia. We found *B. quintana*–infected body lice and seroprevalence of this microorganism in 19% of homeless persons and typhus group rickettsiae in 56%. Public health professionals should start preemptive measures and active vector control.

Homeless persons make up part of the population at highest risk for infectious diseases because of factors such as deficient hygiene habits, infrequent washing and changing clothes, and overcrowding (*1*). Within this group, vector-borne diseases caused by bacteria of the genera *Bartonella*, *Rickettsia*, and *Borrelia* are of great importance; louseborne *B. quintana* is the main microorganism associated with infections in homeless persons (*1*). In Colombia, the presence of *R. prowazekii*, a typhus group rickettsiae (TGR), in lice and related human infections in Bogotá was evident only during 1918–1922 and 1941 (*2*). We investigated the presence of *B. quintana* and TGR in body lice collected from homeless persons in Bogotá and these persons' exposure to such microorganisms.

The Study

The Research and Ethics Committees of the Facultad de Ciencias of the Pontificia Universidad Javeriana (Bogotá, Colombia) approved this study (February 14, 2013). All participants read, accepted, and signed the informed consent form.

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During May–September 2015, we enrolled a total of 153 persons from a homeless shelter in Bogotá in the study and obtained serum samples from each person. We also collected lice from participants' clothing or body (below the neck) and considered these lice a positive indication of body louse infestation. Of the study participants, 132 were men, 17 were women, and 4 were transgender (for the study, people who had a gender identity or gender expression that differs from their assigned sex). Participants' mean age was 39.6 (SD ± 11.65) years. Eighteen (11.7%) were infested with body lice; lice were preliminarily identified as *Pediculus humanus humanus*, according to standard taxonomic keys (*3*), and all had the same light color (Figure 1).

To detect specific IgG to *Bartonella* spp. (*B. quintana* antigen) and TGR (*R. typhi* antigen) in serum samples, we used commercially sourced indirect fluorescent antibody (IFA) kits (Bartonella IFA IgG and Rickettsia IFA IgG; Focus Technologies, Cypress, CA, USA). We screened serum at a dilution of 1:64. Among the 153 study participants, 29 (19.0%) had IgG that reacted exclusively against *Bartonella* spp. at a titer of ≥64, and 86 (56.2%) had IgG that reacted exclusively against TGR at a titer of ≥64. Twenty (13.1%) participants had IgG against both *Bartonella* spp. and TGR.

For identification of *Bartonella* spp. and *Rickettsia* spp. from louse samples, we organized 201 body lice into 39 pools and extracted DNA from each pool (DNeasy Blood and Tissue; QIAGEN, Valencia, CA, USA). We screened all louse pools by standard PCR for *Bartonella* spp. (citrate synthase gene [*gltA*] and 16S–23S rRNA intergenic transcribed spacer region [ITS-1]) and *Rickettsia* spp. (*gltA*, 16S RNA, and *ompB* rickettsial genes) as described (*4*). Eleven (28%) louse pools were positive for *Bartonella* spp., of which 7 were positive for *gltA*, 10 were positive for ITS-1, and 6 were positive for both genes. We found no evidence of *Rickettsia* spp. infection in body lice. For *Bartonella* spp., sequence of the ITS-1 fragment amplified by standard PCR and phylogenetic analysis with maximum-likelihood method and 1,000 bootstrap replicates, performed using MEGA software version 6 (*5*), confirmed the bacteria as *B. quintana* (GenBank accession no. KY605045) (Figure 2, panel A).

To examine the mitochondrial clade in *P. h. humanus* captured in this study, we tested DNA samples from 2

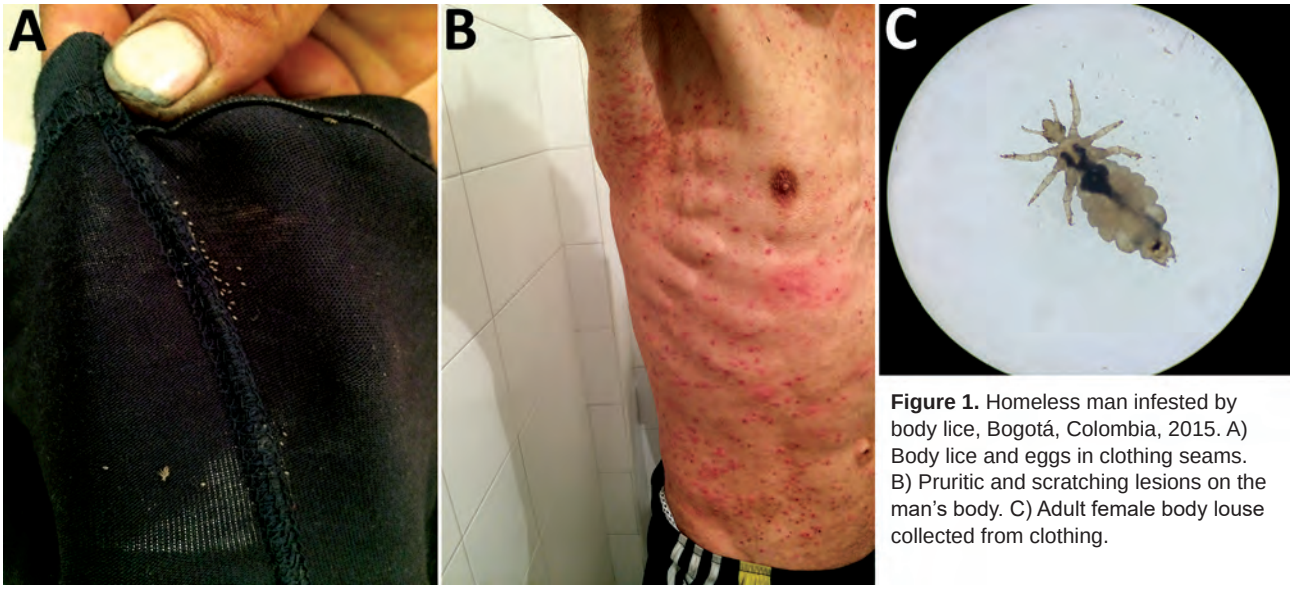

Figure 1. Homeless man infested by body lice, Bogotá, Colombia, 2015. A) Body lice and eggs in clothing seams. B) Pruritic and scratching lesions on the man's body. C) Adult female body louse collected from clothing.

randomly selected lice and PCR amplified the *cytb* genes as reported (*6*). We compared the *cytb* sequences obtained in our study with known head and body louse sequences from the 5 *P. h. humanus* clades (A–E) (*6*). Phylogenetic analysis using the maximum-likelihood method demonstrated that the 2 sequences obtained in our study belong to clade A (GenBank accession nos. KY605043 and KY605044) (Figure 2, panel B).

Conclusions

Our study demonstrates evidence of infestation by the body louse (*P. h. humanus*) infected with *B. quintana* and exposure to TGR in homeless persons in Bogotá. The rate of body louse infestation in the studied population (11.7%) was within the range reported elsewhere (7%–30%) (*1*,*7*), confirming that homeless persons are among the population groups most vulnerable to parasitism by this arthropod and associated infectious agents (*1*). We also found seroprevalence for *Bartonella* spp. (19.0%) in line with the range reported in other studies worldwide (0.4%–62%) (*8*,*9*). Although cross-reactions in the IFA between different *Bartonella* species are possible (highlighting those associated with homeless persons: *B. quintana*, *B. elizabethae*, and *B. henselae*) (*8*,*9*), we consider that the seroprevalence detected in homeless persons in Bogotá probably is due to *B. quintana* because it is the microorganism most frequently associated with homeless persons (*1*), and we detected it in 28.2% of body lice collected from persons sampled, again in agreement with previous studies (1.4%–94%) (*10*).

On the other hand, the level of seropositivity against TGR found in our study (56.2%) was considerably higher than levels reported in previous studies (≈0.54%–22%) (*9*). We were not able to perform a Western blot–associated cross-adsorption test to distinguish the specific *Rickettsia* species involved in the TGR-positive serum (*11*). However, we consider that *R. typhi* was probably the predominant species responsible for the seropositivity for the following reasons: *R. prowazekii* was not detected in collected body lice; infection with *R. typhi* is frequent in homeless persons (*11*); and no records from healthcare government entities in Colombia, whether local (Bogotá) or national, suggest the occurrence in this population of febrile illness with high death rates, which would be more compatible with the epidemiology of epidemic typhus (*R. prowazekii* infection) than with murine typhus (*R. typhi* infection) (*12*). Although human body lice are not clearly identified vectors of *R. typhi*, it seems that under certain circumstances they could transmit *R. typhi* (*13*), as well as other rickettsiae (*14*). More work is needed to identify properly the *Rickettsia* and *Bartonella* species involved in this antibody prevalence.

We identified the lice collected from homeless persons in Bogotá as belonging to the haplogroup/clade A, which is distributed worldwide and comprises *P. h. humanus* and *P. h. capitis* (*6*). In our study, given that lice were collected from clothes and body areas below the neck, they probably were *P. h. humanus*. Nevertheless, molecular determination by PCR using the Phum_PHUM540560 gene currently is the only way to distinguish body and head lice (*15*).

Our study is subject to several limitations. The detection of *Bartonella* spp. and *Rickettsia* spp. was based on standard PCR and not on real-time PCR, which is more sensitive. As a result, some samples could have tested negative because of low DNA load. Moreover, we did not test serum or blood samples by molecular assays, nor perform cultures to isolate infectious agents from the lice. Nonetheless, our study results should encourage public health professionals in

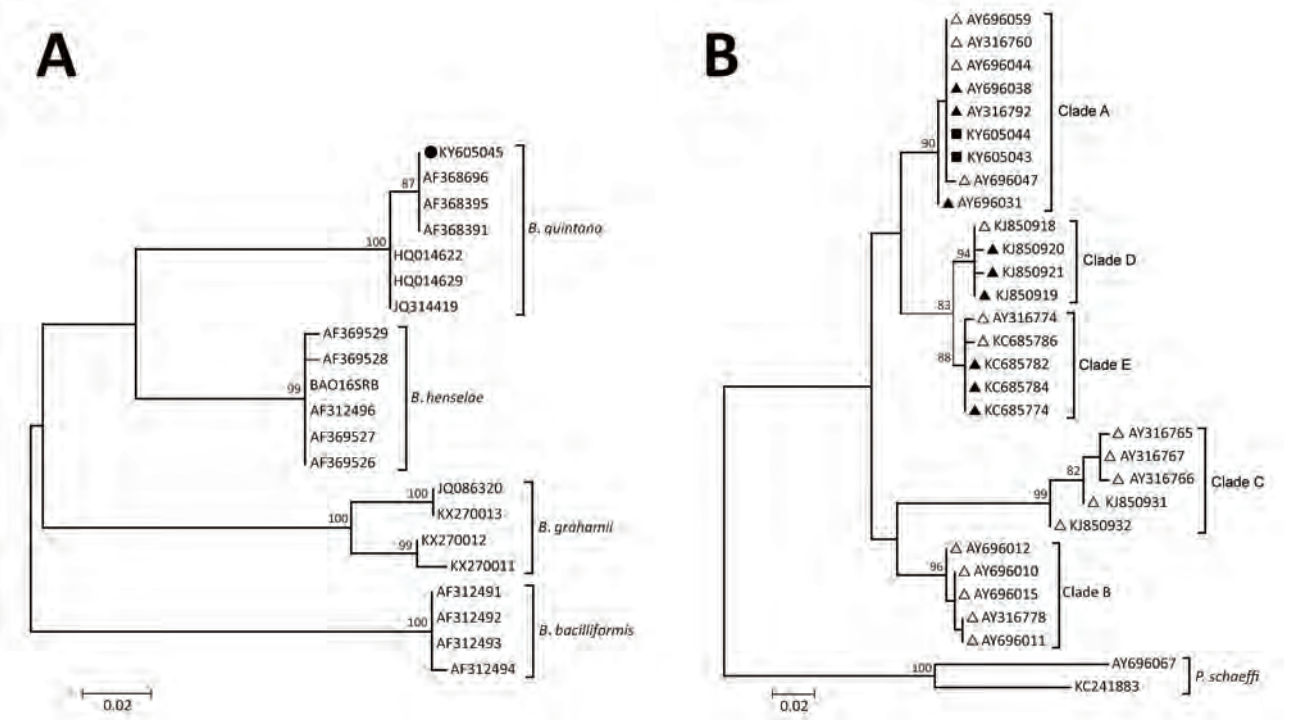

Figure 2. Maximum-likelihood (ML) analyses of intergenic transcribed spacer 1 genes of *Bartonella* spp. and of the *Pediculus humanus humanus* louse mitochondrial cytochrome b (*cytb*) gene. A) *Bartonella* spp. analysis. The tree with the highest log likelihood is shown, and ML bootstrap values >80 are indicated at each node. The tree is drawn to scale. Sequences are indicated by GenBank accession number; solid circle indicates the sequence retrieved in this study. The *Bartonella* species is indicated to the right of each branch. B) *P. h. humanus* analysis. The tree with the highest log likelihood is shown, and ML bootstrap values are located above the node. The tree is drawn to scale. The body louse sequences (solid triangles), head louse sequences (open triangles), and GenBank accession numbers are indicated. Cytochrome b sequences from *Pediculus schaeffi* were used as outgroups. Solid squares indicate the sequences retrieved in this study. The mitochondrial clade is indicated to the right of each branch. Scale bars indicate nucleotide substitutions per site.

Bogotá to start preemptive measures and active vector control (delousing and ivermectim treatment) (*1*), conduct future research evaluating the clinical characteristics of *Bartonella* and *Rickettsia* infections in homeless persons in Bogotá, confirm circulation of specific species of these microorganisms, and screen for *Bartonella* spp. endocarditis by blood culture among homeless persons who have high antibody titers.

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Street Cleaning Trucks as Potential Sources of *Legionella pneumophila*

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In 2015, Legionnaires' disease was diagnosed in a street cleaning worker. We found *Legionella pneumophila* serogroup 1 in the water and internal foam from the tanks of 2 trucks used by the worker during the incubation period. The internal foam was removed, and a *Legionella* prevention program was implemented.

Legionnaires' disease (LD) is an acute pneumonia caused by inhalation or aspiration of aerosols contaminated with *Legionella* bacteria. *Legionella* spp. are ubiquitous in freshwater aquatic habitats. Multiplication of *Legionella* in artificial water systems is facilitated by temperatures around 35°C and factors such as lack of disinfection, water stagnation, and poor maintenance (*1*).

In Spain, 925 cases of LD were reported in 2014, of which 82% were sporadic cases, not related to outbreaks (*2*). LD outbreaks have been related to cooling towers, spa pools, and water distribution systems (*2–4*), which are considered by regulations in Spain to have a high probability of proliferation of *Legionella* (*5*).

The identification of exposure in sporadic cases provides a good opportunity to enhance understanding of reservoirs for *Legionella* in relatively rare sources (*6,7*). This case report summarizes the environmental study conducted to identify the possible source of exposure of a street cleaning worker who contracted LD.

The Study

A 58-year-old man in Barcelona who smoked began to show symptoms compatible with legionellosis on July 27, 2015. On August 11, LD was diagnosed in the patient; this diagnosis was confirmed by a urinary antigen test (UAT). On August 14, the case was reported to the Public Health Agency of Barcelona.

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A trained public health practitioner interviewed the affected patient by using a standardized questionnaire to obtain information on demographic data, personal risk factors, and activities during the 15 days before the onset of illness. The only exposure to sources of aerosolized water highlighted by the epidemiologic survey was an occupational exposure to high-pressure water hoses from street cleaning trucks during the incubation period. The trucks used had 2-m^3 water tanks with an internal foam lining ≈15 cm thick. The purpose of the foam was to help steady the truck when in motion. The truck tanks were filled with groundwater untreated with chlorine or with drinking water from the town's water supply network, depending on their availability during the journey. At the end of the day, the tanks were emptied but the internal foam remained impregnated with water. Once a year, the tanks were disinfected with a 20-ppm chlorine solution for 2 hours; hoses were replaced relatively often because of deterioration. However, the interior walls of the tanks were never cleaned, and the foam lining was never replaced.

Three days after the interview, we took samples from the 4 trucks that the worker used in the 15 days before symptom onset. We took water samples and smears from the hoses and water tanks to test them for *Legionella*. We also took a water sample from the groundwater tank where the trucks were usually filled.

We analyzed the samples in accordance with the ISO 11731 method for counting *Legionella* spp. (*8*). In addition, we obtained counts of *L. pneumophila* serogroup 1 and of serogroups 2–15. We performed molecular typing of the strains of *L. pneumophila* serogroup 1 isolated in the different samples via DNA macrorestriction and subsequent pulsed field electrophoresis. We calculated the similarity coefficient and displayed the results of molecular typing as a dendrogram generated by the FPQuest software package (Bio-Rad Laboratories, Hercules, CA, USA).

Water sample temperatures ranged from 26°C to 28°C. We detected *L. pneumophila* serogroup 1 in the water from 2 of the 4 trucks sampled. *L. pneumophila* serogroup 1 counts were 150 CFU/L for truck 1 and 1,000 CFU/L for truck 2 (Table). We did not detect *Legionella* in the sample of the groundwater tank or in the hose smears of either truck.

After *Legionella* detection, the tanks were disinfected and cleaned following the cleaning procedure described by law for hot water systems (*5*). After 15 days, we drew new

Table. Counts of *Legionella pneumophila* serogroups 1 and 2–15 in the samples of water, foam, and smears analyzed before and after cleaning and disinfection of 2 street cleaning trucks, Barcelona, Spain, 2015*

Location and sample type	Before cleaning and disinfection, CFU/L		After cleaning and disinfection, CFU/L	
	L. pneumophila serogroup 1	*L. pneumophila* serogroups 2–15	*L. pneumophila* serogroup 1	*L. pneumophila* serogroups 2–15
Truck 1				
Water tank	150	<1†	400	<1
Hose water	150	<1	5,700	<1
Foam sample 1	NA	NA	250‡	Not detected
Foam sample 2	NA	NA	5,500‡	Not detected
Tank surface smear	NA	NA	Detected	Not detected
Truck 2				
Water tank	1,000	1,000	125	125
Hose water	<1	50	<1	50
Foam sample 1	NA	NA	275‡	125‡
Foam sample 2	NA	NA	10‡	10‡
Foam sample 3	NA	NA	Not detected	10‡
Foam sample 4	NA	NA	10‡	Not detected
Tank surface smear	NA	NA	Not detected	Not detected

*NA, not analyzed.
†Limit of detection in water samples: 1 CFU/L.
‡Counts for 250 cm^3 of foam.

water samples to assess the effectiveness of the disinfection, and after 23 days, we took samples of the foam and from the internal surfaces of the tanks of both trucks. We obtained samples of foam by cutting out pieces (4 × 4 × 15 mm) with a sterile scalpel and placed them in sterile containers with Ringer's solution for subsequent *Legionella* analysis. The results indicated the presence of *L. pneumophila* serogroup 1 in the foam and water tanks of both trucks and in the hose water sample and the smear from the tank of truck 1 (Table).

The results of molecular typing indicated that clones were detected in truck 1 (Dice similarity coefficient 100%) belonging to the same strain found in the hose water, in the tank water, and in the foam. We also detected another clone, genetically related to the first (Dice similarity coefficient 89%), in the tank water, in the smears of the surface of the tank, and in the foam. We identified 2 different molecular patterns in the strains isolated in the tank water and in the foam in truck 2; both patterns appeared in both tank water and foam (Figure). Regarding the foam, we obtained similar results in the foam underneath decorative stones in an ornamental fountain that caused an LD outbreak in a hospital (*9*).

Weather conditions were warm in July 2015, with an average temperature >25°C even at night (*10*). These temperatures, along with water stagnation, would have favored proliferation of the bacteria in the water tanks.

Although the mode of transmission is not clear, the high-pressure hose used by the worker probably discharged aerosols containing bacteria that could be inhaled (*7*). The worker did not use a protective facemask and was the second employee in the same company who was working with cleaning trucks to contract LD. Four years earlier, another case of LD had occurred in similar conditions (J. Cayla,

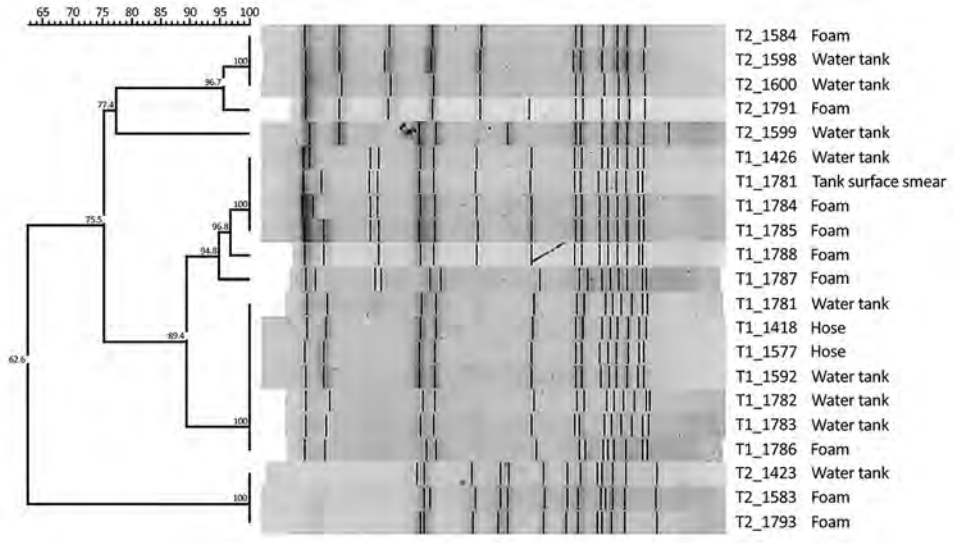

Figure. Dendrogram generated by FPQuest software showing the pattern of SfiI bands for isolates of *Legionella pneumophila* from 2 street cleaning trucks, Barcelona, Spain, 2015. Strains are identified with code of truck of origin (T1, T2) and an internal number. Scale bar represents Dice similarity coefficient percentage.

Public Health Agency of Barcelona, pers. comm., 2011 July 22).

Published criteria (*11*) indicate that cleaning trucks are a potential source of *Legionella*. The level of evidence of this study corresponds to level III, because *L. pneumophila* subgroup 1 was isolated in the trucks used by the worker but clinical and environmental strains could not be compared because he was diagnosed by a UAT. Furthermore, because UAT detects only *L. pneumophila* serogroup 1, lack of respiratory culture can lead to missed diagnoses of other species and serogroups; fortunately, this limitation does not apply here.

A report describing similar exposure concerned an asphalt paving machine causing an outbreak of *Legionella* in 2009 (*6*). Like the trucks in this study, this was a mobile source, and the use of untreated water had also contributed to tank pollution. Although some studies have associated LD with occupations such as gardening (*12*,*13*) and professional driving (*14*), no studies have related LD to street cleaning.

Conclusions

We detected *L. pneumophila* serogroup 1 in 2 of the 4 trucks used by the affected worker during the incubation period; either truck could have been the source of exposure. The results of this study show that the internal foam of the tanks could act as a reservoir for *L. pneumophila* and that maintenance and routine cleaning (without removing the foam) did not prevent *Legionella* proliferation.

After the occurrence of this case, the internal foam in the truck tanks was removed, and the water tanks were cleaned again. The company responsible for the street cleaning trucks adopted a water management plan, with the agreement of the Public Health Agency of Barcelona, based on Hazard Analysis and Critical Control Points methodology (*15*) and stricter control measures. Workers are now required to wear personal protective equipment during work-related exposure.

Ms. Valero is a public health technician in the Barcelona Public Health Agency in Barcelona, Spain. Her main research interests are environmental health protection research and the study of potential sources of *Legionella.*

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Virulence of Japanese Encephalitis Virus Genotypes I and III, Taiwan

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The virulence of genotype I (GI) Japanese encephalitis virus (JEV) is under debate. We investigated differences in the virulence of GI and GIII JEV by calculating asymptomatic ratios based on serologic studies during GI- and GIII-JEV endemic periods. The results suggested equal virulence of GI and GIII JEV among humans.

Japanese encephalitis virus (JEV), a mosquitoborne flavivirus, causes Japanese encephalitis (JE). This virus has been reported in Southeast Asia and Western Pacific regions since it emerged during the 1870s in Japan (*1*). JEVs are divided into 5 genotypes on the basis of envelope structural protein genes for phylogenetic reconstruction. JEV genotype III (GIII) has been the most widely distributed in the temperate zone and is most frequently associated with JEV outbreaks in Asia. JEV genotype I (GI) originated in Indonesia and circulated in Thailand and Cambodia during the 1970s (*1*). Dominance of GIII was replaced by GI during 1992–2001 in Japan, Korea, Thailand, and Vietnam (*1*).

In Japan, the confirmed case incidence of JEV suddenly decreased from 20–50 cases each year during 1980–1990 to <10 cases after 1992 (*2*). This decrease may be related to implementation of JEV vaccine in Japan but also to less virulent GI JE viruses circulating there (*3*–*6*). A similar decrease was not seen in the other countries where genotype replacement had also occurred in recent years (*7*). In Taiwan, JEV GI was first detected in 2008 and became the island-wide dominant circulating genotype within a year (*8*,*9*), which provided an excellent opportunity to study the transmission dynamics and pathogenicity of these 2 JEV genotypes.

A mouse model showed that the pathogenic potential is similar among different JEV genotypes (*10*). However, the pathogenic difference between GI and GIII virus infections among humans remains unclear. Endy et al. reported that the proportion of asymptomatic infected persons among total infected persons (asymptomatic ratio) is an excellent indicator for estimating virulence or pathogenicity of dengue virus infections among humans (*11*). We used the asymptomatic ratio method for a study to determine if GI JEV is associated with lower virulence than GIII JEV among humans in Taiwan.

The Study

JEVs were identified in 6 locations in Taiwan during 1994–2012 (online Technical Appendix Figure, panel A, https://wwwnc.cdc.gov/EID/article/23/11/16-1443-Techapp1.pdf). GIII viruses were the only known circulating JEVs in Taiwan before 2009 (online Technical Appendix Figure, panel B). A genotype shift was complete by 2009, and since then, all JEV isolates in Taiwan have evolved from GI viruses (*8*). To investigate differences in the virulence of GIII and GI viruses in human infections, we conducted a subcohort and cross-sectional combined study to determine the JEV asymptomatic infection ratio. We used serum panels collected during the GIII JEV endemic period (1994–2000 [*12*,*13*]) and during the GI JEV endemic period (2010–2012).

The institutional review boards of the Mennonite Christian Hospital and the Tungs' Taichung Metroharbor Hospital reviewed and approved clinical protocols for the serum sample collection. Approximately 10% of total specimens were paired serum samples. We used the plaque reduction neutralization test (PRNT) and IgM antibody-capture ELISA (MAC-ELISA) to determine the infection status of each serum specimen (*14*). We tested serum panels collected before and after 2009 by using MAC-ELISA and PRNT and used viral antigens and viruses derived from the GIII-T1P1 and GI-YL2009–4 strains, respectively.

We further tested all neutralizing and IgM antibody-positive specimens by using GI YL2009–4, GIII T1P1, and dengue virus 2 (DENV-2) viral antigens to determine the genotype-specific infection status and to exclude false-

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Table 1. Descriptive characteristics of populations for study of virulence of JEV genotypes I and III, Taiwan*

Characteristic	Region								p value†
	Changhua	Taipei	Pingtung	Miaoli	Taichung	Hualien	Taichung	Hualien	
Year	1994	1995	1999	2000	2010	2010	2012	2012	ND
Circulating JEV	GIII	GIII	GIII	GIII	GI	GI	GI	GI	ND
No. participants	795	886	571	274	510	754	527	300	ND
No. (%) vaccinated	36	41	41	33	48	48	49	46	<0.05
Average age, y‡	42.8	40.9	47.1	51.3	49.6	48.1	52.0	49.2	<0.05
Male sex, %	45	52	47	54	50	48	52	49	>0.05

*JEV, Japanese encephalitis virus; ND, no data.
†Analyzed by using 1-way analysis of variance.
‡Range 40.9–52.0 y.

positive results possibly caused by DENV infection. We used SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) for statistical analyses.

We collected 5,557 specimens from 4,617 participants (Table 1). The average participant age by year ranged from 40.9 to 52.0 years ($p<0.05$); overall, 49.6% of participants were male and 50.4% female ($p>0.05$). The proportion of persons receiving JEV vaccinations increased over time from 33% to 49% ($p<0.05$).

The positivity rate for neutralizing antibody, elicited by natural infection or immunization in the past, differed significantly, ranging from 58% to 75% among the 8 serum panels ($p<0.05$) (Table 2). A JEV infection that had been acquired recently was indicated by the presence of IgM among the seronegative population, seroconversion of the neutralizing antibody, and a ≥4-fold increase in the neutralizing antibody titer among the seropositive population. For the 8 serum panels, the IgM positivity rate ranged from 0.90% to 4.91% ($p<0.05$), and the proportion of seroconversion and a ≥4-fold increase in the neutralizing antibody titer ranged from 0% to 1.37% ($p>0.05$). The incidence rate of JEV infection ranged from 1.83% to 5.49% ($p<0.05$) in the 8 serum panels.

We calculated the asymptomatic ratio by dividing the number of confirmed JE cases by the number of JEV infection cases. The number of confirmed JE cases (Table 2) was obtained from the CDC National Infectious Disease Statistics System, Taiwan (https://nidss.cdc.gov.tw/en/Default.aspx), which reported 1–4 cases/year in the regions from which the 8 serum panels were collected (Table 2). The number of JEV infection cases, calculated by multiplying the total population by the incidence of JEV infection, ranged from 10,243 to 53,657 in the 8 serum panels. The asymptomatic ratios (95% CI) for GIII virus infection were 1:6,640 (1:5,102–1:8,000) for Changhua County in 1994; 1:17,886 (1:15,408–1:21,322) for Taipei City in 1995; 1:8,779 (1:8,000–1:9,709) for Pingtung County in 1999; and 1:10,243 (1:8,065–1:13,947), for Miaoli County in 2000. The asymptomatic ratios for GI virus infection were 1:7,029 (1:6,329–1:7,874) for Taichung City and 1:6,063

Table 2. Estimated incidence of infection and asymptomatic ratios of JEV genotypes I and III among study populations, Taiwan*

Characteristic	Region								p value†
	Changhua	Taipei	Pingtung	Miaoli	Taichung	Hualien	Taichung	Hualien	
Year	1994	1995	1999	2000	2010	2010	2012	2012	ND
No. specimens (incidence of infection)	966 (171)	974 (88)	638 (67)	411 (137)	656 (146)	905 (151)	632 (105)	375 (75)	ND
NT positive, %	62	69	60	58	65	75	72	66	<0.05
IgM positive, % (A)	4.91	0.90	1.93	1.10	0.98	2.25	3.23	3.67	<0.05
Seroconversion + ≥4-fold increase in NT titer, %‡ (B)	0.58	1.13	0	0.73	1.37	1.32	0.95	1.33	>0.05
Incidence of infection, % (C = A + B)	5.49	2.03	1.93	1.83	2.35	3.57	4.18	5.00	<0.05
Population§ (D)	483,766	2,643,221	909,778	559,703	598,186	339,659	593,780	337,382	ND
Predicted no. infections (E = C × D)	26,559	53,657	17,559	10,243	14,057	12,126	24,820	16,869	ND
Confirmed cases (F)¶	4	3	2	1	2	2	2	2	ND
Asymptomatic ratio (G = F/E)	1/6,640	1/17,886	1/8,779	1/10,243	1/7,029	1/6,063	1/12,410	1/8,435	>0.05
Asymptomatic ratio, 95% CI	1/5,102–1/8,000	1/15,408–1/21,322	1/8,000–1/9,709	1/8,065–1/13,947	1/6,329–1/7,874	1/5,348–1/6,993	1/10,846–1/14,493	1/7,299–1/10,010	ND

*Serum samples were collected after the Japanese encephalitis season (May–September); multiple serum samples were collected from some participants. JEV, Japanese encephalitis virus; ND, no data; NT, neutralizing antibody.
†Analyzed by using one-way analysis of variance.
‡Among the IgM-negative population, JEV infection was estimated by the observation of an increase in the NT titer, including seroconversion and ≥4-fold increase in the NT titer among subjects with multiple serum samples.
§In Taichung, only residents of the districts around the serum-collecting hospital were included.
¶According to the official report of the Taiwan Centers for Disease Control.

(1:5,348–1:6,993) for Hualien County in 2010; ratios were 1:12,410 (1:10,846–1:14,493) for Taichung City and 1:8,435 (1:7,229–1:10,010) for Hualien County in 2012.

We applied the log-linear Poisson regression model to adjust the asymptomatic ratio by the number of patients who had encephalitis and were infected by other pathogens, and also by age, sex, and vaccination status (online Technical Appendix Table) (*15*). The results revealed that non–JEV-specific encephalitis symptoms (fever, headache, convulsion, and seizure) (intercept, $p<0.05$) influenced the calculation of asymptomatic ratios but not age, sex, or vaccination status ($p>0.05$).

We calculated the overall GI JEV– and GIII JEV–specific asymptomatic ratios by using the adjusted asymptomatic ratios of the 8 serum panels collected during the GI JEV– and GIII JEV–endemic periods (Figure). The GI-specific asymptomatic ratio was 1:15,378 (1:6,168–1:24,588) and the GIII-specific ratio was 1:18,842 (1:6,624–1:30,260); these ratios were not significantly different ($p>0.05$).

Conclusions

The possible effects of JEV genotype replacement remain unclear, particularly in terms of disease burden, virulence, vaccine efficacy, and policy decisions. In this study, we combined the IgM seropositivity rate, the proportion of seroconversion, and paired samples displaying a ≥4-fold increase in the neutralizing antibody titer to calculate incidence of the infection (Table 2). The results showed that the incidence of JEV infection fluctuated over years and in different regions in Taiwan. Nevertheless, genotype replacement had no significant effect on this fluctuation (GI:GIII = 3.78 ± 1.25:2.82 ± 3.18; $p>0.05$).

The dramatic decline observed in the number of clinical JE cases after the genotype replacement from GIII to GI in Japan suggested that GI viruses were less virulent than GIII viruses (*3*–*6*). However, we found that the asymptomatic ratio of GI and GIII JEV infections was similar, indicating equal virulence of GIII and GI JEVs. These results were also supported by the mouse virulence and neurovirulence experiments and disease burden estimation (*10*).

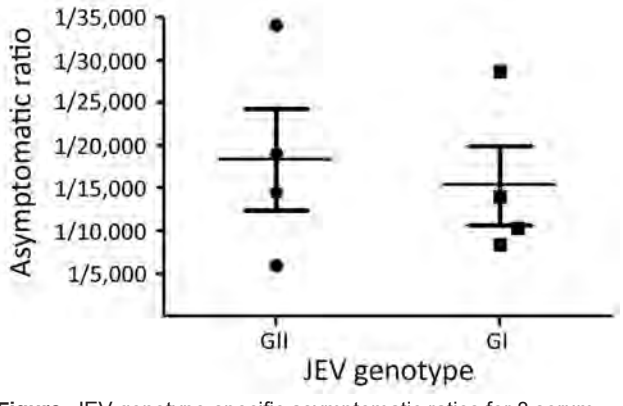

Figure. JEV genotype-specific asymptomatic ratios for 8 serum panels collected during the GIII- and GI-endemic periods (1994–2000 and 2010–2012) in Taiwan. The adjusted asymptomatic ratios estimated from genotype-representing populations were included to calculate JEV genotype-specific asymptomatic ratios. Horizontal lines indicate mean; error bars indicate SEM. JEV, Japanese encephalitis virus.

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Polyclonal Pulmonary Tuberculosis Infections and Risk for Multidrug Resistance, Lima, Peru

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Because within-host *Mycobacterium tuberculosis* diversity complicates diagnosis and treatment of tuberculosis (TB), we measured diversity prevalence and associated factors among 3,098 pulmonary TB patients in Lima, Peru. The 161 patients with polyclonal infection were more likely than the 115 with clonal or the 2,822 with simple infections to have multidrug-resistant TB.

Within-host heterogeneity of *Mycobacterium tuberculosis* infection is increasingly recognized as an obstacle for the accurate diagnosis (*1*) and effective treatment (*2*) of tuberculosis (TB) and may complicate the control of TB in communities (*3*). Within-host heterogeneity may arise through 2 mechanisms: 1) by reinfection or simultaneous infection with multiple strains, which results in a polyclonal (mixed) infection, or 2) by accumulation of mutations, which results in clonal heterogeneity (*4*). The treatment challenge posed by within-host heterogeneity has been most clearly demonstrated for infections with drug-susceptible and drug-resistant variants (*5*). The relatively high prevalence of multidrug-resistant (MDR) TB in Peru (≈6% among new case-patients and 21% among retreatment case-patients) (*6*) places increased stress on the healthcare system.

Our main objectives were to estimate the prevalence of within-host *M. tuberculosis* heterogeneity at the time of treatment initiation in a large cohort of pulmonary TB patients in Peru and to determine if factors measurable at the baseline visit were associated with complex infections (*7*). To determine whether our insights were sensitive to the method used for distinguishing between classes of heterogeneous infections, we used a newly described method (classifier of tandem repeats [ClassTR]) (*8*), which uses 24-loci mycobacterial interspersed repetitive units–variable number of tandem repeats (MIRU-VNTR) data to distinguish polyclonal and clonal infections, and we compared these findings with an analysis based on the standard threshold-based approach (*9*).

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The Study

During September 2009–August 2012, we attempted to enroll all adults (>15 years of age) with a diagnosis of incident pulmonary TB from 106 healthcare centers in Lima, Peru; details of the study design have been reported previously (*7*). We recorded baseline data on demographics, medical history, and results of drug susceptibility testing (DST) for rifampin, isoniazid, streptomycin, ethambutol, and pyrazinamide. We restricted our analysis to pretreatment samples and data from participants with culture-positive TB from whom sufficient mycobacterial DNA could be successfully obtained from the baseline sample to perform MIRU-VNTR typing.

All enrolled index case-patients and household contacts evaluated for active TB were assessed by sputum smear microscopy with Ziehl-Neelsen staining and culture on solid Lowenstein-Jensen medium. Initial DST was performed by using the proportion method on Lowenstein-Jensen medium; second-line DST was performed by using the proportion method on Middlebrook 7H11 agar. We shipped 100 μL of the lysate from suspensions of mycobacterial colonies harvested from Lowenstein-Jensen slants to Genoscreen (Institute Pasteur, Lille, France) for 24-loci MIRU-VNTR typing.

The standard threshold approach for classifying complex infections by using MIRU-VNTR data classifies patterns with >1 band (i.e., repeat copy number) at a single locus as clonal infections and patterns with >1 band at multiple loci as polyclonal infections (*9*). To better distinguish between clonal and polyclonal infections, we used an alternative method called ClassTR, which leverages additional information about differences in loci copy numbers and from other strains present in the population (*8*). In simulation studies, ClassTR more accurately distinguished between these 2 mechanisms of within-host diversity than did the threshold approach (*8*).

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To understand whether our findings were robust to the classification approach used, we adopted ClassTR for our main analysis, but we also repeated all analyses with the threshold approach. We used univariable and multivariable multinomial logistic regression to identify baseline factors independently associated with having a clonal or a polyclonal infection, setting simple infection as the referent. Analysis was limited to complete cases. Co-linearity was assessed by calculating variance inflation factors, and $p<0.05$ was considered statistically significant. Co-linear variables were removed to produce the final multivariable model. Statistical analyses were conducted in R version 3.3 (http://www.R-project.org). Research ethics committees in Peru and Boston approved the study.

We analyzed results for 3,098 participants. Most participants were <35 years of age (64.8%) and male (61.9%); 108 (3.5%) were known to be HIV infected. Nearly a fifth (18.8%) of participants reported a prior history of TB, and 78 (2.5%) reported having received a course of isoniazid chemoprophylaxis. A total of 375 (12.1%) participants had MDR-TB, 288 (9.3%) had isoniazid or rifampin monoresistance, and 357 (11.5%) had other resistance patterns (predominantly streptomycin resistance). A total of 2,822 (91.1%) participants had simple infections (i.e., no evidence of within-host heterogeneity by MIRU-VNTR), and the remaining 276 (8.9%) had evidence of within-host heterogeneity. Using ClassTR, we classified 161 (5.2%) infections as polyclonal and 115 (3.7%) as clonal (Table 1).

Multivariable multinomial logistic regression results associated polyclonal infection with multidrug resistance (adjusted odds ratio 1.66, 95% CI 1.05–2.62; p = 0.03) and other drug resistance (adjusted odds ratio 1.97, 95% CI 1.27–3.06; p = 0.002) (Table 2). No factors were significantly associated with clonal infection in either univariable or multivariable analysis. These associations were largely preserved when we repeated the analysis by using the threshold classification approach (online Technical Appendix Tables 1, 2, https://wwwnc.cdc.gov/EID/article/23/11/17-0077-Techapp1.pdf).

Table 1. *Mycobacterium tuberculosis* resistance patterns among patients with pulmonary TB, Lima, Peru, September 2009–August 2012*

Resistance†	Simple, no. (%)	Clonal, no. (%)	Polyclonal, no. (%)
Pansensitive	1,917 (67.9)	73 (63.5)	88 (54.7)
INH or RIF resistance	260 (9.2)	11 (9.6)	17 (10.6)
Multidrug	333 (11.8)	15 (13.0)	27 (16.8)
Other	312 (11.1)	16 (13.9)	29 (18.0)
Total	2,822	115	161

**Mycobacterium tuberculosis* strain type determined by classifier of tandem repeats. INH, isoniazid; RIF, rifampin; TB, tuberculosis.
†Drug susceptibility testing was performed for RIF, INH, streptomycin, ethambutol, and pyrazinamide.

Conclusions

Among a large cohort of pulmonary TB patients in Lima, Peru, we found evidence of within-host *M. tuberculosis* diversity at the time of treatment initiation in ≈9%. The ClassTR approach for classification based on MIRU-VNTR typing indicated that 5.2% of patients had polyclonal infections and 3.7% had clonal infections.

Polyclonal infections were positively associated with multidrug resistance and other drug-resistance patterns. When we used a 2-sided exact binomial test to test the hypothesis that the risk for multidrug resistance among participants with polyclonal infection (using the observed fraction 27/161) differed from that expected with infection by 2 randomly selected strains, calculated as $1 - (1 - 348/2{,}937)^2$, we obtained a p value of 0.11. This p value suggests that, at least in this setting, the association between polyclonal infection and multidrug resistance cannot be attributed to

Table 2. Factors associated with clonal and polyclonal *Mycobacterium tuberculosis* infection among patients with pulmonary TB, Lima, Peru, September 2009–August 2012*

Characteristic	Clonal aOR (95% CI), n = 115	p value	Polyclonal aOR (95% CI), n = 161	p value
Age, y				
15–24	Referent		Referent	
25–34	1.21 (0.75–1.96)	0.44	1.40 (0.93–2.11)	0.11
35–44	1.16 (0.64–2.11)	0.62	1.42 (0.87–2.31)	0.16
≥45	1.33 (0.79–2.23)	0.29	1.22 (0.77–1.95)	0.40
Male sex	0.93 (0.63–1.38)	0.72	1.10 (0.78–1.55)	0.59
Previous TB	0.82 (0.48–1.37)	0.45	1.27 (0.86–1.86)	0.23
Previous INH receipt	1.51 (0.54–4.27)	0.44	1.84 (0.82–4.15)	0.14
HIV infection	0.99 (0.35–2.78)	0.98	1.12 (0.50–2.49)	0.79
≥1 chronic disease	0.90 (0.55–1.46)	0.66	0.97 (0.64–1.46)	0.88
Hospitalized	1.01 (0.58–1.75)	0.97	0.98 (0.61–1.57)	0.93
Resistance pattern				
Pansensitive	Referent		Referent	
INH or RIF resistance	1.11 (0.58–2.13)	0.76	1.38 (0.81–2.37)	0.24
Multidrug resistance	1.24 (0.70–2.22)	0.46	1.66 (1.05–2.62)	0.03
Other	1.34 (0.77–2.33)	0.31	1.97 (1.27–3.06)	0.002

*Results of multivariable regression analysis using classifier of tandem repeats method. aOR, adjusted odds ratio; INH, isoniazid; RIF, rifampin; TB, tuberculosis.

more than the increased risk that would accrue from multiple exposures.

A review of the literature on factors associated with within-host diversity revealed substantial variability between settings. Studies from Botswana and Taiwan found a higher prevalence of polyclonal infection among patients with MDR-TB (*10*,*11*); however, studies from Vietnam and KwaZulu-Natal (South Africa) did not find this association (*12*,*13*). It is possible that this association may be modified in the presence of HIV coinfection or that the ability to identify such an association is easier in areas where the prevalence of multidrug resistance is higher.

The main strengths of this study relate to the large prospective cohort of pulmonary TB patients evaluated in a study area with a population of 3.3 million persons. However, 30% of enrolled participants did not have culture-confirmed TB, precluding MIRU-VNTR analysis on all participants. Use of the MIRU-VNTR assay on cultured specimens to detect within-host heterogeneity was motivated by practical considerations. Because MIRU-VNTR typing is unable to identify all minority variants, and some diversity may be lost during culture (*14*), our categorization of infections into simple, clonal, and polyclonal may be subject to misclassification, which would be differential (i.e., complex infections are more likely to be misclassified as simple than the reverse) and could lead to bias. The use of a high number of MIRU-VNTR loci also reduces the likelihood of homoplasy. Furthermore, although the biological clock of the MIRU-VNTR marker seems to be relatively stable (recently estimated MIRU-VNTR mutation rate for TB is 2.70×10^{-3} mutations/locus/year [*15*]), changes accruing in the marker could lead to misclassification of clonal strains as polyclonal strains; we used the ClassTR method in an attempt to minimize such misclassification.

We found complex infections attributable to multiple infection events to be associated with increased risk for MDR TB. This finding further emphasizes the value of efforts to mitigate the transmission of MDR TB.

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Long-Term Viruria in Zika Virus–Infected Pregnant Women, Brazil, 2016

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During the 2016 Zika virus outbreak in Brazil, we detected Zika virus RNA in urine samples collected from Zika virus–positive pregnant women during different stages of pregnancy. Women had positive and negative intervals of viruria; 3 newborns had adverse outcomes. Further research is needed to clarify the relationship between viruria and outcomes for newborns.

Zika virus is a reemerging flavivirus that was first isolated in Uganda in 1947 during the course of a yellow fever virus survey (*1*). The virus remained at low transmission levels until the first Zika virus outbreak in Micronesia in 2007, spreading to the Pacific Islands in 2013–2014 and reaching Brazil in 2015 (*2*). Before the outbreak in Brazil, Zika virus infection was considered a mild febrile illness that did not produce severe outcomes. However, the cases of microcephaly, fetal abnormalities, and Guillain–Barré syndrome reported in Brazil reshaped our knowledge of the course of infection with this flavivirus, and demand for a rapid molecular approach to diagnose Zika virus infection began (*2*).

Molecular diagnosis of flavivirus infections is usually performed with blood or serum samples during the viremic period, which is sustained for ≈5–7 days (*3*). In the case of Zika virus infection, urine, saliva, and semen have been identified as additional sources that can be used for viral RNA detection. Urine and semen are especially useful; detection in these fluids is prolonged, being present even after the clearance of viremia (*4*,*5*). We describe the detection of Zika virus RNA in the urine from a group of Zika virus–positive pregnant women.

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The Study

During the February–October 2016 Zika virus outbreak, pregnant patients with Zika-like symptoms were treated at the Public Health Authority in São José do Rio Preto, Brazil. We collected serum and urine samples from these women during their first visit to the facility after symptom onset (Table) and tested their samples for Zika virus RNA (*6*). We extracted viral RNA from 140 μL of each sample using the QIAamp Viral RNA Mini Kit (QIAGEN, Valencia, CA, USA) according to the manufacturer's instructions. We performed a 1-step, quantitative, real-time, fluorescent probe–based PCR with primers targeting the Zika virus envelope gene as previously described (*6*).

Pregnant women who were Zika virus positive by quantitative reverse transcription PCR (RT-qPCR) (*6*) were monitored by a multidisciplinary medical team at the Criança e Maternidade Teaching Hospital in São José do Rio Preto, and viruria levels in urine were measured until delivery. RT-qPCR results were considered positive if the cycle threshold (C_t) was ≤38.5 and negative if the C_t was >38.5, as described previously (*6*). The pregnant women considered for enrollment in this study were 4–38 weeks into their pregnancies. This work was approved by the institutional review board of the São José do Rio Preto School of Medicine.

RT-qPCR results showed that urine samples taken from patients at different weeks of gestation were positive for Zika virus RNA; however, some of the consecutively collected samples were negative, showing positive and negative intervals (Table). Although not all samples were positive, we detected viral RNA in urine samples acquired sequentially over a course of ≥4 weeks for 6 of 13 patients (nos. 2, 4, 6, 8, 12, 13). The maximum period of detection was 7 months (patient no. 2). The number of collections varied depending on when during the pregnancy the patient started treatment at the facility and when the last urine sample was collected before delivery. Of the 12 newborns for which outcomes were known, 3 (25%) had adverse outcomes: 1 had subependymal cysts and 2 had unilateral abnormal otoacoustic emissions (Table).

Conclusions

We report findings of long-term viruria among Zika virus–positive pregnant women, with Zika virus RNA

Table. Long-term viruria in 13 Zika virus–positive pregnant women, São José do Rio Preto, Brazil, 2016*

Pt. no.	Week of gestation at virus detection	Days until first collection	Sample no., week of gestation/sample type and result/C_t									Week of gestation at birth	Adverse newborn outcome
			1	2	3	4	5	6	7	8	9		
1	16	2	16/S+/ 29.34; 16/U+/ 31.31	17/U+/ 33	19/U–	22/U+/ 38.01	24/U–	27/U–	31/U–	36/U+/ 37.24		38	No
2	4	3	4/S+/ 35.62; 4/U+/ 36.39	6/U–	10/U–	12/U–	17/U–	23/U+/ 38.21	28/U+/ 37.63	32/U+/ 37.58	36/U–	39	NA
3	30	5	30/S+/ 37.33; 30/U+/ 25.08	32/U–	34/U+/ 35.79	37/U+/ 36.42						38	No
4	17	4	17/S+/ 33	18/U–	23/U–	28/U+/ 38.06	32/U+/ 37.82	34/U–	36/U+/ 37.03			39	No
5	27	4	29/S+/ 37.35	31/U+/ 36.86	33/U–	35/U–						36	Unilateral abnormal OAE
6	14	1	14/S+/ 33.51; 14/U+/ 32.75	14/S–; 15/U+/ 37.72	21/U–	27/U–	31/U+/ 36.59	35/U+/ 36.98				38	No
7	14	39	20/U+/ 38.5	22/U–	27/U+/ 34.88	31/U–	36/U–					39	No
8	15	6	16/S+/ 37.28; 16/U+/ 34.56	18/U–	23/U+/ 36.6	27/U–	32/U+/ 32.68	36/U+/ 36.64				39	No
9	17	16	18/U–	22/U+/ 37.58	27/U–	32/U+/ 37.62	38/S–					38	No
10	21	32	26/U–	28/U–	32/U–	35/U+/ 38.1						37	Subependymal cysts
11	5	12	6/S+/ 37.85	14/U–	19/U–	23/U–	27/U+/ 37.71	31/U–	35/U–			38	No
12	19	47	25/U+/ 36.87; 25/S–	30/U+/ 36.81	32/U+/ 38.2	36/U–						38	No
13	24	41	25/U–	27/U–	31/U+/ 38.15	36/U+/ 33.9						37	Unilateral abnormal OAE

*Plus signs (+) indicate positive test results (C_t ≤38.5) and minus signs (–) indicate negative test results (C_t >38.5 or undetermined) (*6*). All pregnant women initially tested positive for Zika virus RNA. For some women (patient nos. 7, 8, 9, 10, 12, 13), data from the initial samples were not available. C_t, cycle threshold; NA, not available; OAE, otoacoustic emissions; pt., patient; S, serum; U, urine.

being detectable in urine during different stages of pregnancy. Plasma and serum are considered the biologic fluids of choice for the molecular diagnosis of Zika virus for ≤5 days after illness onset (*6*,*7*). However, after this short viremic period, these fluids are no longer ideal for diagnosis, although whole blood has been found to be more sensitive than plasma for Zika virus detection (*6*–*8*). A diagnosis on the basis of viremia is not recommended for cases in pregnant women >1 week after the onset of symptoms (*7*). In this study, Zika virus RNA was detected in the urine samples from pregnant women collected 1–7 months after the onset of symptoms; however, viruria was not detected after the women delivered. We suspect that this phenomenon might occur because virus replication is maintained in fetal tissues (e.g., brain, placenta, umbilical cord, liver, lung, spleen, muscles) at different viral loads (*7*,*9*) and that these tissues, therefore, act as virus reservoirs (*9*). The intermittence of Zika virus RNA detection in urine samples (fluctuation between negative and positive C_t results) was likely due to the sensitivity of the test. More studies are necessary to understand the dynamics of the replication of Zika virus in fetal tissues and its connection to viruria in pregnant women.

Urine, semen, and saliva have been described as specimens that can be used in the molecular detection of Zika virus after the clearance of viremia (*4*,*5*). The detection of RNA of other flaviviruses in urine and semen samples has been previously described (*5*,*10*), and for these viruses, viral RNA is detectable for longer durations and at higher viral loads in semen and urine samples than they are in plasma samples (*5*,*11*). The presence of Zika virus RNA in semen samples supports the theory of sexual transmission and has led to discussions

of Zika virus replication in the genitourinary tract (*5*). This hypothesis is supported by West Nile virus literature showing the detection of virus RNA in the renal cells of experimentally infected hamsters (*10*). Zika virus viruria and the presence of the virus in the peritubular cells of the testes (*12*,*13*) suggests that renal tissue might be a repository of the virus. However, in our study, Zika virus RNA was not detected in urine samples at all stages of pregnancy.

The use of urine for diagnosis represents an additional tool for virus detection. Urine is easy to obtain from pregnant women (because collection of this sample does not induce physiologic stress) and can be taken >5 days after the onset of clinical symptoms (*14*,*15*). The detection of Zika virus RNA (particularly in blood, urine, and semen) >5–7 days after symptom onset is a new feature in the body of knowledge available on flaviviruses. The spread of Zika virus and its consequences have led to a paradigm shift and a race to develop new technologies to help understand the virus and prevent infection.

Considering that some mothers had viruria just before delivery and newborns without adverse outcomes, the adverse outcomes of the 3 newborns might or might not have been related to the viruria. Because microcephaly and fetal abnormalities have been attributed to Zika virus, continuing to monitor women who had Zika virus infections during their pregnancies will be essential to manage adverse fetal outcomes. The monitoring of Zika virus viruria in pregnant women through the regular collection of urine samples over the course of the pregnancy proved to be a workable approach; however, the meaning of viruria and the consequences for newborns will need to be evaluated further. Whether virus replicates in fetal tissues and uses them as reservoirs remains to be determined.

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Changing Demographics and Prevalence of Body Lice among Homeless Persons, Marseille, France

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The prevalence of body lice among 2,288 sheltered homeless persons in the city of Marseille during 2000–2017 was 12.2% and significantly decreased over time. We report a positive association between body lice infestations and older age, duration of stays in France for migrants, frequent consumption of alcohol, and tobacco smoking.

Homeless persons are predisposed to infections because of their poor physical state and lack of hygiene; therefore, outbreaks of contagious diseases are more prevalent among them (*1*,*2*). Body lice infestation prevalence in homeless populations has been shown to be 19.0%–68.0% (*3*–*7*). However, despite the capacity of these ectoparasites to be vectors of several diseases, study of infestation has been minimal among the homeless. To identify potential risk factors for body lice infestation, we analyzed the demographics and chronic medical conditions of the homeless population from Marseille and their variations during 18 years.

The Study

The protocol for this study was reviewed and approved by the Institutional Review Board and Ethics Committee of Assitance Publique Hôpitaux de Marseille (2010-A01406–33). We conducted cross-sectional, 1-day surveys during 2000–2017 in 2 Marseille shelters (A and B) housing a limit of 300 homeless persons each with a high turnover. Most persons in shelters A and B stay for nights only with no time limitation, but shelter A has a special day/night unit with a 35-bed capacity, dedicated to high-risk sedentary homeless persons whose characteristics include a high level of poverty, poor hygiene, alcoholism, and mental illness. We informed participants of our ongoing study of infectious diseases and that they could receive a complete medical examination free of charge and treatment when needed. Participants volunteered and signed informed consent documents. A medical team interviewed participants by using a standardized questionnaire and physically examined them for the presence of ectoparasites (online Technical Appendix, https://wwwnc.cdc.gov/EID/article/23/11/17-0516-Techapp1.pdf). Several delousing measures were administered over time at the 2 shelters (online Technical Appendix).

We recruited 2,288 persons, 57% of whom were enrolled in shelter A (Table 1). Most participants were middle-aged men, most of whom were originally from North Africa and settled in France >12 years before the survey was done. A total of 334 (33.2%) migrants reported recurrent travel to their country of origin. Homelessness lasting >1 year accounted for 44.1% of cases.

The proportion of France- and Eastern Europe–born homeless persons decreased significantly over time (R^2 = 0.68 and 0.55, respectively) and the proportion from North Africa increased (R^2 = 0.64) (online Technical Appendix Figure 1). The mean duration of stays in France for migrants and the mean duration of homelessness decreased significantly over time (R^2 = 0.71 and 0.53, respectively; online Technical Appendix Figure 2).

Among participants, 75% reported smoking tobacco, 60% consuming alcohol, and 20% consuming cannabis (online Technical Appendix Figure 2). In addition, 25% of participants had an elevated body mass index; 5.8% were underweight. Tobacco smoking and frequent alcohol consumption decreased significantly during the study period (R^2 = 0.61 and 0.94, respectively; online Technical Appendix Figure 3).

We recorded a high prevalence of pruritus (548 persons [26.9%]), associated with scratch lesions in 306 (17.4%) persons. Overall, 382 (23.3%) participants had lice. The prevalence of differing types of lice was body lice, 12.2% (n = 242); head lice, 4.5% (n = 87); crab lice, 3.2% (n = 53); and scabies, 2.8% (n = 50). The prevalence of body lice decreased significantly during the study period (R^2 = 0.58) (online Technical Appendix Figure 3).

Body lice prevalence was higher in shelter A than in shelter B, increased with age and duration of homelessness, and was higher among persons born in France compared with others (Table 1). Body lice were less common in persons born in North Africa than in those born elsewhere. Body lice prevalence in migrants increased with duration

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Table 1. Demographics and results of univariate and multivariate analysis of risk factors for body lice infestation among homeless persons, Marseille, France, 2000–2017*

Demographics	Total	Body lice prevalence	Odds ratio (95% CI), p value	
			Univariate analysis	Multivariate analysis
Shelter used				
A†	1,305 (57.0)	90 (10.5)	1.33 (1.01–1.76), 0.041	3.45 (1.37–8.66), 0.008
B	983 (43.0)	152 (13.5)		
Sex				
M	2,161 (95.4)	236 (12.6)		
F	105 (4.6)	5 (5.7)		
Age, y				
Mean (SD)	43.1 (15.0)	NA		
Range	18–86	NA		
<25	221 (10.0)	4 (2.1)		
25–50	1,291 (58.5)	105 (9.5)		
>50	694 (31.5)	121 (19.8)	2.70 (2.04–3.57), <0.0001	2.47 (1.14–5.34), 0.022
Birthplace				
France mainland	597 (26.4)	95 (19.2)	2.18 (1.64–2.89), <0.0001	
France overseas territories	23 (1.0)	6 (30.0)		
North Africa	1,109 (49.0)	95 (9.6)	0.62 (0.47–0.81), <0.0001	
Sub-Saharan Africa	123 (5.4)	14 (13.0)		
Eastern Europe	270 (11.9)	16 (7.0)		
Western Europe	77 (3.4)	10 (14.3)		
Asia	51 (2.3)	3 (6.7)		
Other	11 (0.5)	0		
Duration of residence in France, y				
Mean (SD)	12.2 (16.8)	NA		
Range	0–75	NA		
<1	390 (38.8)	8 (2.3)		
1–5	176 (17.5)	4 (2.6)		
>5‡	438 (43.6)	41 (11.6)	5.46 (2.83–10.55), <0.0001	4.28 (1.79–10.23), 0.001
Visited country of origin since immigration	334 (33.2)	20 (7.4)		
Total no. visits to country of origin since immigration	672 (66.8)	27 (4.8)		
Duration of homelessness, y				
Mean	3.8	NA		
Range	0–57	NA		
<1	1,218 (55.9)	66 (6.3)		
1–5	443 (20.3)	52 (13.4)		
>5	518 (23.8)	112 (24.7)	3.67 (2.77–4.88), <0.0001	

*Values are no. (%) persons except as indicated. Blank cells indicate statistically insignificant results; variables with a prevalence <5.0% were not included in the univariate model. NA, not applicable.
†Includes high-risk homeless special unit (33 of the 300 beds in the shelter).
‡North African migrants only: body lice prevalence was 1.6% in persons living in France for <5 y vs. 9.3% in those living in France for >5 y (OR 6.39, 95% CI 2.41–16.95; p<0.0001).

of stay in France. Consuming alcohol frequently, smoking tobacco, and being underweight were associated with an increased risk for body lice (Table 2). In multivariate analyses, only housing in shelter A, older age, duration of stay in France for migrants, frequent consumption of alcohol, and smoking tobacco remained associated with an increased prevalence of body lice. Smoking, alcohol consumption, and underweight prevalence varied according to place of birth (online Technical Appendix).

Conclusions

In this survey, we observed significant changes over time in the demographic characteristics of the homeless population in Marseille. Overall, France-born, long-term homeless persons were progressively replaced by migrants of North Africa origin, who had a shorter duration of homelessness. Concurrently, the prevalence of frequent alcohol consumption and tobacco smoking decreased over time. France-born homeless persons were more prone to alcoholism and smoking habits, and those originating from North Africa were less likely to be frequent consumers of alcohol.

The decrease over time in overall body lice prevalence could be attributed to the changes in the characteristics of the population and also to the effects of delousing interventions conducted in the shelters. We identified several independent risk factors for body lice, including older age, residence duration in France of migrants, frequent alcohol consumption, and tobacco smoking. The latter 2 factors are likely correlative because they are markers of poor self-care, which may be associated with risk of body lice infestation.

Table 2. Results of univariate and multivariate analysis of risk factors for body lice infestation among homeless persons, Marseille, France, 2000–2017*

Risk factor	Total	Body lice prevalence	Odds ratio (95% CI), p value	
			Univariate analysis	Multivariate analysis
Substance use				
Alcohol				
Never	892 (39.8)	29 (3.7)		
Sometimes	623 (27.8)	55 (9.8)		
Frequently	729 (32.5)	155 (25.1)	5.01 (3.77–6.68), <0.0001	3.93 (1.85–8.36), <0.0001
Tobacco				
Never	576 (25.5)	20 (4.0)		
Yes	1680 (74.5)	218 (14.9)	4.16 (2.60–6.65), <0.0001	2.46 (1.04–5.79), 0.04
Cannabis (never)	921 (82.1)	70 (8.1)		
Cannabis	201 (17.9)	23 (11.9)		
Injected substances	19 (1.1)	5 (27.8)		
Nasally inhaled substances	40 (3.0)	7 (24.1)		
Drug substitutes	25 (1.5)	5 (21.7)		
Medical conditions				
COPD	38 (9.9)	11 (5.6)		
Asthma	63 (6.1)	3 (6.5)		
Bronchitis	44 (4.3)	4 (12.1)		
Cancer	6 (0.9)	0		
Diabetes	47 (5.8)	1 (2.2)		
Hepatitis	22 (2.8)	7 (33.3)		
History of pulmonary TB	55 (4.2)	3 (7.1)		
Weight				
Mean BMI (SD)	23.8 (4.2)			
BMI range	13.5–65.0			
Underweight	97 (5.8)	14 (17.9)	2.998 (1.53–5.90), 0.001	
Normal weight	1,009 (60.3)	93 (10.8)		
Overweight	439 (26.2)	23 (6.0)		
Obesity	129 (7.7)	11 (9.6)		

*Only statistically significant results are reported; blank cells indicate statistically insignificant results. Variables with a prevalence <5.0% were not included in the univariate model..

These results correlate with the observation of very high prevalence of body lice found in a specific survey by our team of 33 high-risk homeless persons from shelter A in which an 84.9% prevalence was found, compared to an estimated 22.0% prevalence in the overall population of shelter A over the same period of time (*8*). Those results corroborate our observation of a higher overall prevalence of body lice in shelter A than in shelter B. The subpopulation of the homeless persons with an elevated level of high-risk behaviors, housed in the special sector of shelter A, may have acted as a source of reinfestation for the other persons in this shelter. Unfortunately, being housed in shelter A's special unit was not documented on a regular basis in our surveys.

We found no other published study addressing risk factor analysis for body lice among sheltered homeless persons. In a Paris survey, A. Arnaud et al. conducted a risk factor analysis among homeless persons sleeping in public areas only, and body lice prevalence was associated with a history of pubic lice, begging, and not attending municipal showers (*9*). In a survey of homeless persons in San Francisco who were consulting for possible lice infestation, male gender, African American ethnicity, and sleeping outdoors were significantly associated with having body lice (*7*).

Our observation that the strongest determinant of body lice in the homeless was alcoholism correlates with previous observations that trench fever is associated with a history of alcoholism (*10*–*13*). Because body lice are known vectors of trench fever, which was the most frequently reported vectorborne infection identified in homeless persons in Europe and the US during 1990–2014, targeted removal of lice should specifically reduce its incidence in this population (*14*,*15*).

Our survey has several limitations. The homeless persons were not randomly selected, so those who had skin disease symptoms might have been more prone to enroll in the survey because a free medical examination was offered. Our results represent only homeless persons provided with shelter and cannot be extrapolated to those sleeping outside, where a higher prevalence of body lice has been reported (*9*).

Notwithstanding these limitations, these results demonstrate the value of investigating the homeless in shelters directly to estimate the prevalence of body lice and its risk factors. Our survey indicates that demographic factors, addictions, and being underweight are factors associated with body lice risk, which may be used to better target populations for delousing measures.

Dr. Ly is a medical doctor and a PhD student at Aix-Marseille University. His main research interest is epidemiology of diseases in mobile populations.

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Pulmonary versus Nonpulmonary Nontuberculous Mycobacteria, Ontario, Canada

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In Ontario, Canada, during 1998–2010, nontuberculous mycobacteria (NTM) from pulmonary sites comprised 96% of species/patient combinations isolated; annual rates of isolation and cases increased steadily. NTM isolates from nonpulmonary sites comprised 4% of species/patient combinations; annual rates and cases were temporally stable. NTM increases were driven exclusively by pulmonary isolates and disease.

Nontuberculous mycobacteria (NTM) cause pulmonary and nonpulmonary disease, but most isolates and disease cases are pulmonary (*1*). Studies have demonstrated temporal increases in pulmonary NTM isolation and disease (*2*,*3*). To determine trends in nonpulmonary NTM, we compared annual observed prevalence of pulmonary versus nonpulmonary NTM in Ontario, Canada, and compared the spectrum of NTM species isolated by body site.

The Study

We retrospectively reviewed positive NTM culture results obtained during 1998–2010 by the Public Health Ontario Laboratory, which identifies ≥95% of NTM isolates in Ontario (*4*). Until mid-2000, cultures were performed by using a Bactec 460 TB system, after which a BACTEC MGIT 960 system (Becton Dickinson, Franklin Lakes, NJ, USA) was used. DNA probes (AccuProbe; Hologic Inc., Marlborough, MA, USA) were used for speciation of *Mycobacterium avium* complex (MAC) and *M. gordonae* isolates; during 1998–2007, high-performance liquid chromatography was used to speciate others; thereafter, DNA probes (AccuProbe and GenoType line-probe assays [Hain Lifescience GmbH, Nehren, Germany]) were used. Because MAC was not speciated before 2008, we used this designation throughout the study.

We counted the persons for whom ≥1 positive culture for each NTM species/complex per year per body site was reported. Outcomes were pulmonary isolation (≥1 positive culture from sputum, bronchoscopy samples, pleural fluid/tissue, or lung tissue); pulmonary disease (≥2 positive sputum cultures of the same species within the calendar year, or ≥1 positive culture from bronchoscopy, pleural fluid/tissue, or lung tissue (by American Thoracic Society microbiological definition, positive predictive value 70%–100%) (*5*–*8*); and nonpulmonary isolation (≥1 positive culture from other sources). *M. gordonae* was considered a contaminant and excluded from pulmonary disease (*9*) but included in pulmonary isolation and nonpulmonary case counts. Outcomes were not mutually exclusive; we considered persons with NTM pulmonary disease to have pulmonary isolates and counted persons with pulmonary and nonpulmonary isolates in both groups. Careful electronic and manual selection ensured that each patient/species/anatomic site could be represented only once per year. We calculated prevalence of annual NTM isolation and NTM pulmonary disease as the number of persons from whom NTM was isolated or who had disease in a calendar year divided by the contemporary population (Statistics Canada, http://www.statcan.gc.ca/tables-tableaux/sum-som/l01/cst01/demo02a-eng.htm), expressed per 100,000 population. We used a generalized linear model with negative binomial distribution to assess annual rate changes and performed analyses with SAS version 9.4 (SAS Institute, Inc., Cary, NC, USA). All tests were 2-tailed with a type 1 error (α) rate of 5%. The University of Toronto Research Ethics Board approved this study.

During the study period, NTM was isolated from 26,067 patients. Pulmonary isolates predominated: mean annual unique species/patient/body site combinations were 2,631 pulmonary (96%) and 103 nonpulmonary (4%) (Table). Pulmonary and nonpulmonary NTM was isolated from 169 (0.6%) patients. Species distributions from pulmonary versus nonpulmonary sources were similar: MAC, followed by *M. xenopi*, from pulmonary and nonpulmonary sites, except for *M. marinum*, which was rarely pulmonary.

Annual rates of pulmonary NTM isolation and disease prevalence were 11.4 isolates and 4.65 cases per 100,000 population in 1998 and 22.2 and 9.08 per 100,000 in 2010 (Figure 1). As reported, frequency of pulmonary isolation and disease increased steadily (*3*,*4*). Annual prevalence of nonpulmonary NTM was 0.65–0.79 isolates/100,000 and did not change appreciably over time (Figure 2).

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Table. Average number of patients per year who had nontuberculous mycobacteria isolated, by body site and species/complex, Ontario, Canada, 1998–2010*

Species	Pulm	Skin/soft tissue	MS	Lymph	Blood/marrow	GI/GU	CNS	Other
MAC	1,328	8	1	2	17	18	0.8	3
Mycobacterium xenopi	568	0.2	0.3	0	0.3	10	0.2	0.5
M. gordonae†	338	0.2	0	0	0	6	0.1	0.3
M. fortuitum	131	2	0.8	0	2	3	0.2	1.2
M. abscessus	58	2	0.4	0.1	0.5	0.3	0	0.8
M. chelonae	40	3	1	0.2	0.5	0.7	0	1.0
M. simiae complex	42	0.6	0.3	0.1	0.5	0.7	0	0.5
M. kansasii	34	0.3	0.2	0	0.2	0.6	0	0.1
M. marinum	0	6	1.5	0	0	0	0	0.6
Other‡	92	0.8	0.5	0.1	1	2	0.1	0.3
All	2,631	22	6	3	22	41	1	8

*Contemporary Ontario population 11.3–13.2 million. CNS, central nervous system; GI, gastrointestinal system; GU, genitourinary system; lymph, lymphatic system; MAC, *Mycobacterium avium* complex; MS, musculoskeletal system; pulm, pulmonary system.
†*M. gordonae* was excluded from the number of pulmonary disease cases but included in pulmonary and nonpulmonary isolation cases.
‡Other species most commonly identified (% of grand total over entire study period, average no. patients per year), for pulmonary isolates were *M. mucogenicum* (0.93%, 24.5), *M. terrae* complex (0.43%, 11.4), *M. scrofulaceum* (19%, 5), *M. peregrinum* (0.17%, 4.4), *M. neoaurum* (0.14%, 3.8), *M. shimoidei* (0.13%, 3.3), *M. szulgai* (0.12%, 3.2), *M. celatum* (0.09%, 2.4), *M. malmoense* (0.08%, 2.2), *M. elephantis* (0.08%, 2.1), and *M. margeritense*/*M. smegmatis* (0.06% each, 1.5 each) and for nonpulmonary isolates included *M. mucogenicum* (1.3%, 1.38), *M. smegmatis* (1.0%, 1.08), *M. terrae* complex (0.5%, 0.54), *M. genavense* (0.4%, 0.38), *M. senegalense* (0.2%, 0.23), *M. malmoense*/*M. scrofulaceum*/*M. szulgai* (0.15% each, 0.15 each), *M. margeritense*/*M. shimoidei* /*M. elephantis* (0.07% each, 0.08 each).

Conclusions

Contrasting with documented increased pulmonary NTM isolation and disease in Ontario, rates of nonpulmonary NTM isolation are stable or decreasing, regardless of species or body site. The difference in trends indicates that pulmonary and nonpulmonary NTM represent different diseases with different risk factors. NTM pulmonary disease generally occurs in persons with preexisting structural lung damage (*8*) or abnormal mucociliary function (*10*) and is strongly associated with increasing age (*5*). Nonpulmonary NTM disease occurs in persons with generalized immunosuppression or after NTM entry into breached tissue (*9*). We considered the possibility that after the introduction of antiretroviral medication for HIV in the 1990s, a reduction in disseminated NTM may have masked an increase in nonpulmonary NTM infection in non–HIV-infected patients. However, the absence of decreased isolation from blood or bone marrow in the first several years and the steady number of cases (Figure 2) do not support this possibility. Although we lacked data regarding immune status, the small proportion of patients from whom pulmonary and nonpulmonary NTM were isolated (0.6%) does not suggest a large proportion of severely immunosuppressed patients.

There are several possible explanations for discordant temporal trends between pulmonary and nonpulmonary NTM. A selective increase in respiratory exposure would

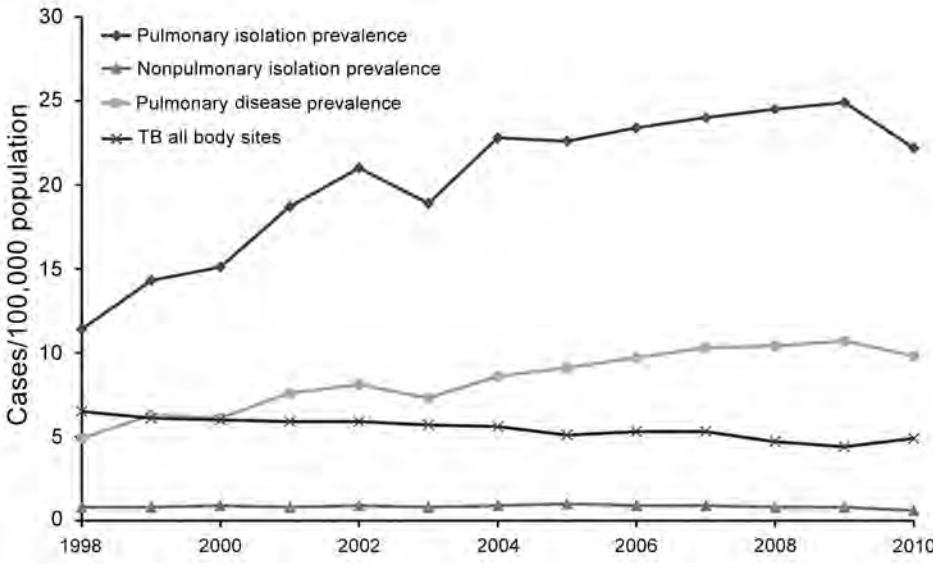

Figure 1. Prevalence of pulmonary and nonpulmonary nontuberculous mycobacteria (NTM) isolation and pulmonary NTM disease in Ontario, Canada, 1998–2010. Annual increase and modeled annual change were 6.3% (*3,4*) and 1.04 (95% CI 0.696–1.38)/100,000 population (p<0.001) for pulmonary isolation and 8.0% (*3*) and 0.402 (95% CI 0.307–0.497)/100,000 population (p<0.001) for pulmonary disease. Significant increases occurred in *Mycobacterium avium* complex (annual change 0.291 [95% CI 0.236–0.346]/100,000 population; p<0.001); *M. xenopi* (annual change 0.059 [95% CI 0.015–0.103]/100,000 population; p = 0.002); and *M. abscessus* (annual change 0.019 [95% CI 0.015–0.024]/100,000 population; p<0.001). TB (all body sites) isolation decreased by an average of 2.2% annually (6.5 to 4.9/100,000 population) during the study period. TB, tuberculosis.

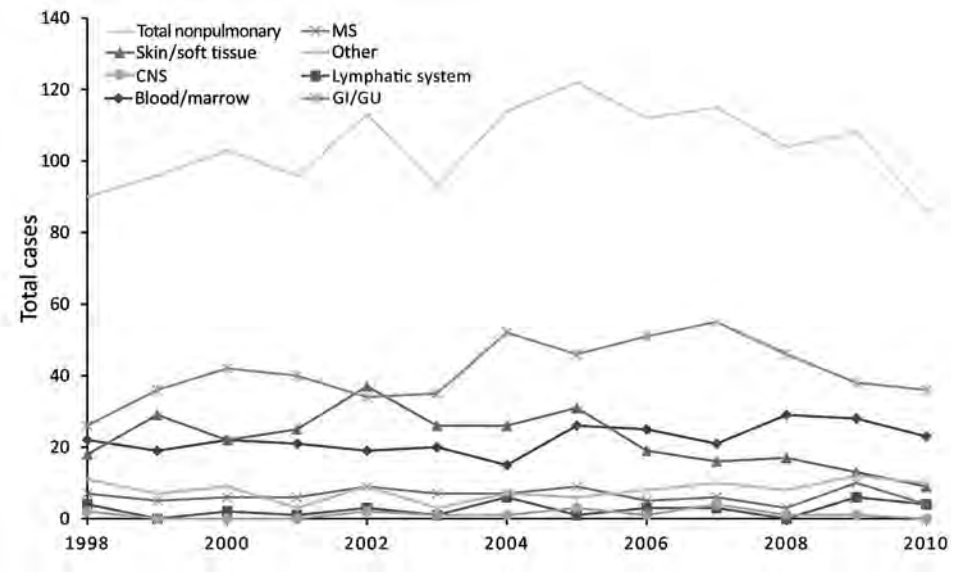

Figure 2. Isolation of nonpulmonary nontuberculous mycobacteria by body site, Ontario, Canada, 1998–2010. There was no significant temporal change by anatomic site except for a decrease in skin/soft tissue infections (modeled annual change –0.011 [95% CI –0.020 to –0.003]/100,000 population; p = 0.001). *Mycobacterium marinum* significantly decreased over time (modeled annual change -0.003 [95% CI –0.007 to 0.001]/100,000 population; p = 0.0480); isolation of other species from nonpulmonary sites was unchanged. Overall nonpulmonary isolation modeled annual change was -0.004 (95% CI –0.019 to 0.010)/100,000 population (p = 0.410). CNS, central nervous system; GI, gastrointestinal system; GU, genitourinary system; lymph, lymphatic system; MS, musculoskeletal system.

preferentially drive pulmonary NTM. However, although exposure to respirable waterborne NTM is undoubtedly widespread, data identifying recent increases are lacking. Other possibilities include changes in risk factors for NTM pulmonary disease (e.g., aging, chronic obstructive pulmonary disease, and iatrogenic causes [medications]) and improved diagnostic modalities (e.g., increased use of computed tomography in the United States [*11*]). Reduced turberculosis incidence, leading to reduced cross-immunity to NTM, has been proposed as an explanation for the reciprocal trends in tuberculosis and NTM infections observed in many areas (*2*); however, it is not known why waning immunity would result in increased pulmonary NTM only.

A previous population-based study compared the epidemiology of pulmonary and nonpulmonary NTM by examining isolates referred to the Netherlands national reference laboratory during 2000–2007 (*12*). The study indicated large increased numbers of NTM isolates, mostly *M. avium*. The average annual percentage increase was similar for pulmonary (31.3%) and extrapulmonary (33.0%) *M. avium*, differing markedly from our results. The differences could reflect referral bias (the Netherlands national reference laboratory received 85% of NTM isolates; the Public Health Ontario Laboratory received >95%), improvements in laboratory methods in the Netherlands potentially increasing detection of both pulmonary and nonpulmonary NTM, or differences in NTM epidemiology by region (*13*,*14*).

In Olmsted County, Minnesota, USA, incidence of cutaneous NTM infections apparently tripled from 1980–1999 to 2000–2009, driven partly by increased *M. abscessus/chelonae*, often from surgical/cosmetic procedures (*15*). Contrasting with our observation of reduced skin/soft tissue infections, their observations could result from differences in methods, geography, or number of surgical/cosmetic procedures.

A study limitation is lack of clinical data to confirm NTM pulmonary disease. Although our definition of NTM pulmonary disease has acceptable positive predictive value (*5*–*8*), it misclassifies some patients as having disease. By contrast, we underestimated NTM pulmonary disease because we counted only persons who met the definition each calendar year; there were probably persons with prevalent disease that was undiagnosed or diagnosed in a previous year but lacked ongoing sputum collection or for whom NTM isolation was staggered over 2 calendar years. The net effect of over/underestimating NTM pulmonary disease is unclear. We also classified all nonpulmonary isolates as representing disease, overestimating nonpulmonary disease, especially because feces and urine comprise most gastrointestinal/genitourinary sources. Excluding gastrointestinal/genitourinary isolates would increase the proportion of pulmonary isolations from 96.0% to 97.7%. Although gastrointestinal/genitourinary isolates comprise the largest nonpulmonary group, the trend for this group is similar to that for others (Figure 2), so its inclusion does not affect our conclusions. The lack of data regarding the number of samples submitted prevents assessment of the effect of sampling on NTM isolation. However, we previously identified an increased number of pulmonary samples submitted during 1997–2002, leveling off during 2002–2007 (*4*). Given the steady increase in pulmonary isolates and disease throughout the study

period, increased sampling does not explain our findings. Our lack of clinical data prevents comparison of features of pulmonary versus nonpulmonary NTM, and our lack of detailed epidemiologic data prevents assessment for regional or temporal nonpulmonary outbreaks from common point sources. The increase of NTM in Ontario reflects only pulmonary NTM.

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High-Level Fosfomycin Resistance in Vancomycin-Resistant *Enterococcus faecium*

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Of 890 vancomycin-resistant *Enterococcus faecium* isolates obtained by rectal screening from patients in Pittsburgh, Pennsylvania, USA, 4 had MICs >1,024 μg/mL for fosfomycin. These isolates had a Cys119Asp substitution in the active site of UDP-N-acetylglucosamine enolpyruvyl transferase. This substitution increased the fosfomycin MIC ≥4-fold and rendered this drug inactive in biochemical assays.

Vancomycin-resistant enterococci can cause nosocomial bacteremia, infective endocarditis, and intraabdominal and urinary tract infections that have limited treatment options. Fosfomycin is an antimicrobial drug that shows a wide spectrum of activity that includes enterococci, staphylococci, and many gram-negative species (*1*). Fosfomycin inactivates UDP-N-acetylglucosamine enolpyruvyl transferase (MurA) by covalent modification of a highly conserved cysteine residue in the active site of MurA (*2*). Some bacterial species, such as *Borrelia burgdorferi* and *Mycobacterium tuberculosis*, are naturally resistant to fosfomycin because they encode an aspartic acid residue instead of cysteine in the active site of MurA. Furthermore, in *Escherichia coli*, substitution of this cysteine at position 115 by aspartic acid results in fosfomycin resistance (*3*).

Fosfomycin has historically shown excellent in vitro activity against vancomycin-resistant enterococci, and therefore might be considered as a treatment option for urinary tract infection caused by this organism (*4*). However, information regarding the activity of fosfomycin against vancomycin-resistant enterococci in the setting of increasing fosfomycin use is limited (*5*). We tested vancomycin-resistant enterococcal isolates obtained from rectal screening cultures at the University of Pittsburgh Medical Center (Pittsburgh, PA, USA) during 2012–2016 for fosfomycin resistance.

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The Study

We tested 890 vancomycin-resistant enterococcal isolates by growth on Mueller-Hinton agar plates containing 100 or 200 μg/mL fosfomycin and 25 μg/mL glucose-6-phosphate (Sigma-Aldrich, St. Louis, MO, USA). Isolates that grew on both selective plates were subjected to determination of MIC by using the agar dilution method on Mueller-Hinton agar plates supplemented with 25 μg/mL glucose-6-phosphate (*6*).

Of 234 isolates that grew on fosfomycin-containing selective plates, MICs were 32 μg/mL for 10 (4.3%), 64 μg/mL for 92 (39.3%), 128 μg/mL for 120 (51.3%), 256 μg/mL for 7 (3.0%), 512 μg/mL for 1 (0.4%), and >1,024 μg/mL for 4 (1.7%). When we used the Clinical and Laboratory Standards Institute breakpoint for urinary tract infections (*6*), we found that 12 isolates (1.3%) had MICs ≥256 μg/mL and were considered resistant; an additional 120 isolates were considered to have intermediate resistance. The estimated resistance rate of 1.3% is consistent with that reported in a recent surveillance study conducted in the United States (*7*). However, the resistance rate would be much higher if we applied the European Committee on Antimicrobial Susceptibility Testing (Växjö, Sweden) breakpoint of ≤32 μg/mL for susceptible isolates and >32 μg/mL for resistant isolates.

The 4 vancomycin-resistant enterococci isolates with MICs >1,024 μg/mL were *Enterococcus faecium*. We subjected these isolates and a representative fosfomycin-susceptible *E. faecium* isolate (kindly provided by L. Harrison) to high-throughput paired-end sequencing by using NextSeq (Illumina, San Diego, CA, USA). We performed de novo assembly by using CLC Genomics Workbench version 10.0 (QIAGEN, Valencia, CA, USA). We deposited assembled genome sequences in GenBank (accession nos. SAMN07274321–5).

The 4 fosfomycin-resistant *E. faecium* isolates belonged to sequence type (ST) 17 (n = 2), ST18 (n = 1), and ST233 (n = 1) on the basis of in silico multilocus sequence typing and all had the *vanA* gene. These STs belong to clonal group 17, which is a prominent hospital-adapted vancomycin-resistant *E. faecium* clonal lineage associated with outbreaks in healthcare environments (*8*). None of the isolates had *fosB*, a transferable bacillithiol S-transferase gene associated with fosfomycin resistance (*9*). However, *murA* of the 4 fosfomycin-resistant isolates had a codon change of $TGT_{Cys119} \rightarrow GAT_{Asp119}$ at nucleotide position 355–357, which was not present in the fosfomycin-susceptible control isolate

or any of the available *E. faecium* genome sequences and was confirmed by Sanger sequencing (online Technical Appendix Figure, https://wwwnc.cdc.gov/EID/article/23/11/17-1130-Techapp1.pdf). The *murA* gene of the 8 remaining fosfomycin-resistant isolates with lower MICs of 256 or 512 μg/mL did not contain the nonsynonymous mutations corresponding to C119D. Therefore, the C119D substitution was specific to isolates with a fosfomycin MIC >1,024 μg/mL.

We amplified wild-type and mutant (C119D) *murA*, with their native promoters, by PCR with primers murA-F-*Eco*RI (5′-GAGA**GAATTC**CATAAAATGAGATGCG-GATG-3′) and murA-R-*Bam*HI (5′-GAGA**GGATCC**TTA-AGCAATCGTTTGTGCTG-3′) (bold indicates restriction endonuclease site sequences) and cloned them into the shuttle vector pTCV-*lac* (*10*). We selected *E. coli* TOP10 transformants by using kanamycin and erythromycin. After confirmation of sequences, we transformed recombinant plasmids into *E. coli* SM10, subsequently transferred them into *E. faecium* D344S by conjugation, and performed selection by using kanamycin, fusidic acid, and rifampin. The baseline fosfomycin MIC of the host strain was 128 μg/mL. Introduction of pTCV-lac-*murA*^{WT} resulted in a 4-fold increase in the MIC to 512 μg/mL, which might be caused by increased expression of WT MurA produced as a result of complementation. Nonetheless, introduction of pTCV-lac-*murA*^{C119D} yielded a higher MIC of >1,024 μg/mL, which indicated a ≥4-fold increase in the MIC compared with the *murA*^{WT} control (online Technical Appendix Table). This finding provided phenotypic evidence that C119D MurA is less susceptible to inhibition by fosfomycin.

We determined steady-state Michaelis-Menten parameters for recombinant purified wild-type and C119D MurA (Table; online Technical Appendix). The C119D substitution in MurA increased the mean ± SD Michaelis constant (K_m 803.2 ± 180.0 μmol/L) for UDP-N-acetylglucosamine compared with the wild-type enzyme (K_m 382.8 ± 79.5 μmol/L; p = 0.02), but did not affect the catalytic turnover (k_{cat}). This increase in K_m resulted in an ≈2-fold decreased catalytic efficiency (k_{cat}/K_m) for C119D MurA with respect to UDP-N-acetylglucosamine. In contrast, C119D had no major effect on the kinetic parameters for phosphoenolpyruvate as a substrate (Table). The mean ± SD 50% inhibitory concentration of fosfomycin for wild-type MurA was 176.8 ± 38.3 nmol/L; no inhibition of C119D MurA was observed at concentrations ≤100 μmol/L fosfomycin (Figure).

Our finding that high-level fosfomycin resistance in vancomycin-resistant enterococci can be conferred by substitution of the active site cysteine in MurA is consistent with the mode of action of fosfomycin, which covalently and irreversibly binds to the thiol group of this residue. The MurA enzymes in *M. tuberculosis* and *B. burgdorferi* are refractory to fosfomycin inhibition and naturally possess aspartic acid at the equivalent position (*11*,*12*). Previous site-directed mutagenesis–based studies of *E. coli* MurA showed that aspartic acid and glutamic acid substitutions, although conferring fosfomycin resistance, had a major effect on catalytic functioning of the enzyme (*3*). Specifically, the catalytic efficiency of C115D *E. coli* MurA was reported to be ≥10-fold less than the wild-type enzyme (*3*).

In our study, the C119D substitution had only minimal effect on vancomycin-resistant enterococci MurA activity. The reason for these differences in kinetic activity is unclear, and additional structure–function studies will be required to elucidate differences between these MurA proteins. Nevertheless, our kinetic data help explain why this substitution was selected in *E. faecium*, whereas *E. coli*–producing C115D MurA has not been identified clinically. The nonsynonymous mutations associated with the C119D substitution of MurA were observed only in isolates that had an MIC >1,024 μg/mL and not in any isolates with lower-level resistance to fosfomycin. Therefore, the mechanisms underlying low-level fosfomycin resistance in enterococci need to be determined.

Conclusions

In this study, fosfomycin maintained activity against most contemporary vancomycin-resistant enterococci isolates, but we identified high-level resistance caused by substitution of the active site cysteine in MurA, which made it refractory to inhibition by fosfomycin but retained its catalytic activity. Our finding that high-level resistance to fosfomycin might arise through mutations of the target enzyme MurA, accompanied by modest impairment of the catalytic activity, indicates the need for ongoing surveillance activities to ensure its activity against vancomycin-resistant enterococci is maintained. In addition, this finding highlights the potential relevance of aspartic

Table. Michaelis-Menten steady-state kinetic parameters for vancomycin-resistant *Enterococcus faecium* wild-type and C119D MurA*

	UNAG (p value)				PEP (p value)			
Enzyme	K_m, μmol/L	V_{max}, μmol/L/min	k_{cat}/min	k_{cat}/K_m, μmol/L/min	K_m, μmol/L	V_{max}, μmol/min	k_{cat}/min	k_{cat}/K_m, μmol/L/min
WT MurA	382.8 ± 79.5	13.9 ± 1.6	138.7 ± 16.4	0.4	229.0 ± 87.201	29.5 ± 8.4	294.5 ± 83.8	1.3
CD119D MurA	803.2 ± 1,780 (0.02)	11.9 ± 2.2 (NS)	119.4 ± 22.2 (NS)	0.2	304.6 ± 35.2 (NS)	28.6 ± 3.2 (NS)	285.5 ± 32.1 (NS)	0.9

*Values are mean ± SD for ≥3 independent experiments unless otherwise indicated. Statistical differences between kinetic parameters for vancomycin-resistant enterococci WT and C119D MurA were assessed by using a paired *t*-test. MurA, UDP-N-acetylglucosamine enolpyruvyl transferase; NS, not significant; PEP, phosphoenolpyruvate; UNAG, UDP-N-acetylglucosamine; WT, wild type.

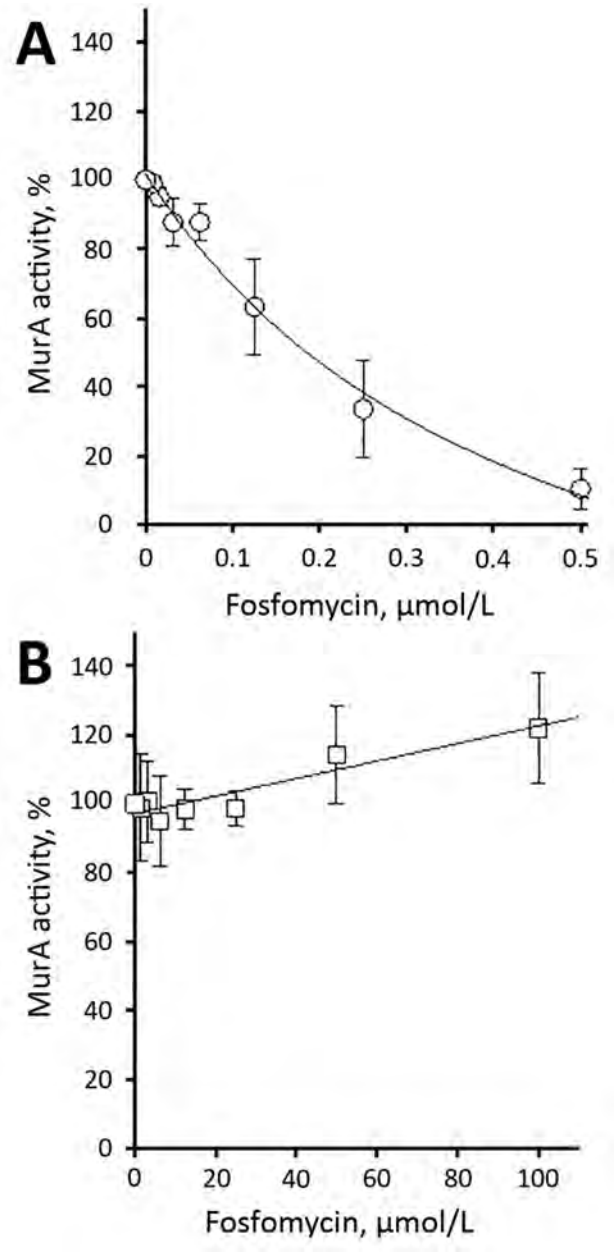

Figure. Inhibition of recombinant purified vancomycin-resistant *Enterococcus faecium* wild-type (A) and C119D (B) MurA by fosfomycin. The 50% inhibitory concentration was 176.8 ± 38.3 nmol/L for wild-type MurA and >100 μmol/L for C119D MurA. Error bars indicate mean ± SD of ≥3 independent experiments. MurA, UDP-N-acetylglucosamine enolpyruvyl transferase.

acid–substituted, catalytically active MurA enzymes as a target for inhibitor development.

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Prevention of Legionnaires' Disease in the 21st Century by Advancing Science and Public Health Practice

Ruth L. Berkelman, Amy Pruden

A dramatic shift in emphasis by public health officials from detection, investigation, and control of outbreaks of Legionnaires' disease to primary prevention strategies is underway in the United States. Expansion of primary prevention efforts is occurring with remarkable speed and is marked by new public health guidance and policy aimed at ensuring adequacy of water systems management (*1*–*5*). Professional societies are also providing more rigorous and detailed guidance. The American Society of Heating, Refrigerating and Air-Conditioning Engineers approved an industry standard in 2015 and established minimum management requirements to reduce the risk for infection with *Legionella* spp., the bacteria that cause Legionnaires' disease, in building water systems (*6*). In 2015, the American Industrial Hygiene Society also released specific guidance on control of *Legionella* spp. in engineered water systems and focused on validation of water management efforts and testing water for viable *Legionella* spp. counts as the key tool to validate effectiveness of these efforts (*7*). In June 2017, the Centers for Medicaid and Medicare Services issued a requirement to reduce risk for infection with *Legionella* spp. in healthcare facilities (*8*).

This sea of change in *Legionella* spp. program focus in public health is occurring because of a convergence of factors. The >5-fold increase in cases of disease caused by *Legionella* spp. during 2000–2015 has mandated a substantive public health response. The increase has been widely recognized as likely to continue unabated in the absence of more effective prevention strategies. Recent large outbreaks, such as those in the Bronx, New York, USA (*9*), have been critical in garnering public attention and generating momentum for more robust prevention efforts. The fact that analysis by the Centers for Disease Control and Prevention indicated that only 4% of Legionnaires' disease cases were outbreak-associated has further illustrated limitations of outbreak detection and control as the primary prevention tool (*1*). Supported by the Alfred P. Sloan Foundation, the first public health conference on *Legionella* spp. in 25 years identified gaping holes in our knowledge of prevention of infections with *Legionella* spp. and indicated the need for robust federally sponsored research (*10*).

Numerous factors are contributing to the increase in incidence of Legionnaires' disease. Increasing numbers of persons are at increased risk because of aging of the population, greater use of immunosuppressant drugs, and higher prevalence of comorbid conditions. Changing environmental conditions are also facilitating human exposure to aerosolized water containing *Legionella* spp. There is a growing dependence on heating, ventilation, and cooling systems, as well as increased complexity of indoor plumbing systems in large buildings, which have a labyrinth of water lines and features ranging from hundreds of showerheads in rooms along lengthy corridors to spas and indoor decorative fountains. Potential new sources, such as street cleaning machines reported by Valero Munoz et al. in this issue (*11*), continue to be identified. Inadequate maintenance of public water supplies might result in increased risk for contamination of building water systems and other water devices or equipment. Also in this issue, Lapierre et al. suggest potential new ways water systems can become contaminated; this article describes the potential for cross-contamination of cooling towers (*12*).

Healthcare providers play an essential role in diagnosing disease; diagnosis assists with targeting antimicrobial drug therapy, as well as playing a critical role in public health surveillance. The urine antigen test identifies only *L. pneumophila* serogroup 1, and its use minimizes the role of other *Legionella* subtypes and species (*13*). Respiratory diagnostic panels are increasing in number and need to include the capacity to identify all species of *Legionella* (*14*).

Ideally, a combined approach of surveillance and improved fundamental understanding of the factors triggering proliferation of *Legionella* spp. will inform optimal engineering design and industrial hygiene practice. *Legionella* spp. are known to have a symbiotic relationship with certain protozoa that graze on drinking water biofilms, and infection of these hosts can enhance virulence of the bacteria. Premise (i.e., building) plumbing can be a reservoir for pathogens such as *Legionella* spp., even in the presence of

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high disinfectant residuals (e.g., chlorine), and different microbes display distinct responses to various disinfectants, pipe materials, and water ages and their interactions.

Water-conserving technologies, such as reuse practices and high-volume water storage, can result in high water age, diminished protective disinfectant residuals, and degraded water quality. Low-flow velocities of certain water-conserving fixtures, now popular in many "green" buildings, might fail to adequately flush out biofilms and other particulates conducive to *Legionella* spp. growth. Similarly, reducing water heater temperatures may play a role in saving energy and reducing scalding, but if these benefits are found to be marginal relative to the potential to spread disease, these technologies should be reevaluated. Thus, as we as a society strive for new water- and energy-conserving building designs, the potential to stimulate *Legionella* spp. growth warrants serious consideration.

Respiratory pathogens other than *Legionella* spp., including nontuberculous mycobacteria (NTM) and *Pseudomonas* spp., are also found in engineered water systems. The prevalence of cases of infection with NTM is increasing dramatically and resulting in the hospitalizations of thousands of patients each year and months to years of multiple antimicrobial drug use (*15*). Whether current measures for prevention of *Legionella* spp. amplification will control other waterborne pathogens, such as NTM, is unclear. The US Environmental Protection Agency Safe Drinking Water Act, which has been in effect since the 1970s, is geared toward risk for fecal pathogens being ingested. This act does not take into account pathogens that adhere to biofilms in pipes and grow in various conditions.

Collaboration is needed across scientific disciplines and agencies to fill gaps in our collective knowledge of modern water pathogens, weaknesses in our physical water infrastructure, and inadequacies of our existing diagnostic and regulatory approaches to effectively identify and control disease risks. *Legionella* spp. illuminate many of the challenges posed by water pathogens in the 21st century. With a growing national interest in a safe water supply, it is an opportune time for public health and medicine to come together with industrial hygiene, engineering, microbiology, and environmental protection to understand the problems and ensure effective risk management of *Legionella* spp. and other pathogens inhabiting our water systems.

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Blood Culture–Negative Endocarditis, Morocco

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We investigated the microorganisms causing blood culture–negative endocarditis (BCNE) in Morocco. We tested 19 patients with BCNE by serologic methods, molecular methods, or both and identified *Bartonella quintana*, *Staphylococcus aureus, Streptococcus equi*, and *Streptococcus oralis* in 4 patients. These results highlight the role of these zoonotic agents in BCNE in Morocco.

Blood culture–negative endocarditis (BCNE) can occur when the patient has previously received antibiotic drugs or in the presence of slow-growing or intracellular microorganisms (*1*,*2*). In Morocco, epidemiologic data on endocarditis are fragmentary and show that this disease is frequently associated with rheumatic heart disease (*3*). The aim of this multicenter preliminary study was to investigate the microorganisms causing BCNE in Morocco by using a multimodal strategy.

During June 1, 2009–September 1, 2010, we prospectively included all patients with BCNE seen in 3 health centers in the Morocco regions of Casablanca and Marrakech. The specimens were then referred to the Institut Pasteur du Maroc in Casablanca before being transported to Marseille, France, where diagnostic assays were performed as described by Fournier et al. (*4*). For each studied patient, the physician in charge completed a questionnaire. Answers were not obtained for all questions from all patients.

We used indirect immunofluorescence assays to detect significant levels of antibodies to *Coxiella burnetii* IgG titer to phase I ≥1:800), *Bartonella quintana, B. henselae* (IgG titer ≥1:800), and *Legionella pneumophila* (total antibody titer >1:256), as previously described (*4*). We detected specific antibodies to *Brucella melitensis* by using an immunoenzymatic antibody test (titer >1:200) and to *Mycoplasma pneumoniae* by using the Platelia *M. pneumonia* IgM kit (Bio-Rad, Marnes-la-Coquette, France). When results of first-rank tests were negative, we systematically performed the Western blot test by using *Bartonella* antigens (*4*).

We extracted bacterial DNA from excised valves or EDTA blood by using the QIAmp Tissue kit (QIAGEN, Hilden, Germany) and performed PCR (*5*). We examined paraffin-embedded heart valves and used hematoxylin and eosin stain for histopathologic features (*5*). To detect microorganisms within tissues, we systematically performed the Giemsa, Gram (Brown-Brenn and Brown Hopps), periodic-acid Schiff, Grocott-Gomori, Warthin-Starry, Gimenez, and Ziehl-Nielsen stains (*4*). For patients for whom results of all other techniques remained negative, we performed autoimmunohistochemistry as described by Lepidi et al. (*6*).

Our prospective study enabled the identification of 19 patients (Table 1): 11 men, 7 women, and 1 person of unspecified gender. Mean age was 40.26 years (range 22–57 years). Among these, 6 lived in urban areas (socioeconomic conditions unknown) and 7 in periurban communities under conditions of poverty. No information on residential environment could be obtained for the remaining 6 patients. All patients except 1 had received antibacterial drugs before blood sampling.

Samples from all 19 BCNE patients were tested by serologic methods, molecular methods, or both. Among

Table. Demographic and clinical features of patients with blood culture–negative endocarditis included in the study.*

Features	Value	% Patients
Sex		
M	11	61%
F	7	38%
Unspecified	1	
Mean age, y	40.26	
Valve involved		
Native valve	18/19	94.7
Valvular bioprosthesis	1/19	5.3
Aortic	4/17	23.5
Mitral	10/17	58.8
Aortic and mitral	1/17	5.9
Tricuspid	1/17	5.9
Echocardiographic signs of endocarditis		
Left-sided endocarditis	2/17	11.8
Right-sided endocarditis	10/17	58.8
Pacemaker infection	1/17	5.9
Valvular vegetation	12/17	70.6
Valvular abscess	6/17	35.9
Clinical symptoms		
Fever (temperature >38.5°C)	17/17	100
Cardiac murmur	16/17	94.1
Glomerulonephritis	1/17	5.9
Other data		
Drug abuse	1/17	5.9
Alcohol dependence	3/17	17.6

*Values are no. patients/no. for which data were available except as indicated.

these, we identified an etiologic agent for 4 patients. A 48-year-old man living in impoverished conditions had a positive *Bartonella* serologic test result (IgG 1:6400); Western blot analysis of the serum sample resulted in the specific diagnosis of *B. quintana* infection. In addition, 3 cardiac valves from 3 patients tested by using 16S rRNA PCR were positive, 1 each for *Staphylococcus aureus*, *Streptococcus equi*, and *Streptococcus oralis*. PCR performed with a second gene confirmed all 3 PCR results. None of these 3 patients lived in impoverished areas.

In Morocco, cases of BCNE represent two thirds of all cases of infectious endocarditis and constitute a major problem of diagnosis and management of patients (*1*). In other countries, the negativity of blood cultures in BCNE may be explained mostly by the administration of antimicrobial drugs before blood culture collection or by the causative role of fastidious microorganisms, as is the case for zoonotic pathogens, such as *C. burnetti*, *B. quintana*, and *B. henselae* (*7*). However, in Morocco, cases of endocarditis caused by these zoonotic pathogens are poorly diagnosed. Previous studies have demonstrated the role of *C. burnetii*, but we could find no study that directly detected *Bartonella* in BCNE.

In the neighboring country of Algeria, *B. quintana* is considered to be the most common agent of infectious endocarditis, with a prevalence of 15.6%, compared with 5% in countries in Europe (*8*). This difference is likely explained by differences in living conditions (*9*). *B. quintana* infections occur preferentially in disadvantaged populations (homeless) infected by body lice. In our study, 7 patients for whom information on their residential environment was available lived in conditions of poverty and poor hygiene, including the patient who had *B. quintana* endocarditis.

Bartonella spp. can infect any heart valve and cause destructive valvular lesions often requiring surgical replacement. Despite the high antimicrobial drug susceptibility of these bacteria, the mortality rate from *Bartonella* endocarditis may reach 31% (*10*). In addition, the recommended treatment for these infections is a combination of doxycycline and gentamicin, which is different from that for endocarditis caused by other common bacterial species, and therefore a specific diagnosis is highly desirable (*10*). However, the small number of patients included in our study precludes the issuance of a definitive recommendation to include serologic testing, molecular testing, or both for zoonotic pathogens in patients with endocarditis in Morocco.

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Zika Virus Persistence and Higher Viral Loads in Cutaneous Capillaries Than in Venous Blood

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We collected venous and capillary serum samples from 21 Zika virus–infected patients on multiple days after symptom onset and found RNA load was higher and median duration of virus detection significantly longer in capillary than in venous blood. These findings raise questions about the role of the capillary compartment in virus transmission dynamics.

Zika virus, belonging to the family *Flaviviridae* and genus *Flavivirus*, is transmitted to humans by mosquito bites but can also be contracted through sexual and vertical transmission (*1*). Zika virus was first detected in Brazil in 2015 and has since spread with the same speed as chikungunya virus through South and Central America and the Caribbean Islands, despite a shorter and lower viremia in humans and a longer replication period in the vector (*2*,*3*). As with other arboviruses, Zika virus viremia is commonly measured in venous blood, even though mosquitoes introduce virus into cutaneous capillary blood. We conducted a prospective descriptive study with Zika virus patients during the Zika virus epidemic in French Guiana to evaluate the kinetics of Zika virus RNA load in serum samples collected sequentially from venous and skin capillary blood.

The study population comprised 21 symptomatic and consenting Zika virus patients infected during March–September 2016. We confirmed Zika virus infection by real-time reverse transcription PCR (RT-PCR) of serum and urine samples provided by patients during the first few days after symptom onset. We obtained serum samples from the venous and cutaneous capillary blood (collected from the fingertip) sequentially 1–18 days after the onset of symptoms. The median age of the population was 40 (range 28–63) years and the sex ratio (male:female) was 1.6. We observed no co-morbidities, and all participants were found to be free of dengue and chikungunya virus infections by methods previously described (*4*,*5*).

We extracted RNA from 150-µL samples using the QIAamp Viral RNA kit (QIAGEN, Hilden, Germany) and performed Zika virus RNA amplification using the RealStar Zika Virus RT-PCR Kit 1.0 (Altona Diagnostics GmbH, Hamburg, Germany) according to the manufacturer's instructions. We performed Zika virus RNA quantification using a reference strain provided by the European Zika Virus Archive (SKU no. 001N-01648) and estimated the Zika virus RNA load as $\log_{10}$ copies per milliliter (online Technical Appendix Table, https://wwwnc.cdc.gov/EID/article/23/11/17-0337-Techapp1.pdf).

Zika virus RNA loads in capillary blood correlated with those in venous blood (Spearman correlation test, $r = 0.54$, $p<0.0001$) but were significantly higher in the capillary samples (Wilcoxon signed rank test, $p = 0.0003$), except for 3 patients (nos. 2, 13, and 21; online Technical Appendix Table). The median duration of Zika virus detection after symptom onset was significantly greater in capillary blood than in venous blood ($p = 0.005$ by log rank test; hazard ratio = 2.99, 95% CI 1.39–6.43), even though the duration of detection in capillary blood was underestimated; RNA was still detectable in the last capillary blood samples taken from 8 patients (nos. 1, 5, 6, 8, 11, 15, 16, and 19; online Technical Appendix Table). The duration of Zika virus RNA detection was greater in capillary than in venous blood for 12 (57%, 95% CI 34%–78%) of the 21 patients (nos. 1, 3, 5, 6, 7, 8, 11, 14, 15, 16, 19, and 20). The maximum duration of RNA detection in capillary blood samples was 18 days after the onset of symptoms (seen with patient no. 19) with a load of 1.9 $\log_{10}$ copies/mL. The duration of detection was equal between the 2 compartments for 7 (33%, 95% CI 15%–57%) of 21 patients (nos. 4, 9, 10, 12, 13, 17, and 18) and longer in venous blood for 2 (10%, 95% CI 1%–30%) of 21 patients (nos. 2 and 21) (online Technical Appendix Table).

These data raise questions about the consequences of longer persistence and higher loads of Zika virus RNA in the cutaneous capillary blood compartment, although we did not test Zika virus replication capacity. The higher load and longer detection of Zika virus RNA in this compartment might be attributable to Zika virus replication in permissive cells of the skin (e.g., human dermal fibroblasts and epidermal keratinocytes); capillaries; or both (*6*). If the Zika virus RNA observed in the serum samples taken from the capillary compartment reflects the presence of infectious virus particles, symptomatic Zika virus–infected patients would need to be shielded from mosquitoes for a longer period than is currently practiced to limit potential vectorborne transmission.

This study comparing the kinetics of Zika virus RNA load between the venous and capillary compartments

highlights the need to further investigate the infectivity and pathophysiology of the virus located in the often neglected capillary compartment. These findings provide new information on this biologic compartment, which plays a key role in vectorborne transmission and transmission dynamics. Moreover, these observations, if validated with more patients and extended to other vectorborne infections, will be vital for preventing and controlling the transmission of Zika virus and other arboviruses.

Institutional review board approval was granted by the Comité de Protection des Personnes Sud-Méditerranée I corresponding to the following study "Etude descriptive prospective de la maladie à virus Zika au sein de la communauté de défense des Forces Armées en Guyane" and was registered February 2016 under the number RCB: 2016-A00394-47. Written informed consent was obtained from each patient as required by the Comité de Protection des Personnes Sud-Méditerranée I.

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Detection of Spotted Fever Group *Rickettsia* DNA by Deep Sequencing

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After conventional molecular and serologic testing failed to diagnose the cause of illness, deep sequencing identified spotted fever group *Rickettsia* DNA in a patient's blood sample. Sequences belonged to *R. honei*, the causative agent of Flinders Island spotted fever. Next-generation sequencing is proving to be a useful tool for clinical diagnostics.

When conventional laboratory tests cannot identify an etiologic agent, unbiased deep sequencing performed directly on a clinical sample has the potential to identify a probable cause of disease. We used deep sequencing to detect spotted fever group (SFG) *Rickettsia* DNA in the blood of a patient for whom diagnosis was not possible through conventional molecular and serologic testing.

In late 2016, a middle-aged woman was admitted to a regional hospital in Queensland, Australia, after 2 weeks of mild cough, myalgia, fever, and lethargy. The day before admission, she experienced a blanching rash and pains in her feet, after which her condition deteriorated and a definite petechial rash appeared. Chest radiographs showed atelectasis on 1 side. Meningococcal septicemia was suspected, and the patient was transferred to intensive care with septic shock. Despite treatment with inotropes and several antimicrobial drugs (including ceftriaxone, vancomycin, meropenem, doxycycline), the patient died the next morning.

Clinical testing did not identify an infectious disease agent in the patient's blood; serologic test results

for *Rickettsia* were negative. Because a limited amount of specimen remained for testing, we applied an unbiased deep-sequencing approach. We extracted DNA from the blood sample by using the MasterPure Complete DNA Purification Kit (Epicenter, Madison, WI, USA) and sequenced with the Ion Torrent PGM (Personal Genome Machine) workflow by using the Ion PGM IC 200 Kit and the Ion 316 Chip Kit, version 2 (Life Technologies, Carlsbad, CA, USA). A total of 3,627,903 sequences were generated and trimmed by using a minimum quality score of Q15 and minimum length of 50 bp. Of the reads generated, 251 matched bacterial DNA sequences (uploaded to GenBank as Bioproject PRJEB21107). The rest either matched human genome sequences and were filtered out (3,619,386 reads) or were unclassified (8,252 reads).

We analyzed the reads for bacterial DNA by using 3 metagenomics tools: Kraken (*1*), PathoScope (*2*), and One Codex (https://www.onecodex.com). All 3 analyses returned similar results; ≈80% of classified reads (208/251 reads, 53,958 total nucleotides) matched sequences from SFG *Rickettsia* spp.; the remainder gave low-number, low-quality matches to other bacteria. Screening of reads for sequences matching 5 rickettsial genes (*rrs*, *ompA*, *ompB*, *gltA*, and *sca4*) found 1 read mapping to the *ompB* gene (online Technical Appendix, https://wwwnc.cdc.gov/EID/article/23/11/17-0474-Techapp1.pdf). This read was a 100% match (272/272 nt) to *R. honei ompB* (GenBank accession nos. AF123724.1, AF123711.1). The next highest match was to *R. parkerii ompB* (accession no. KY113111.1) at 99% (270/272 nt). We confirmed the presence of SFG *Rickettsia* DNA in the DNA extract of the sample by nested PCR and performed Sanger sequencing by using the Invitrogen SuperScript III One-Step RT-PCR system with primers (*3*) and in-house nested primers.

To narrow down the identification to species level, we further analyzed sequences matching *Rickettsia* spp. We downloaded all *Rickettsia* genomes available at the National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/), complete and draft, and used them as reference sequences for mapping of the reads in CLC Genomics Workbench 8 (QIAGEN Aarhus, Silkeborgvej, Denmark). We discarded reads mapping to >1 genome, collected the remaining reads that mapped uniquely to a single genome, and noted the genome to which they mapped. Of the 208 reads, 67 mapped to >1 genome, 1 did not map to any of the genomes and was subsequently identified as matching that of the human reference genome, 3 were unique matches to *R. conorii* (AJUR01, GenBank accession no. NC_003103), 1 was a unique match to *R. sibirica* (accession no. NZ_AHZB01000018), and 151 were unique matches to *R. honei* (accession no. NZ_AJTT00000000) (online Technical Appendix) (*4*). Mapping of the 208 sequencing reads revealed that 207 (99.6%) reads mapped to the *R. honei* genome, giving 1.43% coverage of the genome, and 168 (80.7%) reads mapped to the *R. australis* (accession no. NC_017058) genome, representing 0.03% coverage of the genome.

The main causes of SFG rickettsioses in Australia are *R. australis* and *R. honei*, which cause Queensland tick typhus and Flinders Island spotted fever, respectively (*5*). The rickettsial DNA in the blood sample we describe most closely matched sequences from *R. honei* and had a relatively low level of similarity to sequences from *R. australis*. *R. honei* was initially reported only in the southern states of Australia; however, a genetic variant known as the "marmionii" strain has since been reported in eastern and northern parts of the country (*6*). Unfortunately, the genome of *R. honei* "marmionii" has not been sequenced, and the genes used to differentiate between *R. honei* and *R. honei* "marmionii" were not covered by the sequences generated from the sample. Therefore, we could not confirm which strain of *R. honei* was in the sample.

Flinders Island spotted fever is reportedly associated with relatively mild illness (*5*). However, our detection of *R. honei* DNA in the blood of a deceased patient, in the absence of positive *Rickettsia* serologic test results, is suggestive of acute infection with this agent. This case demonstrates the potential of deep sequencing for identifying unknown etiologic agents, particularly when other methods have not done so.

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Chlamydia trachomatis Biovar L2 Infection in Women in South Africa

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We detected *Chlamydia trachomatis* biovar L2 in vaginal swab specimens of 7 women with vaginal discharge in South Africa. Whole-genome sequencing directly from clinical specimens identified a closely related cluster of strains. The clinical role of this infection in the context of syndromic management should be clarified.

Infection with *Chlamydia trachomatis* biovar L is known as lymphogranuloma venereum (LGV). This infection usually presents as genital ulcers, followed by an invasion of the lymphatic system resulting in buboes, painful swelling of lymph nodes (*1*). In the past 2 decades, another manifestation of LGV has emerged in North America and Europe: rectal LGV infection causing proctocolitis among men who have sex with men (MSM) (*1*). In this population, urethral LGV also occurs (*2*).

There have been only sporadic reports of rectal and genital LGV infection in women living in the industrialized world (*3*,*4*). Cross-sectional studies from France, Switzerland, and the Netherlands did not detect biovar L in specimens from women with genital or rectal *C. trachomatis* infection (*1*,*5*–*7*). Because lymphatic manifestation has become relatively rare, LGV infection is considered an outbreak mainly among MSM in Europe and North America (*1*). Lymphatic LGV is endemic to Africa, but before our study, it was unknown whether *C. trachomatis* biovar L infections occurred in women in Africa. Thus, we determined the prevalence of this infection in South Africa.

To determine whether genital *C. trachomatis* biovar L infections occur in women living in South Africa, we analyzed 82 DNA samples extracted from vaginal swab specimens that were positive by a molecular detection assay for *C. trachomatis* infection at the Department of Medical Microbiology at the University of Pretoria. The Faculty of Health Sciences Research Ethics Committee at the University of Pretoria approved the studies in which these specimens were collected. These swab specimens had been collected during 2012–2016 from women attending different healthcare settings: a mobile health clinic in rural Mopani District (n = 52) and 3 departments at the academic hospital in Pretoria: obstetrics and gynecology clinic (n = 14), antiretroviral treatment clinic (n = 10), and sexually transmitted infection (STI) clinic (n = 6). We assessed the presence of LGV in these genital specimens by using specific PCRs for *C. trachomatis* serovar L and serovar L2b (*8*). For positive PCR results, we confirmed the diagnosis by conducting whole-genome sequencing (WGS) of *C. trachomatis* directly from the clinical specimen as described elsewhere (*9*).

Whereas *C. trachomatis* biovar L–specific PCR showed positive results for 7 specimens obtained from women at the antiretroviral treatment (n = 5) and STI (n = 2) clinics in Pretoria, we did not detect LGV in any of the 52 specimens from women in Mopani District. All PCR test results for serovar L2b were negative. The 7 women with genital LGV all had vaginal discharge and were co-infected with another STI (Table).

WGS confirmed LGV (*ompA* sequence identical to those of the *C. trachomatis* L2 434/BU reference strain) in 4 cases with good mean read depth (≥12) and high genome coverage (>98%). The 4 sequences clustered well with the L2 sequences previously published and away from L1 and L2b sequences. For 1 specimen, the mean read depth

[1]These authors contributed equally to this article.

Table 1. Characteristics of 7 women with vaginal discharge and a positive PCR result for *Chlamydia trachomatis* biovar L, Pretoria, South Africa, 2012–2016*

Patient ID	Healthcare setting	HIV status	Co-infection	*C. trachomatis* WGS result	Mean read depth	Genome coverage, %
1	ART clinic	Positive	*Trichomonas vaginalis*	L2 confirmed	41	99.5
2	ART clinic	Positive	*T. vaginalis*	L2 confirmed	12	98.3
3	ART clinic	Positive	*Mycoplasma genitalium*	L2 confirmed	21	98.6
4	ART clinic	Positive	*M.a genitalium*	L2 confirmed	72	99
5	ART clinic	Positive	*T. vaginalis*	Insufficient WGS read coverage	0.5	29
6	STI clinic	Unknown	*Neisseria gonorrhoeae*	Insufficient clinical material	ND	ND
7	STI clinic	Unknown	*N. gonorrhoeae*	Insufficient clinical material	ND	ND

*ART, antiretroviral therapy; ID, identification; ND, not determined; STI, sexually transmitted infection; WGS, whole-genome sequencing.

was insufficient to allocate a biovar; insufficient DNA was available from 2 other samples for WGS.

This report shows emergence of *C. trachomatis* biovar L2 genital infection in women living in South Africa, a region to which lymphatic LGV is endemic (*1*). Instead of serologic analysis, we used molecular testing and WGS of clinical specimens to confirm the diagnosis, determine genetic relatedness, and identify the specific variant of genotype L. We observed LGV in specimens from women at the academic hospital in Pretoria, but not from women living in the Mopani District, ≈400 km away. Although the distribution of risk factors may be different, the close relatedness of LGV strains suggests that this might be a localized outbreak of genital *C. trachomatis* L2 infection among women living in Pretoria.

The clinical role of genital *C. trachomatis* biovar L infection in women remains to be determined. Analogous to non-LGV *C. trachomatis* infection in women and rectal LGV in MSM, the clinical spectrum of genital LGV in women may vary from a mucosal ulcer with intrapelvic lymphadenopathy to cervicitis with vaginal discharge, or it may manifest without any symptoms at all as persistent asymptomatic infection. Although rectal *C. trachomatis* infections have been reported in African women, the occurrence of rectal LGV is unknown (*10*).

The emergence of genital LGV in women poses a concern in our setting, which uses syndromic management for STIs, because it is unclear whether the infection would be treated adequately with the empirical regimen of azithromycin and ceftriaxone. The main limitation of this report is the lack of follow-up data to confirm whether syndromic management was effective for these biovar L *C. trachomatis* genital infections.

In conclusion, this report shows the emergence of genital *C. trachomatis* L2 infection in South African women. Further research about its distribution in the general population, clinical role, and the occurrence of rectal infections is warranted because it is unclear whether this STI is managed adequately under the current syndromic management guidelines.

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Unrecognized Dengue Virus Infections in Children, Western Kenya, 2014–2015

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We detected a cluster of dengue virus infections in children in Kenya during July 2014–June 2015. Most cases were serotype 1, but we detected all 4 serotypes, including co-infections with 2 serotypes. Our findings implicate dengue as a cause of febrile illness in this population and highlight a need for robust arbovirus surveillance.

Due to lack of national surveillance programs, the extent of infection with dengue viruses (DENV) among children is largely unknown in much of sub-Saharan Africa (*1*). Uncovering this hidden burden is critical for making informed public health decisions that affect populations that are most vulnerable to vectorborne disease. To address this knowledge gap, in January 2014, we initiated a 4.5-year study of the transmission and extent of arbovirus infection in children in Kenya. Although the study continues through June 2018, we identified a cluster of DENV infections among febrile children in western Kenya, prompting this report to raise awareness of DENV as a cause of acute febrile illness (AFI) among children in this country.

We describe results from a cohort of children with AFI who came to 1 of 2 regional health centers that serve the communities of Chulaimbo (a rural village) and Kisumu (an urban city), both located in western Kenya. The cohort is part of a larger ongoing parent study that enrolls additional child cohorts at other study sites or with other study designs and collects vector and environmental data. Details of the parent study are beyond the scope of this report and will be described elsewhere. The study is being conducted under the supervision of the institutional review board of Stanford University (Stanford, CA, USA; IRB-31488) and the scientific and ethics review unit of the Kenya Medical Research Institute (Kisumu, Kenya; SSC 2611).

For this study, we enrolled febrile children 1–17 years of age. At the enrollment and 1-month follow-up visits, we collected information on demographics and risk factors, performed a physical examination, and obtained blood samples. We tested the blood samples for DENV RNA by reverse transcription PCR (RT-PCR) (*2*).

We enrolled 1,258 children with AFI during January 2014–April 2016. We tested blood samples from 1,104 study participants (87.8%) by RT-PCR. Of those, 82 (7.4%) were positive for DENV RNA: 58 (70%) were serotype 1 (DENV-1), 2 (2.4%) were serotype 2 (DENV-2), 13 (15.9%) were serotype 3 (DENV-3), and 2 (2.4%) were serotype 4 (DENV-4) (Figure). We also detected co-infection with 2 serotypes in 6 participants: 2 children had DENV-1 and -3; 3 children had DENV-1 and -4; and 1 child had DENV-2 and -3.

Most DENV cases occurred during July 2014–June 2015. The likelihood of testing positive for DENV by PCR did not differ by age or sex. The median age of DENV PCR-positive participants was 3.5 years (IQR 2.0–5.2 years); 43.9% of participants were female. We did observe a higher likelihood of testing positive by PCR for children from the rural site, Chulaimbo (9.6%), compared with those from the urban site, Kisumu (5.8%) ($p = 0.017$ by Fisher exact test; odds ratio 1.97, 95% CI 1.14–3.43, adjusted for age and sex). Continued surveillance is needed to investigate whether DENV is endemic to these areas and if there are differences in regional endemicity.

Although reports of DENV infection in Kenya remain sparse, several sero-epidemiologic studies have produced evidence of DENV transmission by demonstrating serum DENV IgG in patients of all ages (*3*–*6*). In 2013, an outbreak of DENV-2 in Mombasa was detected, in part, by RT-PCR (*7*). More recently, DENV infections were described in 43 adult patients with fever who resided in Mtwapa, on the coast of Kenya near Mombasa; these cases occurred February 2014–January 2015 (*8*), which overlapped with the period in which we observed the DENV cases we report in this

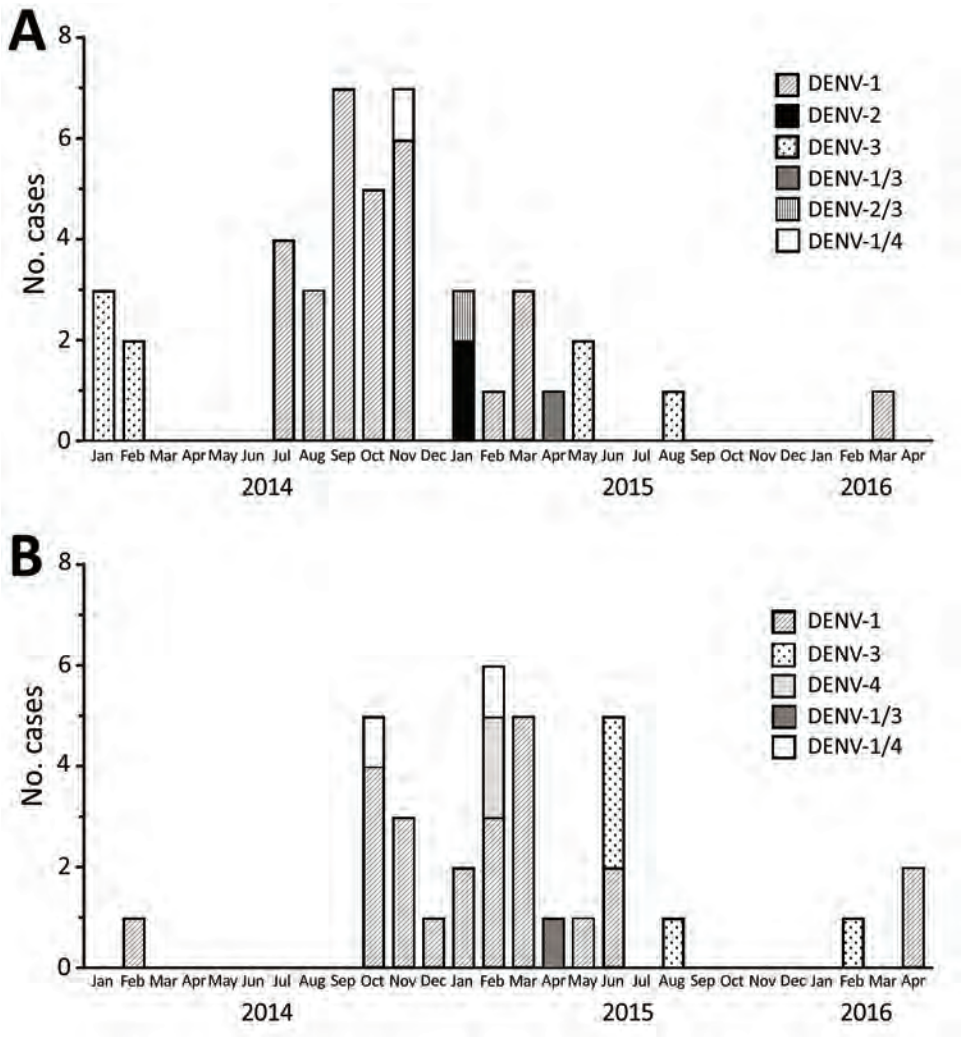

Figure. Epidemic curve for DENV infection in children in 2 locations in western Kenya, 2014–2016. A) Chulaimbo, a rural village; B) Kisumu, an urban city. Serotypes are indicated; some children were infected with multiple serotypes. DENV, dengue virus.

study. All of the DENV PCR-positive subjects from the 2016 study were infected with DENV-2, which is consistent with the DENV serotype observed in the 2013 Mombasa outbreak (*7*). In contrast, most DENV PCR-positive subjects from our study were infected with DENV-1, which suggests regional variation in DENV strain predominance.

DENV viremia during acute infection is known to be transient (*9*); thus, our definition of DENV cases, based on RT-PCR detection of RNA, probably underestimated the true incidence of DENV infection. Data from testing of serum samples for DENV IgM and IgG seroconversion were not available as we wrote this report but would provide confirmation of our PCR results by a second method of detecting DENV infection. IgM testing, although less specific than PCR (*10*), may identify additional cases of DENV infection and could alter the descriptive statistics we present by enhancing our sensitivity for detecting DENV infection.

Improving efforts to detect DENV infections will raise awareness of DENV and increase the likelihood that healthcare providers will suspect it in patients with AFI. Correct diagnoses improve the ability of public health ministries to detect and react to outbreaks of DENV or other arboviral illnesses, rather than contributing to cycles of missed opportunities for preventive interventions.

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Paracoccidioidomycosis after Highway Construction, Rio de Janeiro, Brazil

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Transmission of *Paracoccidioides* spp. fungi to humans is usually related to manipulation of soil. Rural workers are the most affected group. We report an outbreak of paracoccidioidomycosis after deforestation and massive earth removal during construction of a highway in Rio de Janeiro, Brazil. Extensive environmental disturbances might be involved in fungal transmission.

Paracoccidioidomycosis is the major systemic mycosis in Latin America and the leading fungal cause of death in immunocompetent persons in Brazil (*1*,*2*). Paracoccidioidomycosis is a neglected disease whose prevalence and incidence rates are underestimated because of lack of mandatory reporting. Infection follows inhalation of *Paracoccidioides* spp. conidia in the soil (*3*,*4*) and can progress to disease, typically manifested in 1 of 2 clinical forms. The first form is chronic (adult type), which accounts for ≈80% of paracoccidioidomycosis cases, mostly in rural workers who show fungal endogenous reactivation in the lungs and other organs later in life. The second form is acute/subacute (juvenile type), which occurs primarily in young patients and is more severe because of progressive reticuloendothelial involvement, which results in high rates of complications, including death (*5*).

There have been reports of *Paracoccidioides* spp. infections after disturbances of soil that resulted in aerial dispersion of fungal propagules. Native indigenous populations in Latin America changed their ancient livelihood practices to cultivate coffee after deforestation of the Amazon rainforest, which resulted in paracoccidioidomycosis infections (*6*,*7*). In addition, climate changes related to the El Niño events, such as a high rainfall index followed by increased storage of water by soil and higher humidity, have been shown to occur before an increase of acute/subacute paracoccidioidomycosis cases (*8*).

We report an outbreak of paracoccidioidomycosis after deforestation and massive earth removal during construction of a highway in Rio de Janeiro, Brazil. The study protocol was approved by the Evandro Chagas National Institute of Infectious Diseases Research Ethics Committee (register CAAE 42590515.0.0000.5262).

The Evandro Chagas National Institute of Infectious Diseases in Rio de Janeiro is a reference center for paracoccidioidomycosis. This disease is endemic to the state of

[1]All authors contributed equally to this article.

Rio de Janeiro (*5*). During 1988–2015, the annual average number of acute/subacute cases of paracoccidioidomycosis at this institution was 2.3 cases/year for this state and 1.4 cases/year for Baixada Fluminense, a region composed of 12 municipalities in the metropolitan area of Rio de Janeiro (Figure). However, during December 2015–December 2016, a total of 8 cases were diagnosed at this center, all from Baixada Fluminense, a rate ≈5.7 times higher than that expected for this period. The most recent (2016) census in Brazil estimated that there were 968,680 persons <30 years of age living in the affected municipalities (*9*).

Case definition was based on clinical and laboratory criteria: reticuloendothelial involvement in young patients; laboratory test results confirming the presence of multibudding yeast-like *Paracoccidioides* cells by direct microscopy or histopathologic analysis; fungal isolation in culture; or a positive serologic result for paracoccidioidomycosis (*3*). Data for the 8 case-patients (4 males and 4 females) are provided (online Technical Appendix Table 1, https://wwwnc.cdc.gov/EID/article/23/11/17-0934-Techapp1.pdf). Mean age was 22 (range 10–28) years. Median time for diagnosis was 7 (range 4–16) months. Predominant clinical manifestations were cervical lymph node enlargement (100%), hepatomegaly (25%), and splenomegaly (25%). Serious complications occurred in 5 patients: adrenal insufficiency (3 patients), cholestasis (1 patient), esophageal fistula (1 patient), and acute upper airway obstruction (1 patient). A 19-year-old patient died because of complications of paracoccidioidomycosis.

The Raphael de Almeida Magalhães Highway, also known as Arco Metropolitano, is a new highway in the study region (Figure). During its construction (2008–2014), large areas were deforested and massive amounts of earth were removed, which resulted in discovery of 62 archeological sites through 2012 (*10*). Two thirds of this highway (97 km) was constructed during 2012–2014. The highway was complete for 1 year before the number of new cases of paracoccidioidomycosis increased. Residences of patients were 0.1 km–16.6 km from construction areas. The increase in the number of acute/subacute cases of paracoccidioidomycosis, with temporal and geographic relationships to this construction, suggests a possible new risk for outbreaks of paracoccidioidomycosis.

Other hypotheses for this cluster are clearing of forests, soil humidity, and the El Niño phenomenon (*3*,*8*). It is noteworthy that the highway crosses a native Atlantic forest area. Moreover, over several months in 2013, this region had high rainfall indexes (online Technical Appendix Table 2), which presumably contributed to retention of moisture in the soil. A previous study showed that soil humidity favors sporulation and dispersal of *Paracoccidioides* spp. (*3*). Also, a high-intensity El Niño phenomenon occurred during May 2015–March 2016.

The incidence of acute paracoccidioidomycosis in the affected area after highway construction (8.25 cases/1 million persons/y, 95% CI 4.18–16.3 cases/1 million persons/y) was higher than that before highway construction (1.29 cases/1 million persons/y, 95% CI 0.74–4.03 cases/1 million persons/y). More persons were probably exposed to *Paracoccidioides* conidia, but these persons did not show progression/development of disease, did not seek medical attention, and did not have

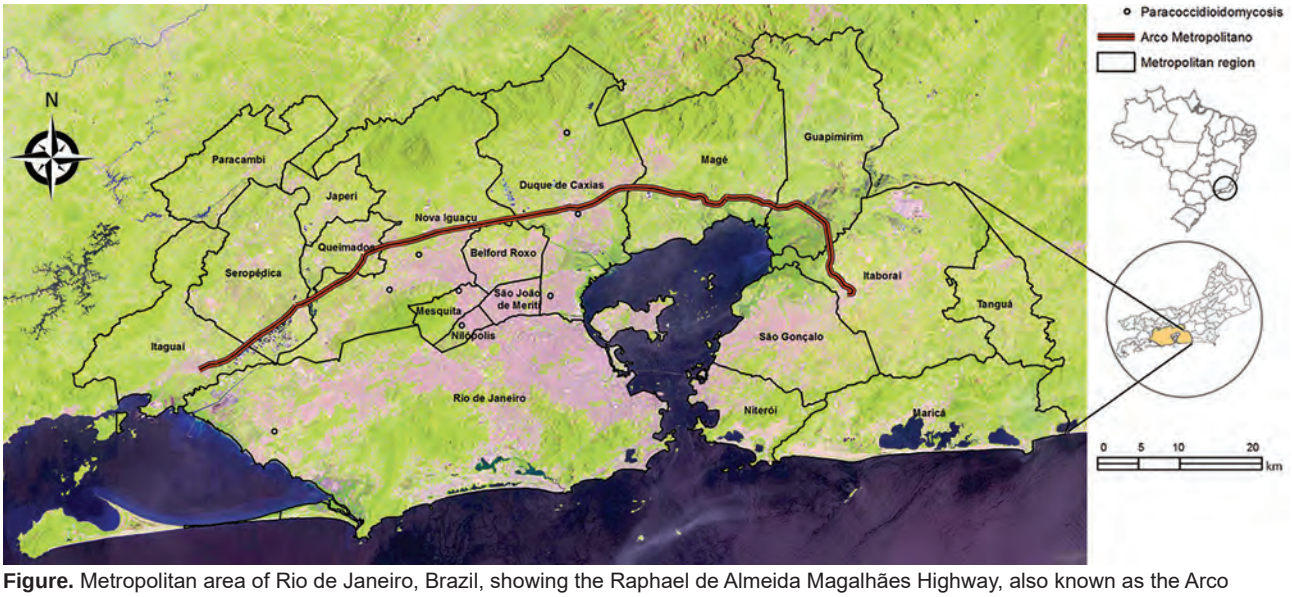

Figure. Metropolitan area of Rio de Janeiro, Brazil, showing the Raphael de Almeida Magalhães Highway, also known as the Arco Metropolitano, and georeferenced cases of paracoccidioidomycosis (open circles) during highway construction. Inset shows location of metropolitan area in Rio de Janeiro State and of Rio de Janeiro State (circle) in Brazil. Source: Landsat 8 Images (https://earthexplorer.usgs.gov/).

cases reported to health authorities. The chronic form of paracoccidioidomycosis will probably develop in some of these patients.

This study underscores the need for paracoccidioidomycosis surveillance, especially in the context of environmental alterations enhanced by climate change and affected by construction, deforestation, and other human interventions. Enhanced surveillance will more fully identify relative risks of different human enterprises and facilitate interventions for at-risk populations to reduce and prevent future outbreaks of paracoccidioidomycosis.

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Mycobacterium shimoidei, a Rare Pulmonary Pathogen, Queensland, Australia

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Nontuberculous mycobacteria are human pathogens with increasing incidence and prevalence worldwide. *Mycobacterium shimoidei* is a rare cause of pulmonary disease, with only 15 cases previously reported. This series documents an additional 23 cases of *M. shimoidei* from Queensland, Australia, and highlights the pathogenicity and clinical role of this species.

Nontuberculous mycobacteria (NTM) are prominent human pathogens, with >150 species reported worldwide (*1*). *Mycobacterium shimoidei* is a slow-growing NTM that was first isolated in Japan in 1968, successfully gaining species status in 1975 (*2*). Since then, only 15 cases have been reported worldwide (*3*–*10*).

In Queensland, Australia, NTM is a reportable condition, requiring all isolates to be reported to the Queensland Mycobacterium Reference Laboratory. This series examines all *M. shimoidei* cases in Queensland during January 1, 2000– December 31, 2014.

We extracted data from the Queensland Notifiable Condition System with ethics approval obtained from the Metro North Human Research Ethics Committee (HREC/15/QPCH/65). Confirmatory testing was conducted at the Queensland Mycobacterium Reference Laboratory using 2 methods: the Hain Genotype CM/AS Line Probe Assays (Hain Lifescience, Nehren, Germany) and 16S rRNA sequencing.

We obtained clinical information from treating physicians and patient medical records. We recorded each isolate as being likely clinically significant, possibly significant, or unlikely significant, and as being consistent or not with NTM lung disease according to the 2007 American Thoracic Society and Infectious Disease Society of America criteria.

Specimens from 23 patients (35 total isolates) cultured *M. shimoidei* in Queensland during the study period. Individual clinical characteristics, treatment, and outcomes can be seen in the Table. Previously reported cases are summarized in the online Technical Appendix (https://wwwnc.cdc.gov/EID/article/23/11/17-0999-Techapp1.pdf).

Sixteen (69.6%) patients were male (mean ± SD age 66.2 ± 12.6 years), consistent with previous case reports (*3*–*10*). Nine (39.1%) were classified as being likely clinically significant and 7 (30.4%) possibly significant. Ten patients (43.5%) met the 2007 American Thoracic Society and Infectious Disease Society of America criteria for having NTM lung disease. All isolates were cultured from respiratory specimens, with 15 (65.2%) isolated from sputum; 2 (8.7%) from bronchial washings; 2 (8.7%) from both bronchial washings and sputum; and 2 (17.4%) from lung tissue either with computed tomography–guided biopsy or at autopsy. Only 4 (17.4%) specimens were smear-positive by microscopy.

Table. Clinical characteristics, treatment, and outcomes of *Mycobacterium shimoidei* isolates, Queensland, Australia*

Specimen (isolates)	Age, y/sex	Significant	Signs/ symptoms	Radiology	Concurrent conditions	Management (time)	Outcome
Sp and Br (×4)	60/M	Likely	C, Sp, WL	Cavities, nodule	COPD, asthma	Observed	Stable
LTis (×1)	56/M	Likely	Died	Unknown	Unknown	None	Died
Sp (×1)	75/F	Likely	C, Sp, WL	Cavities, nodules	COPD, HF, AF, GERD	None	Died of other cause
Sp (×3)	72/M	Likely	C, D, WL	Cavity, nodules	COPD, bronchiectasis, IHD	Observed	Died of lung disease
LTis (×1)	62/F	Likely	C, WL, NS	Cavity	None	INH, RFP, PZA, EMB (6 mo)	Stable
Sp (×2)	68/M	Likely	C, Sp, H, WL, Fa	Cavities, consolidation	COPD, aspergillus, HTN	CLA, MFX, SMX (12 mo)	Improved
Sp and Br (×4)	70/M	Likely	C, Sp, CP	Cavities	Lung cancer, COPD, bronchiectasis	CLA, RIF, EMB (12 mo)	Died of lung disease
LTis (×1)	77/F	Likely	C, WL, Fa	Cavity, nodules	COPD, GERD	CLA, RFP, EMB (18 mo)	Improved
Sp (×3)	68/M	Likely	C, Sp, WL	Cavity, consolidation	COPD, RA, anemia	Observed	Stable
Br (×1)	76/M	Possibly	D, WL	Nodules	COPD, anemia	None	Unknown
Br (×1)	84/M	Possibly	C, Sp	Mass, effusion	Lung cancer, GERD	Observed	Died of lung disease
Sp (×1)	84/M	Possibly	C, D, Fa	Consolidation	COPD, bronchiectasis	Observed	Improved
Sp (×1)	29/M	Possibly	C, D, WL	Nodules	CF, bronchiectasis	AMK, CFX, AZA, CFZ (24 mo)	Improved
Sp (×1)	74/F	Possibly	C, Sp	Nodules, consolidation	Bronchiectasis	Observed	Improved
Sp (×5)	84/F	Possibly	C, Sp, H, WL	Nodules	Bronchiectasis, type 2 diabetes, HTN	CLA (2 mo)	Improved
Sp (×1)	58/M	Possibly	C, Sp	Normal	Obesity, HTN	Observed	Stable
Sp (×1)	57/M	Unlikely	Unknown	Unknown	Unknown	Unknown	Unknown
LTis (×1)	55/F	Unlikely	Unknown	Unknown	Unknown	Unknown	Unknown
Sp (×1)	67/M	Unlikely	Unknown	Unknown	Unknown	Unknown	Unknown
Sp (×1)	60/M	Unlikely	C, D	Normal	Asthma	None	Unknown
Sp (×1)	59/F	Unlikely	C	Normal	Asthma, GERD	None	Unknown
Sp (×1)	73/M	Unlikely	Unknown	Unknown	Unknown	Unknown	Unknown
Sp (×1)	54/M	Unlikely	Unknown	Unknown	Unknown	Unknown	Unknown

*AF, atrial fibrillation; AMK, amikacin; AZA, azithromycin; Br, bronchoscopic washing; C, cough; CF, cystic fibrosis; CFX, cefoxitin; CFZ, clofazimine; CLA, clarithromycin; COPD, chronic obstructive pulmonary disease; CP, chest pain; D, dyspnea; EMB, ethambutol; Fa, fatigue; GERD, gastroesophageal reflux disease; H, hemoptysis; HF, heart failure; HTN, hypertension; IHD, ischemic heart disease; INH, isoniazid; LTis, lung tissue; MFX, moxifloxacin; NS, night sweats; PZA, pyrazinamide; RA, rheumatoid arthritis; RFP, rifampin; RIF, rifabutin; SMX, sulfamethoxazole, Sp, sputum; WL, weight loss.

The most common symptoms were cough or sputum (16; 69.6%); weight loss (9; 39.1%); dyspnea (5; 21.7%); fevers or sweats (4; 17.4%); and fatigue (2; 8.7%). Cough and sputum predominated in previous cases, but not weight loss (*3–10*). Radiology demonstrated cavitary disease in 9 patients (39.1%). Similar to our cohort, 9 of the 15 previously reported cases had cavities, highlighting a potentially distinguishing feature of *M. shimoidei* lung disease (*3–7*).

The most common associated concurrent conditions were obstructive airway disease (10; 43.5%), bronchiectasis (6; 26.1%), gastroesophageal reflux disease (4; 17.4%), and malnutrition (3; 13.0%). Underlying chronic lung disease was also present in previously reported cases and included chronic obstructive pulmonary disease, past tuberculosis, pneumoconiosis, and bronchiectasis (*3–10*).

Although 16 patients (69.5%) were deemed to have either likely or possibly clinically significant disease, only 6 (26.0%) underwent medical treatment, with 7 (30.4%) being actively observed. These low treatment numbers may reflect a lack of knowledge in relation to *M. shimoidei*; however, they may also be an indirect result of the underlying comorbidities and poor functional status of infected patients.

When medical treatment was offered, however, 5 of the 6 patients improved or had stable disease, with the sixth patient dying of lung cancer while undergoing antimicrobial therapy. Of the 7 patients who were observed, 3 remained stable, 2 improved, and 2 died of either chronic lung disease or progression of their *M. shimoidei* infection. In comparison, 6 of the 15 previous cases in the literature improved with medical treatment, with 4 dying during treatment and 1 remaining stable with observation alone (*3–10*). Although this relatively high death rate may reflect the nature of the patients' comorbidities, it still highlights the clinical significance of *M. shimoidei* if isolated.

Although none of the Queensland cohort underwent drug susceptibility testing, review of previous cases suggests that a combination of rifabutin, ethambutol, and clarithromycin may be an effective drug regimen, with moxifloxacin/levofloxacin, sulfamethoxazole, pyrazinamide, and linezolid as other potential agents (*3–7*).

Our study has several limitations. First, it is a retrospective case series with data extracted from a passive surveillance system. Even though all laboratory-confirmed cases were captured, it is possible that not all patients with *M. shimoidei* infection received this diagnosis or were able to provide an appropriate specimen for identification. Futhermore, due to both the clinical characteristics being reported by various treating physicians and a large proportion not having complete clinical or follow-up data available, we may have captured inaccurate or inconsistent data.

This case series highlights the clinical significance and pathogenicity of *M. shimoidei.* Cases have been isolated only from respiratory specimens, occur predominantly in male patients with underlying chronic lung disease, and commonly present with cavitary disease. Although illness and death are associated with *M. shimoidei* infection, a reasonable outcome can be achieved with treatment. Possible drug regimens involve a combination of rifabutin, ethambutol, and clarithromycin, with moxifloxacin/levofloxacin, sulfamethoxazole, pyrazinamide, and clofazimine also potentially being useful. Increased recognition and understanding of this pathogenic organism are necessary to improve patient outcomes.

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The Breadth of Viruses in Human Semen

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Zika virus RNA is frequently detected in the semen of men after Zika virus infection. To learn more about persistence of viruses in genital fluids, we searched PubMed for relevant articles. We found evidence that 27 viruses, across a broad range of virus families, can be found in human semen.

The finding by Atkinson et al. that Zika virus RNA is frequently detected in the semen of men after infection (*1*) highlights our knowledge gaps regarding the persistence of viruses in genital fluids, especially semen. Replicating Zika virus (*2*), like Ebola and Marburg viruses (*3*), has been isolated from semen and has been sexually transmitted. However, it is probable that many more viruses capable of causing viremia (presence of virus in the blood) can be found in semen. Seeding to the male reproductive tract may frequently occur in the context of viremia because the blood–testes/deferens/epididymis barriers are imperfect barriers to viruses, especially in the presence of systemic or local inflammation (*4*). Virus may persist even if incapable of replicating within the male reproductive tract because the testes are immunologically privileged (*4*); that is, within the testes, the immune response is restricted to enable the survival of sperm, which are immunogenic. Virus may also be transmitted to semen as a result of survival and replication within the accessory glands (*5*).

To investigate the breadth of viruses in semen, we performed a PubMed search by using the terms "virus* AND semen OR sperm* OR seminal." We imposed no date or language restrictions. This search returned 3,818 results. We screened the titles, abstracts, and full text articles for data that described detection of viruses in semen by nucleic acid amplification or detection, antigen detection, replication in cell culture, or replication in an animal system. We restricted the results to viruses capable of causing viremia. Where we found evidence for virus in semen, we then searched PubMed for evidence of sexual transmission by using the terms "(name of virus) AND sex* AND Transm*."

Our search revealed that 27 viruses that can result in viremia have been found in human semen (Table). For many of these, data on sexual transmission are lacking. Of these 27 viruses, many cause chronic or latent infection (e.g., HIV virus, cytomegalovirus). However, several cause acute infections, including Lassa fever, Rift Valley fever, and chikungunya viruses. Of those causing acute infections, only Zika and Ebola viruses have been systematically screened for in semen (i.e., in case series or cohort studies rather than case reports). These 27 viruses come from diverse families, suggesting that the presence of many viruses in semen is unlikely to be exclusively dependent on specific or conserved viral epitopes, ability of virus to replicate within the male reproductive tract, or common mechanisms of immune evasion. Other factors that may also influence whether viruses exist in semen are level of viremia, inflammatory mediators (altering blood-barrier permeability), systemic immunosuppression, male reproductive tract immune responses, presence of sexually transmitted diseases, and virus structural stability. In mammals, numerous viruses are detectable in semen, including viruses that can cause disease in humans, such as Japanese encephalitis virus, foot and mouth disease virus, parainfluenza virus, and paravaccinia virus (*6*). Several other viruses that result in viremia can cause orchitis and have been detected in human testes, suggesting the possibility that these viruses may also be detectable in semen. These viruses include influenza virus, lymphocytic choriomeningitis virus, phlebotomus fever virus, cocksackie B virus, echovirus, dengue virus, systemic acute respiratory syndrome virus, parvovirus, smallpox virus, vaccinia virus, and rubella virus (*7*).

Given these findings, the following questions need to be addressed: which viruses are shed and remain viable in semen, for how long, and at what concentrations? The answers to these questions have implications for risks for sexual transmission and, therefore, embryonic infection, congenital disease, miscarriage, and effects on epidemiologic and transmission models. The presence of virus in the male reproductive tract may increase the risk for acquisition of sexually transmitted infections and may reduce male fertility through spermatogonial stem cell infection or local inflammation. Infection of spermatozoa could result in transmission of virus-induced mutations to subsequent

Table. Viruses that are capable of causing viremia and found in human semen*

Virus	Family	Detection in semen, maximum detection time, d	Isolation from semen, maximum detection time, d	Evidence for sexual transmission within same cohort
Adenoviruses	*Adenoviridae*	AD	RCC	Unknown
Transfusion transmitted virus	*Anelloviridae*	NAA	No data found	Unknown
Lassa fever virus†	*Arenaviridae*	NAA, 103	RCC, 20	Unknown
Rift Valley fever virus†	*Bunyaviridae*	NAA, 117	No data found	Unknown
Ebola virus	*Filoviridae*	NAA, 531	RCC, 82	Epi + mol + sem
Marburg virus†	*Filoviridae*	AD, 83	RAS, 83	Epi + sem
GB virus C	*Flaviviridae*	NAA	No data found	Epi + mol
Hepatitis C virus	*Flaviviridae*	NAA; AD	No data found	Epi + mol
Zika virus	*Flaviviridae*	NAA, 188	RCC, 7	Epi + mol + sem
Hepatitis B virus	*Hepadnaviridae*	NAA; AD	RAS	Epi + mol
Cytomegalovirus	*Herpesviridae*	NAA	RCC	Epi + mol + sem
Epstein Barr virus	*Herpesviridae*	NAA	No data found	Epi and semen
Human herpes virus 8	*Herpesviridae*	NAA	RCC	Epi + mol
Human herpes virus 7	*Herpesviridae*	NAA	No data found	Unknown
Human herpes virus 6	*Herpesviridae*	NAA	No data found	Unknown
Human simplex viruses 1 and 2	*Herpesviridae*	NAA; AD	RCC	Epi + mol + sem
Varicella zoster virus	*Herpesviridae*	NAA	No data found	Unknown
Mumps virus†	*Paramyxoviridae*	NAA, 40	RCC, 14	Unknown
Adeno-associated virus	*Parvoviridae*	NAA	RCC	Unknown
BK virus	*Polyomaviridae*	NAA	No data found	Unknown
JC virus	*Polyomaviridae*	NAA	No data found	Unknown
Simian virus 40	*Polyomaviridae*	NAA	No data found	Unknown
HIV	*Retroviridae*	NAA; AD	RCC	Epi + mol + sem
Human T-cell lymphoma virus 1†	*Retroviridae*	No data found	RAS	Epi + mol
Simian foamy virus	*Retroviridae*	NAA	No data found	Unknown
Chikungunya virus†	*Togaviridae*	NAA, 30	No data found	Unknown

*Presence of nucleic acid or antigen in semen does not represent the presence of replication-competent or infection-competent virus, which can generally only be demonstrated by isolation and culture of virus. Maximum detection time refers to time from symptom onset (only in viruses that cause acute only, not chronic, infection). A complete table with references is provided in the online Technical Appendix (https://wwwnc.cdc.gov/EID/article/23/11/17-1049-Techapp1.pdf). AD, antigen detection; Epi, epidemiologic evidence of sexual transmission; mol, molecular/phylogenetic evidence of sexual transmission; NAA, nucleic acid amplification or detection; RAS, replication in animal system; RCC, replication in cell culture; sem, isolation from semen.
†Data found only in the context of case reports and not case series, case control, or cohort studies.

generations, thereby elevating risks for cancer and other disorders. Indeed, when virus has been detected in human semen, the extent to which virus existence and replication occurs within spermatozoa is unclear (*8*). Not all therapeutics will cross the male reproductive tract–blood barriers, and viruses may persist in semen despite systemic clearance of virus, highlighting the need to consider the male reproductive tract–blood barriers when choosing therapeutic agents in clinical trials. Virus within the male reproductive tract can also be genetically distinct from virus in other compartments, including blood (*9*), which has implications for gene-based vaccines and therapeutics.

The presence of viruses in semen is probably more widespread than currently appreciated, and the absence of virus in genital secretions should not be assumed for traditionally non–sexually transmitted viruses. The investigation of virus detection and persistence in semen across a range of viruses is useful for clinical and public health reasons, in particular for viruses that lead to high mortality or morbidity rates or to epidemics.

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Legionella pneumophila Serogroup 1 in the Water Facilitites of a Tertiary Healthcare Center, India

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Proactive environmental surveillance for *Legionella pneumophila* in hospitals that treat immunocompromised patients is a useful strategy for preventing nosocomial Legionnaires' disease. We report the presence of *L. pneumophila* serogroup 1 in 15.2% of the water systems of our tertiary healthcare center, which should prompt health officials to formulate mitigation policies.

Legionella pneumophila, the causative agent of Legionnaires' disease (LD), is a bacterium omnipresent in aquatic environments and increasingly recognized as a major cause of community- and hospital-acquired pneumonia. *L. pneumophila* serogroup 1 (*Lp*1), the dominant serogroup, accounts for ≈84% of human infections worldwide (*1*,*2*). Hospital-acquired LD has been reported globally, and routine use of environmental cultures is recommended as a useful strategy to prevent infections (*3*). Although proactive environmental surveillance of *Legionella* and regular treatment of cooling tower installations are recommended in many countries, these practices are not routine in India, and limited studies have been conducted in this country for monitoring *Legionella* contamination in hospital water systems (*4*). We conducted a study to detect *L. pneumophila* and to identify *Lp*1 in the water systems of a tertiary healthcare center in northern India that has organ transplantation and cancer treatment facilities.

We collected 79 water samples (41 potable, 38 nonpotable) from the hospital and general areas of the healthcare center during an 18-month period (May 2015–October 2016). Of 79 samples, 27 were collected from patient areas (wards, intensive care units, outpatient departments, emergency units, and procedure rooms); 14 from residential areas; 15 from cooling towers; and 23 from other buildings (e.g., laboratory divisions, teaching departments, library, and recreational zones). We followed guidelines issued by the US Centers for Disease Control and Prevention regarding isolation of *Legionella* (*5*). In brief, we concentrated 500 mL of water samples and decontaminated 1 part by using heat treatment (in water bath at 50°C for 30 min) and 1 part by acid (in equal volume of HCl-KCl acid buffer [pH 2.2]). We then inoculated 0.1-mL samples onto buffered charcoal yeast extract agar (Becton Dickinson, Sparks, MD, USA) supplemented with glycine, vancomycin, polymyxin B, and cycloheximide (Oxoid, Basingstoke, UK). We presumptively identified colonies growing only on buffered charcoal yeast extract but not on blood agar as *Legionella* species and confirmed the presence of *L. pneumophila* by amplification of a 375-bp region of the *mip* gene using previously published primers (*6*). We identified *Lp*1 by using a real-time PCR (rPCR) assay targeting the *wzm* gene (*7*). We used genomic DNA isolated from *L. pneumophila* strain Philadelphia (ATCC 33152) for standardization of PCR and rPCR and *L. pneumophila* strain Knoxville (ATCC 33153) for standardization of culture.

We identified *Legionella* spp. in 21 (26.6%) of 79 water samples (10 potable and 11 nonpotable) by culture. We obtained a collection of 28 isolates from the 79 samples and identified all of them as *L. pneumophila* by PCR. Among these 28 isolates, 18 (64.3%) tested positive for *Lp*1 by rPCR, indicating the presence of this pathogenic serogroup in 12 (15.2%) of the 79 water samples (5 potable and 7 nonpotable).

We repeatedly isolated *L. pneumophila* (>4 times) from 2 high-risk sites: a drinking water unit and a cooling tower situated inside the hospital campus. Four water samples collected from patient areas tested positive for *L. pneumophila*, posing a risk for nosocomial infection. We isolated *L. pneumophila* from water bodies with temperatures ranging from 12°C to 57°C but most frequently (11 times) from those with temperatures of 25°C–50°C. We summarized the isolation of *L. pneumophila* with

respect to type of water sample, sampling site, and temperature (Table).

We demonstrated the presence of *Lp*1 in the hospital water systems, indicating that LD might be a common cause of pneumonia in this setting. Previously published reports have documented infections attributable to *L. pneumophila* in patients with community-acquired pneumonia; however, the prevalence of nosocomial legionellosis in India remains unknown (*4*,*8*,*9*).

On the basis of our findings, we initiated infection control measures and created awareness to formulate *Legionella* risk management in this hospital. We ensured that physicians were cognizant of possible *Legionella* colonization in the hospital water supply and advised them to recommend *Legionella* diagnostic testing for patients with suspected nosocomial pneumonia. Underdiagnosed nosocomial legionellosis might become evident as the awareness of clinicians increases and as specialized laboratory testing for the pathogen becomes easily available.

Eradication of *Legionella* from aquatic bodies is a herculean task. Numerous systemic disinfection measures are available, such as super heat and flush, copper-silver ionization, chlorine dioxide, and point-of-use filters, but no method is ideal for complete eradication of the contagion (*10*). We advised the hospital's engineers and healthcare facility managers to install point-of-use filters to the drinking water taps in areas where *Legionella* was isolated. Repeat sampling of 2 sites that had once tested positive for *Legionella* showed that they were culture-negative for the pathogen 6 six months after installation of the filters. However, to evaluate the efficacy of any disinfection method, validation and monitoring over a prolonged period is required.

Our findings might be an important sentinel of an underestimated threat and could be useful for health officials in India to set standards for LD surveillance and control in hospitals. Although we did not perform sequence-based typing in this study, examination of *Legionella* from natural sources and advances in molecular typing might help clarify the distribution and natural history of LD in this region.

Table. *Legionella pneumophila* isolated from 79 water samples collected at a tertiary healthcare center, by type of water, sampling site, and temperature, India, May 2015–October 2016

Characteristic	Samples positive for *L. pneumophila*, n = 21	Samples positive for *L. pneumophila* serogroup 1, n = 12
Type of water		
Potable	10	5
Nonpotable	11	7
Sampling site		
Patient area	4	3
Residential area	6	1
Cooling tower	5	3
Other building	6	5
Temperature, °C		
<25	9	4
25–50	11	7
>50	1	1

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Outbreak of Zika Virus Infections, Dominica, 2016

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In February 2016, the World Health Organization declared the pandemic of Zika virus a public health emergency. On March 4, 2016, Dominica reported its first autochthonous Zika virus disease case; subsequently, 1,263 cases were reported. We describe the outbreak through November 2016, when the last known case was reported.

Zika virus is a flavivirus transmitted primarily by *Aedes aegypti* and *Ae. albopictus* mosquitoes. The rapid spread of Zika virus from Brazil throughout the Americas, and the associated emergence of Zika congenital syndrome, which causes microcephaly and other birth defects, has posed an unprecedented challenge to global health (*1*,*2*). Zika virus spread through the Caribbean region early in the pandemic. In epidemiologic week 19 of 2015, Brazil reported its first confirmed locally acquired cases. Autochthonous transmission in Martinique was first reported in epidemiologic week 51 of 2015, and the first case originating in Puerto Rico was reported in week 52 of 2015 (*3*). Many other islands began reporting cases of Zika virus infection early in 2016. However, case data from several Caribbean countries has yet to be consolidated and described outside of reports by the Pan American Health Organization.

We defined suspected Zika virus disease cases in the Commonwealth of Dominica, on the eastern sector of the Caribbean Sea, by using guidelines provided by the Pan American Health Organization (*4*). Active surveillance of cases (suspected and confirmed) among persons who visited health clinics started as early as January 2016; however, the first laboratory-confirmed autochthonous case of Zika virus disease was identified in March 2016. We collected data from records in the Ministry of Health and Environment, Dominica, describing patients' age, sex, residence, date of illness onset, clinical features, laboratory diagnoses, and travel history.

The first case of laboratory-confirmed Zika virus disease in Dominica was reported on March 4, 2016 (during epidemiologic week 9), in a 28-year-old woman. New cases (suspected and confirmed) were reported that year through November 6 (Figure). The last cases in 2016 were reported in epidemiologic week 44. A total of 1,263 suspected cases of Zika virus disease were reported in Dominica in 2016, of which 79 (6.25%) were confirmed by using reverse transcription PCR. Of these, only 1 specimen tested negative but was classified as a suspected case.

Sex was reported for 1,255 (99.3%) of 1,263 case-patients. Approximately twice as many case-patients were female (863, 68.8%) than male (392, 31.2%), which is consistent with a female bias found in other reports on Zika virus disease outbreaks (*5*).

Age was reported for 1,245 (98.6%) of 1,263 case-patients. Mean age was 28 years (median 27, range <1–94 years). Of those, 217 (17.4%) case-patients were children <10 years of age and 756 were of reproductive age (15–49 years); 555 (73.4%) of reproductive age case-patients were women.

Of the 1,240 case-patients for whom age and sex were reported, 555 (44.8%) were women of childbearing age. Pregnancy status was only reported for 54 (6.3%) of 863 female case-patients; of those, 16 (29.6%) were pregnant. Of the 16 pregnant women, 11 were confirmed case-patients, and disease was suspected in 5.

Of the 1,263 total case-patients, clinic visit dates were recorded for 1,123 (88.9%), and 27 (2.1%) reported hospitalizations. The average number of days between the onset of symptoms and initial clinic visit was 1.96 (n = 1,109; 5%, 95% quantiles = 0, 5); the average number of days between a recorded clinic visit and case reporting was 0.5 (n = 1,096; 5%, 95% quantiles = 0, 10). Of the 27 (15 female/11 male/1 unknown) hospitalizations, 2 were for women reported to be pregnant, and 6 were for children <10 years of age.

Aedes spp. mosquitoes are widespread throughout the Caribbean and are associated with Zika, dengue, and now chikungunya viruses, which are endemic to many islands. As part of the broader pandemic in the Americas, the Zika virus disease outbreak in Dominica highlights that the presence of *Aedes* spp. can be predictive of outbreaks. Dominica has a population of ≈72,000, on an island of 750 km^2, and ≈1.67% of the population were reported to have suspected or confirmed cases of Zika virus disease in 2016.

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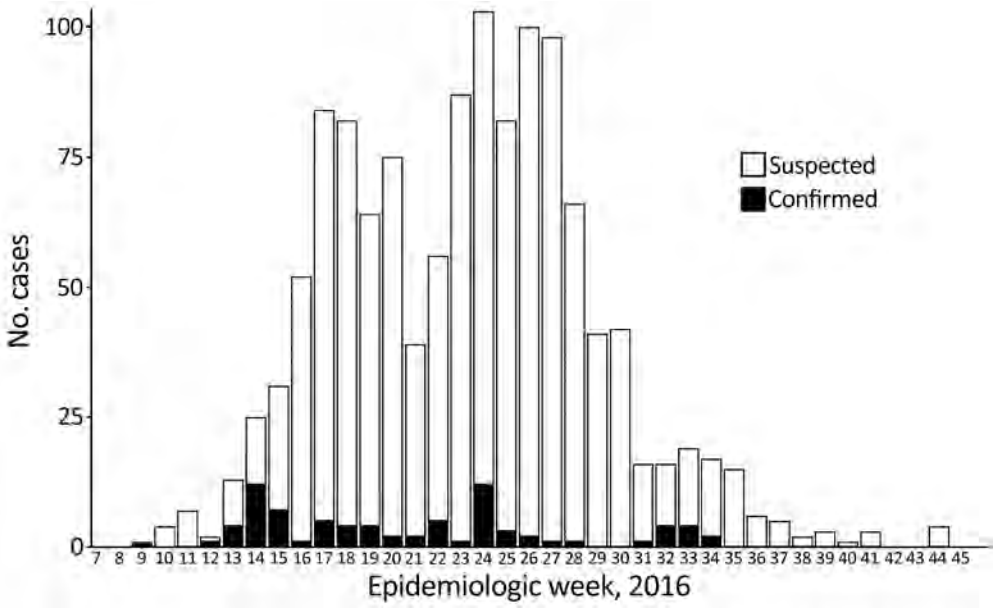

Figure. Suspected and confirmed cases of Zika virus infection reported during outbreak, Dominica, 2016.

We found a bias toward infection in women, including a substantial number of women of childbearing age, highlighting the potential vulnerability of pregnant women and unborn children. As was seen during the recent epidemic of chikungunya in Dominica (*6*), the rapid proliferation of Zika virus disease cases emphasizes the need to strengthen local capacities for targeted vector control and global efforts to support development of effective vaccines, in addition to a better understanding of the role of sexual transmission and the heightened risk to vulnerable populations such as pregnant women.

This study was made possible by a partnership between the Caribbean Institute of Meteorology and Hydrology (CIMH) and an international team of investigators examining the eco-epidemiology and climate drivers of dengue fever, chikungunya, and Zika virus disease in the Caribbean. This project was undertaken through the United States Agency for International Development's Programme for Building Regional Climate Capacity in the Caribbean Programme, executed by the World Meteorological Organization, and implemented by CIMH. This initiative has brought together the national and regional health and climate sectors (Dominica Ministry of Health, Dominica Meteorological Service, Caribbean Public Health Agency, Pan American Health Organization, CIMH). We are grateful for this partnership and cooperation.

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Autochthonous Leprosy without Armadillo Exposure, Eastern United States

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Autochthonous leprosy has been reported in New York City, where there are no wild armadillos. Recent autochthonous cases also have been reported in Georgia and Florida and blamed on armadillos, including cases with no known armadillo exposure. International migration needs to be considered as a cause of autochthonous leprosy.

In 1982, we reported that leprosy in New York City occurred exclusively among foreign-born persons (*1*). In 1991, Mastro et al. reported that leprosy was an epidemic phenomenon without secondary transmission (*2*). In 2000, however, the first autochthonous cases of leprosy in New York City were reported (*3*), and 2 additional autochthonous cases subsequently were reported (*4*,*5*). Autochthonous leprosy has been reported in the eastern United States in Georgia (*6*) and central Florida (*7*); transmission was blamed on armadillos, even though most of these case-patients had no history of exposure to armadillos, and armadillos east of the Mississippi River rarely have leprosy (*8*).

Although the transmission of leprosy is poorly understood, international migration of persons with leprosy is a more likely scenario for autochthonous transmission than contact with armadillos, especially if a case-patient has no history of armadillo exposure. Ramos et al. linked an increase in autochthonous leprosy in Spain to a 5-fold increase in migration from countries where leprosy is prevalent (*9*). There are no wild armadillos in New York City. Autochthonous cases of leprosy reported from the eastern United States should not be assumed to be from armadillos. Physicians throughout the United States need to be aware that leprosy can occur in native-born Americans and that delayed diagnosis, which occurs frequently, can result in unacceptable deformities.

Leprosy most commonly is characterized by an infiltrative dermopathy, which dermatologists and many physicians know is an indication for skin biopsy. Many otherwise highly trained physicians are not aware of this indication for a skin biopsy, which is required to diagnose leprosy. This indication is routinely taught in dermatology clinics, but leprosy is common enough in the United States that it should be incorporated into the core curricula of medical schools. Leprosy also can be characterized by fever and arthritis simulating lupus erythematosus, rheumatoid arthritis, or antiphospholipid syndrome because autoantibodies occur in type II reaction known as erythema nodosum leprosum. Physicians should order a Fite stain on the skin biopsy specimen because *Mycobacterium leprae* is sensitive to the alcohol decolorizing step; if only a routine acid-fast stain (Ziehl-Neelsen) is ordered, the diagnosis is often missed (*10*).

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Diffuse Multibacillary Leprosy of Lucio and Latapí with Lucio's Phenomenon, Peru

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Diffuse multibacillary leprosy of Lucio and Latapí is mainly reported in Mexico and Central America. We report a case in a 65-year-old man in Peru. He also had Lucio's phenomenon, characterized by vascular thrombosis and invasion of blood vessel walls by leprosy bacilli, causing extensive skin ulcers.

In Peru, leprosy has a prevalence of <1/10,000 inhabitants (*1*) and mainly affects people in the Peruvian Amazon (*2*). Leprosy, most commonly caused by infection with the bacterium *Mycobacterium leprae*, can be complicated by lepromatous reactions, which include the unusual manifestation known as Lucio's phenomenon (*3*). Initially described by Lucio and Alvarado in 1852 in Mexico, Lucio's phenomenon was so named in 1948 by Latapí and Zamoro (*3*). This reaction is seen in patients who have pure and primitive nonnodular lepromatous leprosy (diffuse leprosy of Lucio and Latapí [*4*]). This clinical variety of leprosy is most commonly found in Mexico and Central America (*5*–*7*) and is rarely described outside these regions (*8*–*10*). We report a case of multibacillary leprosy, specifically diffuse leprosy of Lucio and Latapí, and Lucio's phenomenon in a patient from the Peruvian Amazon rainforest in Peru.

The patient was a 65-year-old man who worked as a farmer. The disease had an insidious onset and a progressive course over several years, characterized by diffuse erythematous lesions on the skin. Leprosy was diagnosed, and the patient was prescribed treatment, but he did not take it. Two months before being admitted to a hospital, he began to experience a feeling of increased temperature and marked weakness. Multiple reddish-purple lesions with irregular borders appeared on his skin, mostly on the upper and lower extremities, and formed blisters and ulcers. Eventually, bacterial superinfection developed. The patient also experienced loss of appetite, gastric intolerance, and abdominal pain. Possible reasons the patient interrupted treatment were the stigmatization of the disease and a prohibitive distance from the patient's residence to the health center, which complicated clinical supervision.

On physical examination after admission to the hospital, the patient was dehydrated, malnourished, and in poor general condition. There was diffuse infiltration of the skin on his face and ears, with partial loss of eyebrows and eyelashes, and diffuse erythematous infiltrative lesions on the chest. The upper and lower extremities had multiple reddish-purple lesions with irregular borders; multiple ulcers, some with purulent secretion and others with necrotic eschar; and some achromic scars (Figure, panel A). The patient's fingers and toes were shortened and deformed (Figure, panel B) and his hands swollen. The ulcerated lesions were painful.

We requested microscopic analysis (with an oil immersion objective lens at 1,000×) of earlobe skin samples. A minimum of 25 fields/sample were examined. These samples showed positive results (a value of +5, which was defined as 100–1,000 bacilli/observed field). The histopathological analysis, using punch biopsy of the knee, elbow, and shoulder, showed in all 3 fragments a moderate infiltrate of foam cells, which followed the linear paths of the blood vessels and nerves, in some areas that had occlusive vasculopathy and neutrophilic nuclear dust (Figure, panel C). We used Fite-Faraco staining (http://stainsfile.info/StainsFile/stain/micro/afb-fitefaraco.htm) to test for leprosy bacilli. Results were positive for *M. leprae* in the foam cells, interstitial cells, and endothelial cells (Figure, panel D). The anatomic pathology diagnosis was multibacillary leprosy with additional findings of Lucio's phenomenon.

We prescribed the World Health Organization multidrug therapy (MDT) for multibacillary leprosy patients (http://www.who.int/lep/mdt/en/), comprising rifampin, clofazimine, and dapsone. The patient received treatment for 1 month in his home, and the dermal lesions improved considerably. He did not receive corticosteroid treatment. He was readmitted to the hospital with abdominal pain and severe gastric intolerance, which had forced him to discontinue MDT without consulting a doctor, probably within the previous month. The patient died from an undetermined cause.

Lucio's phenomenon is defined as a variety of type 2 lepra reaction. It is a rare event that occurs in people with diffuse leprosy of Lucio and Latapí. It develops

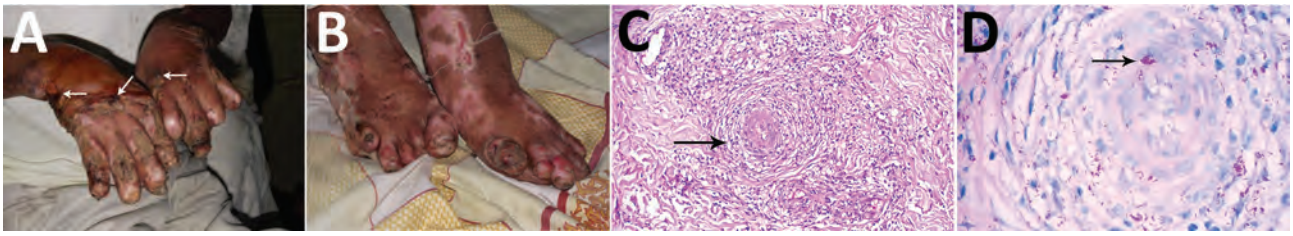

Figure. Diffuse multibacillary leprosy of Lucio and Latapí with Lucio's phenomenon in a 65-year-old man in Peru. A) Vasculitis with necrosis of the superficial vascular plexus. Forearms and dorsum of hands show papulonodular dermotoses infiltrating erythematous lesions (white arrows). B) Patches of scaling skin or necrotic eschar on feet. C) Skin biopsy of leg (hematoxylin and eosin stain, original magnification ×40) showing largely unremarkable epidermis but collection of foamy histiocytes in dermis (arrow). D) Fite-Faraco staining (original magnification ×400) clearly shows large quantity of bacilli (arrow) in the foamy histiocytes, consistent with the theory of infectious vasculitis being an etiopathogenic mechanism of Lucio's phenomenon.

as a result of exacerbated proliferation of leprosy bacilli, which invade the walls of the blood vessels and the endothelial cells, causing endothelial proliferation and reduction of the vascular lumen. These effects, together with the inflammatory reaction and the changes in the coagulation system, lead to vascular thrombosis, ischemia, infarction, and necrosis of the tissues, giving rise to the histopathological features of the phenomenon (*4*,*6*).

The diagnosis of this patient was lepromatous leprosy, confirmed by a positive skin smear from the earlobes (5+). In addition, the patient's condition met the 3 criteria that define Lucio's phenomenon, according to the international literature: skin ulceration, vascular thrombosis, and invasion of blood vessels by leprosy bacilli (*4*,*6*,*9*). The term Lucio's phenomenon should only be used when there is correlation of clinical and anatomic findings and in accordance with strict clinical criteria (*4*) The physiopathological mechanism of Lucio's phenomenon requires further study to be properly understood (*7*). Lucio's phenomenon is a serious condition that can worsen and result in patient death (*4*,*10*), as occurred with this patient.

Lucio's phenomenon is a rare clinical form of multibacillary leprosy that may occur in a severe form in patients outside of Mexico and Central America. An effort must be made to look out for and treat the signs and symptoms as soon as possible to prevent an unfavorable course.

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Dengue Virus Type 2 in Travelers Returning to Japan from Sri Lanka, 2017

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In June 2017, dengue virus type 2 infection was diagnosed in 2 travelers returned to Japan from Sri Lanka, where the country's largest dengue fever outbreak is ongoing. Travelers, especially those previously affected by dengue fever, should take measures to avoid mosquito bites.

In 2009, Sri Lanka experienced an outbreak of dengue fever, which was the largest since dengue fever was classified as a reportable disease in 1996. During that outbreak, 35,008 dengue fever cases and 346 related deaths were reported (*1*). The outbreak and severe dengue were attributed to the new dengue virus type 1 (DENV-1) strain (*1*), which has since remained the predominant serotype in Sri Lanka (*2*).

However, the number of patients with dengue fever has increased drastically during the current and largest outbreak, during January–July 2017, when >90,000 patients were reported in Sri Lanka, particularly in Colombo (*3*). In this outbreak, little is known about the causative virus type. We describe 2 travelers from Japan who were infected with DENV-2 during a late June 2017 visit to Sri Lanka.

In late June 2017, a previously healthy 34-year-old Japanese man (case-patient 1) sought care at the National Center for Global Health and Medicine (Tokyo, Japan) with a 2-day history of a high-grade fever, headache, fatigue, mild stomach ache, and watery diarrhea. His symptoms had begun the day after he returned to Japan after ≈2 months in Colombo, Sri Lanka. He had been bitten by mosquitoes in Colombo. Upon examination, his temperature was 38.9°C, and he had no abnormal findings except congested bulbar conjunctiva. Erythema appeared on his trunk and extremities on day 4 after fever onset. Nonstructural protein antigen positivity (negative for DENV IgM and IgG) and detection of the DENV-2 genome in his serum sample by real-time reverse transcription PCR (rRT-PCR) (cycle threshold 27.8) confirmed dengue fever.

In late June 2017, a previously healthy 56-year-old Japanese woman (case-patient 2) visited the National Center for Global Health and Medicine with a 4-day history of high-grade fever, headache, and arthritis and 3-day history of watery diarrhea. She had visited Colombo for 5 days, and her symptoms began 3 days after she returned to Japan. She also had been bitten by mosquitoes. Upon examination, her temperature was 37.2°C, and she had slight erythema on her face and trunk. Dengue fever was diagnosed on the basis of nonstructural protein antigen positivity (negative for DENV IgM and IgG) and detection of the DENV-2 genome in her serum by rRT-PCR (cycle threshold 23.2).

We amplified virus genome obtained from patients' serum by rRT-PCR and sequenced the E protein coding region of the DENV-2 genome, which revealed that both strains (GenBank accession nos. LC312196 [case-patient 1] and LC312197 [case-patient 2]) belonged to the Cosmopolitan genotype of DENV-2 and shared 99% identity with DENV-2 strains isolated in Singapore in 2014 (accession nos. KX224269 and KX224268) and China in 2015 (accession no. KU504492) (Figure). A phylogenetic tree based on the envelope region of the DENV-2 genome revealed that these 2 isolates belonged not to the branch of Africa strains but to the lower branch, which comprised Asia isolates within the Cosmopolitan genotype.

Dengue fever was first serologically confirmed in Sri Lanka in 1962 (*4*). Since then, although all 4 serotypes (DENV-1–4) were present, epidemics caused by DENV-3 in 1989 and 2002–2004 and by DENV-1 in 2009 were reported nationwide (*1*). After the outbreak in 2009, the annual number of dengue fever patients remained stable at 30,000–50,000 (*3*) (online Technical Appendix Figure, https://wwwnc.cdc.gov/EID/article/23/11/17-1293-Techapp1.pdf). However, the present outbreak situation is more serious because the number of patients has already exceeded the average number of cases during the same interval by >3.5-fold and continues to increase (online Technical Appendix Figure). The Ministry of Health of Sri Lanka reported 93,322 dengue fever cases, including 250 deaths, as of July 17, 2017 (*5*). Our findings indicate that case-patients 1 and 2 were infected with DENV-2 Cosmopolitan genotype during the same period, suggesting that it might be the causative strain in the worst-ever outbreak of this disease in Sri Lanka.

The epidemic strain was highly related to the strains from Southeast Asia because the sequences in current cases were nearly identical to that of a DENV strain isolated in Singapore in 2014 and in Zhejiang Province, China, in 2015. As previously reported, DENV-2 and DENV-3 are associated with severe disease accompanying secondary dengue infections (*6*,*7*), suggesting that the current epidemic of dengue fever could be the worst fatal outbreak in Sri Lanka.

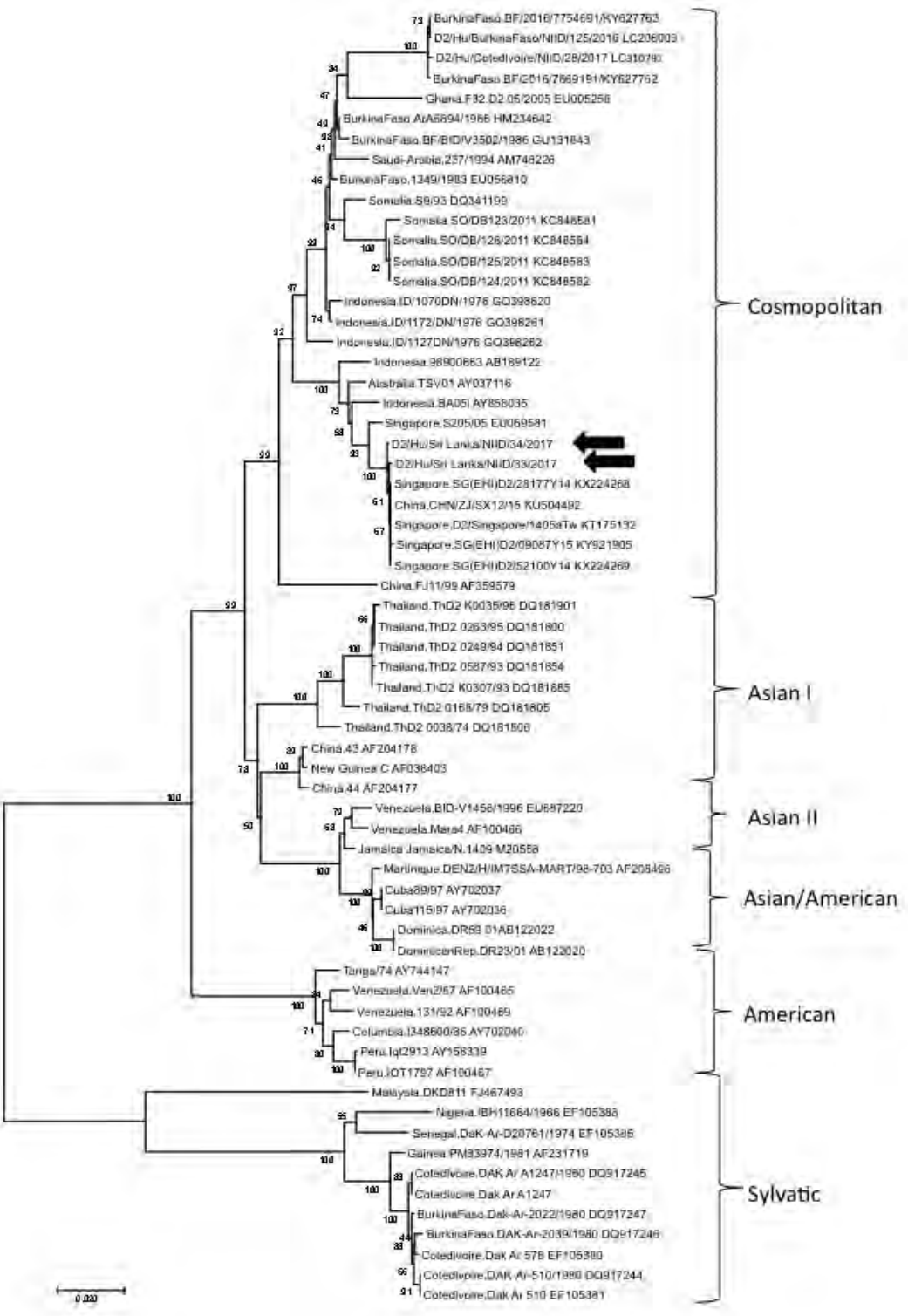

Figure. Phylogenetic analysis of dengue virus type 2 strains obtained from 2 patients who returned to Japan from Sri Lanka in June 2017 (arrows) and a comparison with reference sequences from GenBank. Virus lineages are shown at right. Phylogenetic tree was constructed by using the neighbor-joining method. The maximum composite likelihood method was used, and the rates among sites were uniform. These analyses were performed using MEGA7 (http://www.megasoftware.net). Scale bar indicates nucleotide substitutions per site.

In summary, we report 2 travelers from Japan infected with DENV-2 in Sri Lanka, where the largest reported outbreak in the country's history began in January 2017. Because the virulent DENV-2 strain is considered the causative agent in this epidemic and the number of deaths has been increasing, we encourage travelers, particularly those who have been previously affected by dengue fever, to prepare against vector mosquitos (e.g., by properly using insect repellents) to avoid DENV infection.

Acknowledgments

We thank all the clinical staff at the Disease Control and Prevention Center, Makiko Ikeda, and Ken-ichi Shibasaki for their assistance with the completion of this study.

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The Politics of Fear: Médecins Sans Frontières and the West African Ebola Epidemic

Michiel Hofman and Sokhieng Au, editors; Oxford University Press, New York, New York, USA; ISBN-13: 978-0190624477; ISBN-10: 0190624477; Pages: 304; Price: US $15.72

The Politics of Fear: Médecins Sans Frontières and the West African Ebola Epidemic is an engaging collection of essays that critically evaluates the response of Médecins Sans Frontières (MSF) to the Ebola epidemic in West Africa during 2014 and 2015. It is an appropriate mixture of reflections, vignettes, case studies, and critiques regarding the role of MSF in the epidemic. It does not offer detailed background on the organization itself or on the Ebola epidemic. Thus, the target audience includes readers who understand the role of MSF, as well as the basic principles of Ebola virus disease (EVD). The book describes complexities of management of such a large outbreak and provides context for the difficult decisions made by MSF leadership, as well as clinicians on the ground. Many books and articles have been written about the epidemiology of EVD, but this book is unique in its focus on the politics and ethics of caring for EVD patients in a complex sociopolitical environment.

The content is well balanced, and chapter authors include MSF physicians, MSF nurses, health officers, administrators, social anthropologists, and bioethicists. MSF as an organization is presented as neither savior nor villain in the crisis; it is described objectively as a player whose actions were motivated by the epidemic itself and its decisions as difficult choices at a time where there were no right choices. The essays eloquently describe the motivations behind such decisions as quarantine of patients, participation in clinical trials, allocation of resources, evacuation of medical personnel, and use of experimental treatments. Each account provides a unique perspective of different aspects of the epidemic and response. In these descriptions, the authors highlight the complicated dynamics in navigating limited resources, overwhelming disease, and citizen distrust. Permeating these essays is the humanity and courage of the doctors, nurses, and staff caring for patients.

This book achieves its goal of describing the multifaceted considerations that must be taken into account when designing policy and response to an epidemic and highlighting the vulnerabilities of healthcare systems that must be addressed to achieve more effective response. It also raises the argument that global humanitarianism, although motivated by altruism, must adapt to existing economies, cultures, and politics if there is any hope for a successful outcome. Although the description of the MSF response is powerful, the essay structure of the multiauthored book does not necessarily provide a coherent approach or strategy to avoid and more effectively treat future crises. However, the book does an outstanding job of outlining the problems that must be addressed.

This book is not a detailed description of the science or epidemiology of EVD but provides in-depth explanation and assessment of the actions of MSF in response to the Ebola epidemic. It is an appropriate resource for those readers who wish to better understand the intricacies of a global epidemic humanitarian response in the context of the disparate sociopolitical environments in which they exist. The reader will appreciate the courage of MSF healthcare providers in the response to the Ebola epidemic, as well as the problems that must be addressed to ensure the dedication and sacrifice of these providers translate to favorable outcomes.

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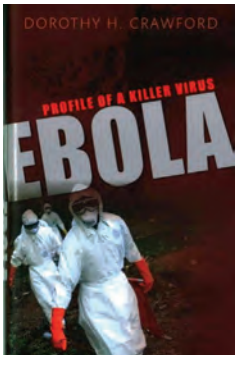
Ebola: Profile of a Killer Virus

Dorothy H. Crawford; Oxford University Press, New York, NY, USA, 2017; ISBN-10: 0198759991; ISBN-13: 978-0-19-875999-7 Pages: 205; Price: US $27.95



Since its first identification in 1976, Ebola has been responsible for about 2 dozen lethal outbreaks in Africa and numerous horrific Hollywood films. Then, in 2014, life seemed to imitate art when Ebola rapidly tore through Guinea, Sierra Leone, and Liberia, causing an unprecedented 11,300 deaths. In this very readable book, Dorothy Crawford, a microbiolo-

gist and the author of several previous books on infectious diseases for the general reader, helps demystify Ebola. The book gives a concise overview of Ebola, beginning with the first identified outbreaks in 1976, and moves on to a summary of what we know about the virus and the disease, clinical care, and attempts to identify the natural reservoir. Another chapter deals briefly with outbreaks after 1976, before moving swiftly to the biggest surprise, Ebola's eruption in West Africa in 2014.

The book is not intended for the technical expert but is an accessible capsule summary for the general reader. Given its origin, the book is also understandably focused on events and perspectives in the United Kingdom. The descriptions in the book are clear and often vivid, and the book provides an accessible overview of Ebola to date. The story has its share of heroes, of course, and is eventful enough for a Hollywood thriller. There are hardworking (and frightened) Western professionals and dedicated nuns. It is especially gratifying to see some of the African heroes recognized, such as Jean-Jacques Muyembe, a microbiology professor in Zaire and the first trained professional to witness the Ebola cases in 1976, who lived to use his knowledge in many later outbreaks. The book is enlivened by memorable vignettes. During the 1995 outbreak in Kikwit (Zaire), Dr. Muyembe explains Ebola and the appropriate precautions to local villagers, translated into their own cultural context as evil spirits that could escape to anyone who touches the victim. Another section tells how a group of young ruffians in a Liberia slum, Moa Wharf, became leaders in the local fight against Ebola.

One of the most disquieting things about the Ebola outbreak in West Africa was the sluggish response of the international community, the subject of much soul-searching ever since. The major response was announced in mid-September and began in October 2014—more than 6 months after the first public report of the outbreak in March. Ironically, newly built Ebola treatment units remained unused as patient numbers dwindled, and by the time vaccine and drug candidates became available, there were no longer enough patients for full-scale randomized trials.

The official goal of "getting to zero" (no Ebola cases) seems implausible, not just because of chronic transmission but because the virus is part of the local ecology. A growing body of circumstantial evidence, including a paper published in Emerging Infectious Diseases a few years ago (*1*), suggests that the virus has been in West Africa for some time. The discoveries of Bundibugyo virus in Uganda, unknown until 2007, and the Taï Forest *Ebolavirus* species in Côte d'Ivoire should have debunked the notion that Ebola could not be present in West Africa.

Crawford does an excellent job of describing the medical response and the urbanization of the outbreak, which probably accounted for much of its devastation and may be a portent of things to come. The usual narrative of the response is generally that "we" came in force and vanquished the outbreak. Because it is obvious and compelling, medical treatment tends to get the most attention, with public health and community participation often relegated to smaller supporting roles. Although Crawford discusses community mobilization, especially late in the epidemic, readers might be interested to know more about this part of the story. Anthropologists and some organizations have been working on documenting and improving grassroots efforts by the people of Africa, including community health workers. As we strengthen health systems and improve access to primary care, we also need to put in place basic public health measures (including water, sanitation, hygiene, and surveillance) that can make communities more resistant to infection, and develop relationships with local leaders before outbreaks occur.

Sustaining the gains achieved has been the hardest part, but is crucial. The author describes a new Ebola Transition Group and Institute for Sanitation, Water, and Public Health in Sierra Leone that appear to be hopeful starts. These measures can help with the next Ebola outbreak but also with the inevitable unexpected epidemic. The author, noting that the Zika virus outbreak in the Western Hemisphere followed shortly after the Ebola outbreak (and was similarly unexpected), rightly argues for more comprehensive global capability. Other infections are waiting for an opportunity to emerge, and the current reactive strategy will likely repeat the same mistakes. George Bernard Shaw wrote: "We learn from history that men [sic] never learn anything from history." As Crawford's book shows, it's time we learned.

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Laurence Stephen Lowry (1887–1976) *Going to Work*, 1943 (detail). Oil on canvas, 179.9 in × 239.8 in/457 cm × 609 cm. ©Imperial War Museums (Art.IWM ART LD 3074), Manchester, Lancashire, England, UK.

Visions of Matchstick Men and Icons of Industrialization

Byron Breedlove

English artist Laurence Stephen Lowry, more commonly referred to as L.S. Lowry, is remembered for being enigmatic and mischievous. Lowry, who rejected 5 different honors during his lifetime—including an Officer of the Most Excellent Order of the British Empire in 1955 and knighthood in 1968—holds the record for the most rejected British honors.

Born in 1887, Lowry lived an upscale area of south Manchester, UK, until financial difficulties forced his parents to relocate to an industrial part of Salford, which he initially hated. His feelings slowly changed: "After a year I got used to it. Within a few years I began to be interested and at length I became obsessed by it."

Though Lowry struggled with schoolwork, he enjoyed drawing and as a youth spent his own money on private art lessons. In 1905, he started taking evening classes in Manchester, where he studied with the French artist Adolphe Valette, who introduced him to Impressionism. Lowry continued attending evening classes throughout the next two decades.

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Despite his enthusiasm for art, Lowry worked as a rent collector for 42 years, retiring from the Pall Mall Property Company on his 65th birthday. His day job accorded Lowry up-close access to everyday life. Jackie Wullschlager, chief art critic of the *Financial Times*, observed that Lowry "knew quotidian dreariness at close hand; he was also used to keeping accounts, and his paintings display an unsentimental, ledger-like notation of pictorial facts even as they become more compositionally complex."

Lowry's best known works depict the environs and icons of industrialization such as mills, manufacturing plants, bridges, and railway stations. Smokestacks and chimneys atop factories and houses belch black smoke into the hazy white sky Lowry favored. His teeming human figures—commonly called "matchstick men" for their singular narrow vertical forms—rigidly move about their activities, appearing disconnected from each other despite their proximity in crowds and long lines.

Focused, perhaps obsessed, with maintaining precision in color and tone, Lowry used only Winsor & Newton Winton Oil Color paints. Further, he famously adhered to a five-color palette of the company's paints, consisting of Ivory Black, Vermillion, Prussian Blue, Yellow Ochre, and Flake White.

Going to Work, this month's cover art, was created to fulfill a short-term commission from the War Artists Advisory Committee. It falls precisely within Lowry's principal oeuvre. The artist fills the foreground with countless anonymous factory workers trudging through the snow, dutifully coalescing into distinct queues as they march toward the bulwark of grim buildings to begin manufacturing machinery and motors at the iconic Mather & Platt foundry, Manchester (which continued operating in some capacity under various owners until July 2017). Crowded between and before the buildings, numerous less-distinct figures swell the ranks of workers toiling to support the war effort.

Viewers, as are Lowry's workers, are inevitably drawn toward the row of buildings dominating the center of the painting. A single smudge of green partly obscured by the double-decker bus suggests a lone tree. Wires stretch across the top of the canvas, and hovering ominously overhead, a pair of barrage balloons offers modest defense from aerial attacks on the factory compound.

Lowry did not consider art a medium for agitation or activism. In his words, "To say the truth, I was not thinking very much about the people. I did not care for them the way a reformer does. They were part of a private beauty that haunted me. I loved them and the houses in the same way, as part of a vision. Had I drawn them as they are, it would not have looked like a vision."

When Lowry died of pneumonia on February 23, 1976, he was both wealthy and well known as an artist and art collector. A few months after his death, the Royal Academy's retrospective exhibition of his works achieved the record number of visitors for any exhibition by a British artist up to that time.

About 5 months after Lowry's death, an outbreak of a new type of pneumonia, now known as Legionnaires' disease, occurred among attendees at an American Legion convention in Philadelphia, Pennsylvania, USA. *Legionella* bacteria can cause Legionnaires' disease or Pontiac fever, collectively known as legionellosis. *Legionella* bacteria are found naturally in freshwater environments but can infect humans when they grow and spread in manufactured water systems.

Viruses, bacteria, and fungi can all cause pneumonia. Many of those factory workers who inspired Lowry's nameless matchstick men may have also died of the disease that ultimately took Lowry. In the 3rd edition of his classic textbook *The Principles and Practice of Medicine* (1898), Sir William Osler describes pneumonia as "the old man's friend," because death from pneumonia seemed to involve less obvious agony than other common causes of death. Despite the availability of vaccines and antibiotics and the remarkable advances in respiratory care, pneumonia continues to affect hundreds of millions of people, old and young, in all parts of the world and also remains the single largest cause of child deaths worldwide.

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Corrections: Vol. 23, No. 9

Norovirus genogroups were referred to incorrectly in the abstract of Norovirus in Bottled Water Associated with Gastroenteritis Outbreak, Spain, 2016 (A. Blanco et al.). The article has been corrected online (https://wwwnc.cdc.gov/eid/article/23/9/16-1489_article).

A link to Table 2 online was incorrect in the print and PDF versions of Real-Time Whole-Genome Sequencing for Surveillance of *Listeria monocytogenes*, France (A. Moura et al.). The PDF of the article has been corrected online (https://wwwnc.cdc.gov/eid/article/23/9/17-0336_article).

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Upcoming Issue

- Fatal Outbreak in Tonkean Macaques Caused by Possibly Novel Orthopoxvirus, January 2015
- Spread of Canine Influenza A(H3N2) Virus, United States
- Group B *Streptococcus* Infections Caused by Handling and Consumption of Raw Fish, Singapore, 2015–2016
- Experimental Infection of Common Eider Ducklings with Wellfleet Bay Virus, a Newly Characterized Orthomyxovirus
- Genomic Analysis of Non–Sorbitol-Fermenting Shiga Toxin–Producing *Escherichia coli* O55:H7
- High Rate of MCR-1–Producing *Escherichia coli* and *Klebsiella pneumoniae* among Pigs, Portugal
- History of *Taenia saginata* Tapeworms in Northern Russia
- *Mycobacterium ulcerans* DNA in Bandicoot Excreta in Buruli Ulcer–Endemic Area, Far Northern Queensland, Australia
- Tick-borne Encephalitis in Sheep, Romania
- West Nile Virus Lineage 2 in Horses and Other Animals with Neurologic Disease, South Africa, 2008–2015
- Lack of Secondary Transmission of Ebola Virus Disease from Healthcare Worker to 238 Contacts, United Kingdom, December 2014
- Genome and Phylogenetic Characterization of Crimean-Congo Hemorrhagic Fever Virus, Spain
- O80:H2 Enteropathogenic *Escherichia coli* in Young Diarrheic Calves, Belgium
- Detection of Zika Virus in April 2013 Patient Samples, Rio de Janeiro, Brazil
- Influenza A(H9N2) Virus, Burkina Faso
- *Angiostrongylus cantonensis* DNA in Cerebrospinal Fluid of Persons with Eosinophilic Meningitis, Laos
- Unexpected Infection with *Armillifer* Parasites

Complete list of articles in the December issue at http://www.cdc.gov/eid/upcoming.htm

Upcoming Infectious Disease Activities

November 5–9, 2017
ASTMH
American Society for Tropical Medicine and Hygiene
66th Annual Meeting
The Baltimore Convention Center
Baltimore, MD, USA
http://www.astmh.org/

December 5–8, 2017
6th National Congress of Tropical Medicine and International Symposium on HIV/AIDS Infection
9th National Congress of Microbiology and Parasitology
80th Anniversary of the Institute of Tropical Medicine Pedro Kourí
Havana, Cuba
http://microbiologia2017.sld.cu/index.php/microbiologia/2017

February 1–3, 2018
8th Advances in Aspergillosis
Lisbon, Portugal
www.AAA2018.org

March 1–4, 2018
18th International Congress on Infectious Diseases (ICID)
Buenos Aires, Argentina
http://www.isid.org/icid/

March 7–9, 2018
ISIRV
2nd International Meeting on Respiratory Pathogens
Singapore
https://www.isirv.org/site/

Announcements

To submit an announcement, end an email message to eideditor@cdc.gov. Include the date of the event, the location, the sponsoring organization(s), and a website that readers may visit or a telephone number or email address that readers may contact for more information.

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You must be a registered user on http://www.medscape.org. If you are not registered on http://www.medscape.org, please click on the "Register" link on the right hand side of the website.

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Article Title

Legionnaires' Disease Outbreaks and Cooling Towers, New York City, New York, USA

CME Questions

1. You are advising a large urban public health department regarding Legionnaires' disease (LD) surveillance. On the basis of the surveillance study by Fitzhenry and colleagues, which one of the following statements about the epidemiological features of 6 community-associated LD outbreaks occurring in New York City since 2006 is correct?

A. No fatalities were reported
B. The largest outbreak occurred in 2007
C. All outbreaks were linked to cooling towers
D. Three outbreaks and 84% (174/207) of outbreak-associated cases occurred in Bronx residents

2. According to the surveillance study by Fitzhenry and colleagues, which one of the following statements about the evolution of investigative methods used by the Department of Health and Mental Hygiene to study 6 community-associated LD outbreaks occurring in New York City since 2006 is correct?

A. Despite improvements in cluster detection tools, small LD clusters are unlikely to be detected
B. Outbreak detection remained constant over time from 2006 to 2015
C. Shoe leather epidemiology identified possible outbreak sources by walking through neighborhoods and interviewing area residents
D. Use of real-time polymerase chain reaction did not change significantly during the study period

3. On the basis of the surveillance study by Fitzhenry and colleagues, which one of the following statements about the public health implications of 6 community-associated LD outbreaks occurring in New York City since 2006 is correct?

A. A new, comprehensive law now regulates the operation and maintenance of New York City cooling towers
B. The concentration of cooling towers predicts whether or where an LD outbreak will occur
C. Widespread availability of clinical cultures for environmental source comparison should facilitate implementation and utility of a cooling tower registry
D. Cluster detection systems are sufficient to identify new outbreaks

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Only one answer is correct for each question. Once you successfully answer all post-test questions, you will be able to view and/or print your certificate. For questions regarding this activity, contact the accredited provider, CME@medscape.net. For technical assistance, contact CME@medscape.net. American Medical Association's Physician's Recognition Award (AMA PRA) credits are accepted in the US as evidence of participation in CME activities. For further information on this award, please go to https://www.ama-assn.org. The AMA has determined that physicians not licensed in the US who participate in this CME activity are eligible for AMA PRA Category 1 Credits™. Through agreements that the AMA has made with agencies in some countries, AMA PRA credit may be acceptable as evidence of participation in CME activities. If you are not licensed in the US, please complete the questions online, print the AMA PRA CME credit certificate, and present it to your national medical association for review.

Article Title

Pregnant Women Hospitalized with Chikungunya Virus Infection, Colombia, 2015

CME Questions

1. You are called to evaluate a 25-year-old woman in her third trimester of an otherwise normal pregnancy. She has multiple symptoms consistent with possible chikungunya virus (CHIKV) infection. Which of the following symptoms was the most common presenting symptom of CHIKV in the current series?

A. Rash
B. Fever
C. Headaches
D. Arthralgia

2. The patient undergoes laboratory testing. What was the most common laboratory finding among patients in the current study?

A. Increased transaminase levels
B. Hyponatremia
C. Leukocytosis
D. Leukopenia

3. The patient is diagnosed with CHIKV infection by reverse transcription polymerase chain reaction. According to the results of the current study, what should you expect regarding clinical outcomes of CHIKV infection during pregnancy?

A. CHIKV sepsis was limited to women in the third trimester
B. Less than 10% of women with CHIKV viremia completed pregnancy during their hospitalization
C. Stage of pregnancy did not influence the rate of admission to the intensive care unit
D. Vertical transmission occurred in 75% of women with CHIKV infection

4. Which of the following symptoms was reported most often among women contacted 1 year after their initial infection with CHIKV?

A. Fever
B. Fatigue
C. Intermittent rash
D. Arthralgias

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Title Page. Give complete information about each author (i.e., full name, graduate degree(s), affiliation, and the name of the institution in which the work was done). Clearly identify the corresponding author and provide that author's mailing address (include phone number, fax number, and email address). Include separate word counts for abstract and text.

Keywords. Use terms as listed in the National Library of Medicine Medical Subject Headings index (www.ncbi.nlm.nih.gov/mesh).

Text. Double-space everything, including the title page, abstract, references, tables, and figure legends. Indent paragraphs; leave no extra space between paragraphs. After a period, leave only one space before beginning the next sentence. Use 12-point Times New Roman font and format with ragged right margins (left align). Italicize (rather than underline) scientific names when needed.

Biographical Sketch. Include a short biographical sketch of the first author—both authors if only two. Include affiliations and the author's primary research interests.

References. Follow Uniform Requirements (www.icmje.org/index.html). Do not use endnotes for references. Place reference numbers in parentheses, not superscripts. Number citations in order of appearance (including in text, figures, and tables). Cite personal communications, unpublished data, and manuscripts in preparation or submitted for publication in parentheses in text. Consult List of Journals Indexed in Index Medicus for accepted journal abbreviations; if a journal is not listed, spell out the journal title. List the first six authors followed by "et al." Do not cite references in the abstract.

Tables. Provide tables within the manuscript file, not as separate files. Use the MS Word table tool, no columns, tabs, spaces, or other programs. Footnote any use of boldface. Tables should be no wider than 17 cm. Condense or divide larger tables. Extensive tables may be made available online only.

Figures. Submit editable figures as separate files (e.g., Microsoft Excel, PowerPoint). Photographs should be submitted as high-resolution (600 dpi) .tif or .jpg files. Do not embed figures in the manuscript file. Use Arial 10 pt. or 12 pt. font for lettering so that figures, symbols, lettering, and numbering can remain legible when reduced to print size. Place figure keys within the figure. Figure legends should be placed at the end of the manuscript file.

Videos. Submit as AVI, MOV, MPG, MPEG, or WMV. Videos should not exceed 5 minutes and should include an audio description and complete captioning. If audio is not available, provide a description of the action in the video as a separate Word file. Published or copyrighted material (e.g., music) is discouraged and must be accompanied by written release. If video is part of a manuscript, files must be uploaded with manuscript submission. When uploading, choose "Video" file. Include a brief video legend in the manuscript file.

Types of Articles

Perspectives. Articles should not exceed 3,500 words and 50 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. Articles should provide insightful analysis and commentary about new and reemerging infectious diseases and related issues. Perspectives may address factors known to influence the emergence of diseases, including microbial adaptation and change, human demographics and behavior, technology and industry, economic development and land use, international travel and commerce, and the breakdown of public health measures.

Synopses. Articles should not exceed 3,500 words in the main body of the text or include more than 50 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (not to exceed 150 words), a 1-line summary of the conclusions, and a brief biographical sketch of first author or of both authors if only 2 authors. This section comprises case series papers and concise reviews of infectious diseases or closely related topics. Preference is given to reviews of new and emerging diseases; however, timely updates of other diseases or topics are also welcome. If detailed methods are included, a separate section on experimental procedures should immediately follow the body of the text.

Research. Articles should not exceed 3,500 words and 50 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. Report laboratory and epidemiologic results within a public health perspective. Explain the value of the research in public health terms and place the findings in a larger perspective (i.e., "Here is what we found, and here is what the findings mean").

Policy and Historical Reviews. Articles should not exceed 3,500 words and 50 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. Articles in this section include public health policy or historical reports that are based on research and analysis of emerging disease issues.

Dispatches. Articles should be no more than 1,200 words and need not be divided into sections. If subheadings are used, they should be general, e.g., "The Study" and "Conclusions." Provide a brief abstract (50 words); references (not to exceed 15); figures or illustrations (not to exceed 2); tables (not to exceed 2); and biographical sketch. Dispatches are updates on infectious disease trends and research that include descriptions of new methods for detecting, characterizing, or subtyping new or reemerging pathogens. Developments in antimicrobial drugs, vaccines, or infectious disease prevention or elimination programs are appropriate. Case reports are also welcome.

Another Dimension. Thoughtful essays, short stories, or poems on philosophical issues related to science, medical practice, and human health. Topics may include science and the human condition, the unanticipated side of epidemic investigations, or how people perceive and cope with infection and illness. This section is intended to evoke compassion for human suffering and to expand the science reader's literary scope. Manuscripts are selected for publication as much for their content (the experiences they describe) as for their literary merit. Include biographical sketch.

Research Letters Reporting Cases, Outbreaks, or Original Research. EID publishes letters that report cases, outbreaks, or original research as Research Letters. Authors should provide a short abstract (50-word maximum), references (not to exceed 10), and a short biographical sketch. These letters should not exceed 800 words in the main body of the text and may include either 1 figure or 1 table. Do not divide Research Letters into sections.

Letters Commenting on Articles. Letters commenting on articles should contain a maximum of 300 words and 5 references; they are more likely to be published if submitted within 4 weeks of the original article's publication.

Commentaries. Thoughtful discussions (500–1,000 words) of current topics. Commentaries may contain references (not to exceed 15) but no abstract, figures, or tables. Include biographical sketch.

Books, Other Media. Reviews (250–500 words) of new books or other media on emerging disease issues are welcome. Title, author(s), publisher, number of pages, and other pertinent details should be included.

Conference Summaries. Summaries of emerging infectious disease conference activities (500–1,000 words) are published online only. They should be submitted no later than 6 months after the conference and focus on content rather than process. Provide illustrations, references, and links to full reports of conference activities.

Online Reports. Reports on consensus group meetings, workshops, and other activities in which suggestions for diagnostic, treatment, or reporting methods related to infectious disease topics are formulated may be published online only. These should not exceed 3,500 words and should be authored by the group. We do not publish official guidelines or policy recommendations.

Photo Quiz. The photo quiz (1,200 words) highlights a person who made notable contributions to public health and medicine. Provide a photo of the subject, a brief clue to the person's identity, and five possible answers, followed by an essay describing the person's life and his or her significance to public health, science, and infectious disease.

Etymologia. Etymologia (100 words, 5 references). We welcome thoroughly researched derivations of emerging disease terms. Historical and other context could be included.

Announcements. We welcome brief announcements of timely events of interest to our readers. Announcements may be posted online only, depending on the event date. Email to eideditor@cdc.gov.

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