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Tuberculosis

March 2013

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EMERGING INFECTIOUS DISEASES®

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EMERGING INFECTIOUS DISEASES

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On the Cover

Alice Neel (1900–1984)
T.B. Harlem (1940)

Oil on canvas
(76.2 cm x 76.2 cm)

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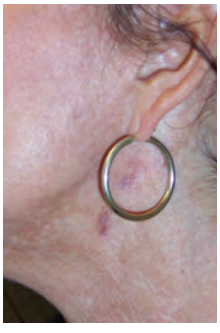

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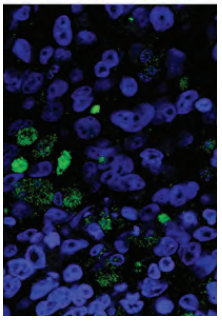

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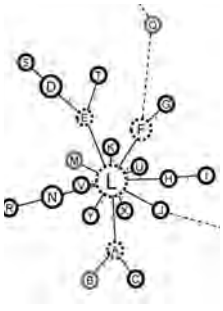

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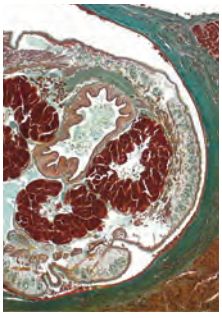

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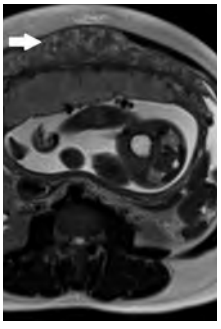

About the Cover

Online Reports

Measles Elimination Efforts and 2008–2011 Outbreak, France

Denise Antona, Daniel Lévy-Bruhl, Claire Baudon, François Freymuth, Mathieu Lamy, Catherine Maine, Daniel Floret, and Isabelle Parent du Chatelet

Although few measles cases were reported in France during 2006 and 2007, suggesting the country might have been close to eliminating the disease, a dramatic outbreak of >20,000 cases occurred during 2008–2011. Adolescents and young adults accounted for more than half of cases; median patient age increased from 12 to 16 years during the outbreak. The highest incidence rate was observed in children <1 year of age, reaching 135 cases/100,000 infants during the last epidemic wave. Almost 5,000 patients were hospitalized, including 1,023 for severe pneumonia and 27 for encephalitis/myelitis; 10 patients died. More than 80% of the cases during this period occurred in unvaccinated persons, reflecting heterogeneous vaccination coverage, where pockets of susceptible persons still remain. Although vaccine coverage among children improved, convincing susceptible young adults to get vaccinated remains a critical issue if the target to eliminate the disease by 2015 is to be met.

In 1983, measles vaccination was introduced into the immunization schedule for toddlers in France; the combined measles-mumps-rubella vaccine (MMR) has been used since 1986. A second MMR dose was added in 1996. Until 2004, recommendation were that the first dose (MMR1) be administered at 12 months of age and the second (MMR2) at 3–6 years of age. A catch-up schedule with 1 dose of MMR was also recommended for unvaccinated children 6–13 years of age.

To meet the World Health Organization (WHO) European Region's goals for measles elimination, a national plan was implemented in 2005. It included bringing forward the administration of MMR2 to a child's second year of life in addition to expanding catch-up to include 2 doses for unvaccinated persons born after 1991 and 1 dose for those born during 1980–1991. Other measures implemented included the vaccination of susceptible health professionals and detailed control measures around suspected cases (*1*,*2*).

Measles was a notifiable disease from 1945 to 1986 in France. From 1986 to 2004, surveillance of the disease was managed through a national sentinel network of general practitioners (*3*). Because cases were becoming rare, mandatory reporting was reintroduced in 2005. Laboratory confirmation by serologic or saliva testing and including virus characterization was simultaneously implemented.

Only 40 and 44 cases were notified in 2006 and 2007, respectively, which placed the incidence of reported cases below the WHO threshold for measles elimination (0.1 cases/100,000 inhabitants). However, in 2008, the number of cases started increasing and rose dramatically thereafter (*4*,*5*). Here, we describe the 2008–2011 measles epidemic in France and the characteristics of cases reported over that period.

Vaccine Coverage

Vaccine coverage is measured through the analysis of infants' health certificates, which are filled in during a mandatory medical examination when children reach their second birthday. The certificates are sent by physicians to the district Maternal and Child Health Offices, then aggregated at a national level and analyzed by the Institut de Veille Sanitaire (InVS). Coverage in older children is assessed through random sampling school surveys conducted among children 6, 11, and 15 years of age (*6*).

The MMR1 coverage at 2 years of age increased steadily in the 1980s, leveling off at 80%–85% of the target population in the 1990s. Although coverage has improved since then, it never exceeded 89%–90% in children born between 2005 and 2008. Furthermore, differences in coverage persist between regions, with many districts in southern France still below 85% (Figure 1). Since 2002, the MMR1 coverage in

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school children has remained consistent at between 93% and 96%, reflecting a significant proportion of catch-up vaccinations being administered to children >2 years of age. Figure 2 shows the increase over time of MMR1 coverage for different birth cohorts. In 2008, the MMR1 coverage reached 96.6% in children 11 years of age.

The same sources were used to monitor the vaccine coverage for MMR2. In 2-year-old children, the MMR2 coverage improved over time, with a 2-fold increase (from 29.3% to 60.9%) between 2006 and 2010. In older children, results from school-based surveys (www.invs.sante.fr/Dossiers-thematiques/Maladies-infectieuses/Maladies-a-prevention-vaccinale/Couverture-vaccinale/Donnees) showed a significant increase over time: from 28.1% in 2003 to 45.1% in 2006 in 6-year-old children; from 56.8% in 2002 to 74.2% in 2005 and to 85.0% in 2008 in 11-year-old children. In 15-year-old children, the MMR2 coverage increased from 65.7% in 2004 to 84.0% in 2009 (provisional data for 2008 and 2009). No vaccine coverage data are available for persons >15 years of age.

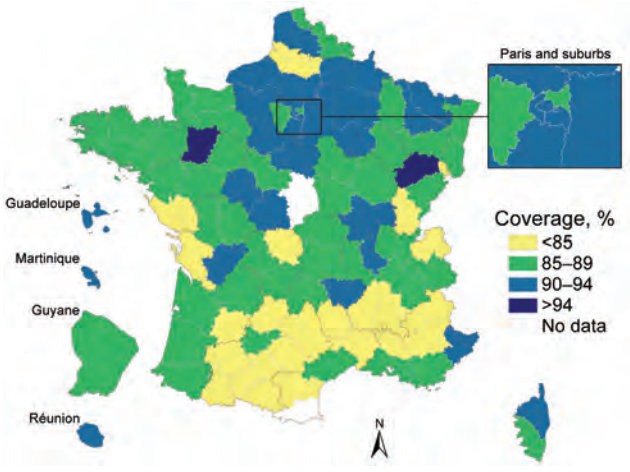

Figure 1. Coverage of initial measles-mumps-rubella vaccination (MMR1) listed in health certificates for children at 24 months of age, by district (département), France, 2003–2008. Data are latest available figures for the period. Sources: Institut de Veille Sanitaire, Ministry of Health statistical department.

Epidemiology of Measles Outbreak

To describe the epidemic, we included cases notified from January 1, 2008, through December 31, 2011, that fulfilled the criteria for reporting. Clinicians and biologists notify cases to the Regional Health Agencies responsible for implementing control measures and sending notification forms to the InVS (notification forms and case definitions available online, www.invs.sante.fr/Dossiers-thematiques/Maladies-infectieuses/Maladies-a-prevention-vaccinale/Rougeole). Cases were classified as clinical or confirmed (biologically or epidemiologically) as described (*4*). Cases in patients without any known exposure to a measles case in France and who had been in a measles-endemic country 7–18 days before the rash onset were considered imported.

Population estimates from the National Institute of Statistics and Economic Studies were used to calculate incidence rates. Proportions were compared by using the χ^2 test. The χ^2 for trend was used to test associations between age groups and types of complications in hospitalized patients. Descriptive analysis was done by using Stata version 11 software (StataCorp LP, College Station, Texas, USA).

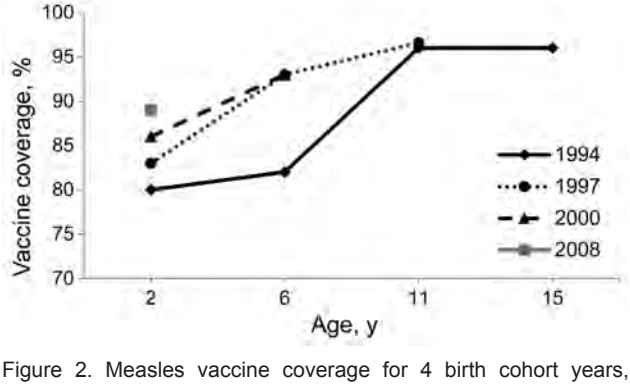

Figure 2. Measles vaccine coverage for 4 birth cohort years, France. Sources: Institut de Veille Sanitaire, Ministry of Health statistical department, Ministry of Education.

The date of the appearance of the rash was used as the disease onset date. Epidemic waves were defined as cases occurring between October and the following September over the 4-year period, resulting in the identification of 3 waves. Notifications to the national nosocomial surveillance system were also included in the analysis.

During spring 2008, several measles clusters were identified among students at private schools operated by a traditionalist religious group; secondary household clusters also occurred (*4*). Ministry of Health (MOH) representatives contacted the group's leaders, but these discussions were unsuccessful in mitigating parents' reluctance to have their children vaccinated. The virus progressively spread out of this community; subsequent outbreaks were reported in both private and public schools. In 2009, community-wide transmission was established.

From January 2008 through December 2011, a total of 22,686 measles cases were notified. We excluded from analysis 84 cases in which postvaccination rash was reported (defined clinically as rash occurring 5–20 days after vaccination in the absence of any known exposure to a measles case); for 15 (18%) patients with known or possible contact with a case, a saliva sample was sent to the National Reference Center (NRC) for virus genotyping, and the vaccine virus was identified. We also excluded 399 cases with negative tests and 29 nonresident patients

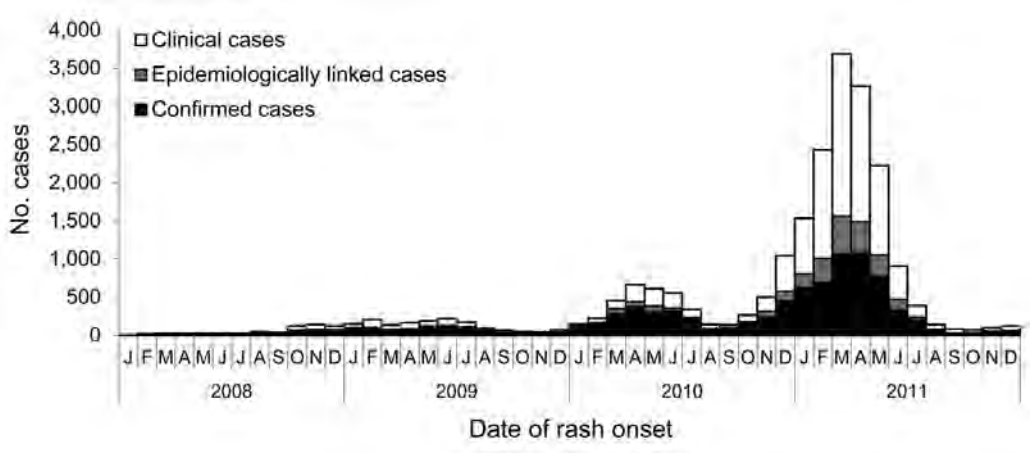

Figure 3. Number of notified measles cases per month, determined by date of rash onset, France, January 2008–December 2011.

exposed to measles during a temporary stay in France. In total, 22,178 cases were included in the analysis, 447 of which were imported (including 230 from Europe).

Spatiotemporal Evolution of the Epidemic

Among the 22,178 cases we analyzed, 603 were reported in 2008, 1,543 in 2009, 5,083 in 2010, and 14,949 in 2011. The epidemic curve (Figure 3) showed that the number of cases started increasing in mid-2008, evolving in 3 epidemic waves. A total of 21,669 cases were reported from October 2008 through September 2011: 1,774 during the first wave, October 2008–September 2009; 3,429 during the second wave, October 2009–September 2010; and 16,466 during the third wave, October 2010–September 2011. Incidence of measles during the study period was 2.7 cases per 100,000 inhabitants during the first wave, 5.2/100,000 during the second wave, and 25.6/100,000 during the third wave. Peaks were observed in April 2010 (659 cases) and March 2011 (3,642 cases).

The geographic distribution of cases was analyzed for the 21,240 (96%) patients who had a documented place of residence. Among them, 14 had been exposed to measles in mainland France but lived in French overseas districts: La Réunion (6), French Guyana (1), Guadeloupe (5), or Martinique (2). The virus circulated nationwide, but southern France was the area most affected, especially during the third wave; incidences in the Rhône-Alpes, Provence-Alpes-Côte d'Azur, and Languedoc-Roussillon regions reached 97.2, 53.9, and 48.3 cases/100,000 persons, respectively (Figure 4).

Case Characteristics

Case Classification and Patient Sex and Age

Of the 22,178 cases analyzed, 10,711 (48.3%) met the clinical case definition, 8,847 (39.9%) were confirmed biologically, and 2,620 (11.8%) were linked epidemiologically to a biologically confirmed case. The male-female ratio for patients was 1.05 and was comparable across all age groups and epidemic waves.

Age was known for 22,087 (99.6%) patients. Median age increased over time: 12 years during the first wave (interquartile range [IQR] 5–18 years), 14.5 years during the second wave (IQR 4–24 years), and 16 years during the third wave (IQR 7–24 years). During the third wave, incidence reached 134.6 cases/100,000 in infants <1 year of age, 68.6 cases/100,000 in children 10–19 years of age, and 46.8 cases/100,000 in persons 20–29 years of age.

Infants <1 year of age were the most affected by the increasing number of cases between waves. Incidence for this age group was 2.6´ higher for the second wave compared with the first and 9.8´ higher for the third wave

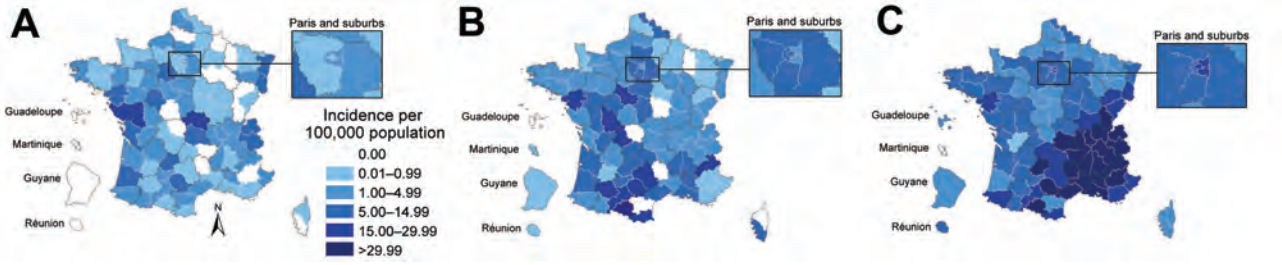

Figure 4. Evolution of geographic distribution of measles cases during 3 epidemic waves, France. A) October 2008–September 2009; B) October 2009–September 2010; C) October 2010–September 2011.

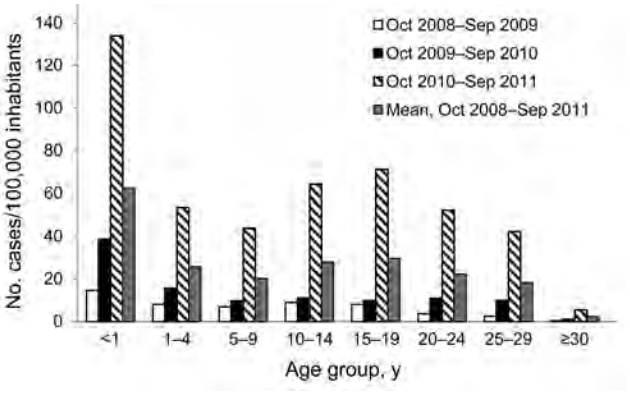

Figure 5. Incidence of measles cases during 3 epidemic waves, by patient age group, France, 2008–2011.

compared with the first. Of 1,572 measles cases reported in this age group (7.5% of all cases), 29 were in infants <1 month of age, including 13 neonatal cases; 269 were in infants 1–5 months of age, 547 in infants 6–8 months of age, and 727 in infants 9–11 months of age. However, increases among all age groups were substantial; from the first to the third waves, incidence increased 7-fold for those 1–9 years of age (from 8.4 to 54.1 cases/100,000 children), 8-fold for those 10–19 years of age (from 8.6 to 68.3 cases/100,000 children), and 15-fold for those 20–29 years of age (from 3.1 to 48.1 cases/100,000 persons) (Figure 5).

Vaccination Status

Patient vaccination status was reported by clinicians for 18,434 (83%) cases; for 6,841 (37.1%) patients, status was verified by a vaccination document listing the date of the last injection. Among these 6,841 patients, 1,375 (20.1%) were vaccinated, 1,041 (15.2%) with 1 dose and 318 (4.7%) with 2 doses. Data on the number of doses were not available for 16 patients (0.2%).

The proportion of vaccinated cases differed significantly between age groups ($p<0.001$). Of persons 20–24 years of age, only 34.8% had been vaccinated: 26.9% with only 1 dose, 4.8% with 2 doses, and 0.1% with an unknown number of doses (Figure 6).

Case Severity

During the 4-year study period, 2,582 (11.6%) measles cases involved complications; most frequently reported were pneumonia (1,375 cases, 6.2%), acute otitis media (321 cases, 1.4%), and hepatitis or pancreatitis (248 cases, 1.1%). Diarrhea was reported in 100 cases (0.4%).

Overall, 4,980 (22.4%) measles patients were hospitalized, with substantial differences in hospitalization rates between age groups. Hospitalization rates were 28% for infants <1 year of age and 31%–38% for adults (Figure 7). Among hospitalized patients, the most frequently reported complication was pneumonia (1,023 cases, 20.6%) (Table). The male-female ratio (1.0) for patients with complications was similar to that for patients without complications; median patient age was 24 years (IQR 11–32 years). The proportion of pneumonia cases increased with age, reaching 28.8% in adults ≥30 years of age ($p<0.001$ for comparison of proportion of pneumonia in adults and overall rate of pneumonia).

Neurologic complications included 1 case of myelitis and 26 cases of encephalitis (rate 0.6/1,000 cases). Of the encephalitis cases, 25 were acute disseminated encephalomyelitis, and 1 was measles-inclusion body encephalitis occurring 4 months after the initial appearance of measles rash. Patient male-female ratio was 0.8; the median age was 16 years (IQR 12–24 years).

Liver and/or pancreatic complications were reported in 5.0% of patients ≥15 years of age ($p<0.001$ for comparison of proportion of liver/pancreatic complications among patients ≥15 years of age and overall rate of liver/pancreatic complications). The proportion of hospitalized patients with otitis media (1.3%) varied significantly, from 5.1% for infants <1 year of age to <0.5% for patients ≥15 years of age ($p<0.001$).

Ten patients died (0.45 deaths/1,000 cases). Causes of death were pneumonia (7), encephalitis (1 acute disseminated encephalomyelitis, 1 measles-inclusion body encephalitis), and myocarditis (1). Nine of these patients were <30 years of age (median 23 years, range 11–68 years); 7 were female (male-female ratio 0.4). Seven patients were immunodeficient; 1 had congenital immunodeficiency and 6 had acquired immunodeficiency (e.g., Hodgkin's lymphoma, Crohn's disease, HIV, immunosuppressive treatment).

Nosocomial Episodes

During the study period, 85 nosocomial episodes involving measles were reported; 73% occurred in emergency, internal medicine, and pediatric wards. These episodes involved 146 cases (most were also notified through mandatory reporting); 1 immunodeficient patient died. Twenty-five of these 85 episodes led to clusters with a median of 3 cases per episode (maximum 16); health care professionals were involved in 75% of those episodes.

Circulating Genotypes

The measles virus genotype D5, predominant in 2008, stopped circulating in mid-2009 and was replaced by genotype D4. The latter accounted for 16.2% of 123 viruses genotyped in 2008, 75.0% of 284 genotyped in 2009, 97.2% of 696 genotyped in 2010, and 90.2% of 529 genotyped in 2011. The D4 genotypes are usually similar to the Montréal.CAN/89xD4 strain and drift from strain MVs/Enfield.GBR/14.07, which was first identified in Great Britain

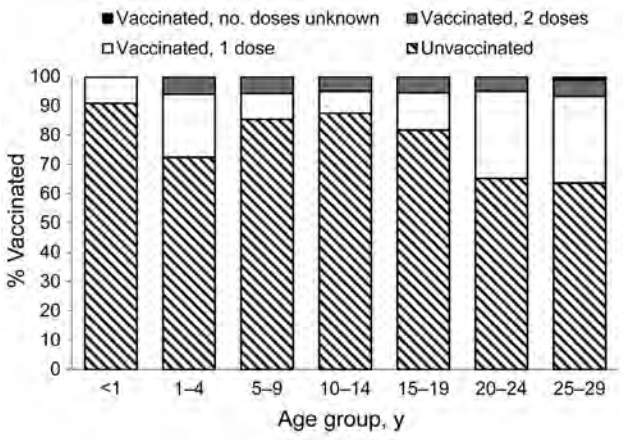

Figure 6. Vaccination status of measles patients, by age, France, January 2008–December 2011. Vaccination status was unknown for 80 patients.

in 2007 (GenBank accession no. EF600554). The epidemic virus in France was the strain MVs/Montaigu.FRA/43.08, first identified during the second half of 2008, and was different from the MVs/Enfield.GBR/14.07, which was found in March 2008 during a nosocomial outbreak in Reims (Champagne-Ardenne region). Circulating to a lesser extent, other genotypes identified by the NRC were A, B3, D8, D9, H1, H2, and, recently, G3 (7.5% in 2011).

Discussion

We describe an explosive outbreak of measles in France, with >22,000 cases reported during 2008–2011. Almost 5,000 persons were hospitalized, including >1,000 who had severe pneumonia and 27 who had encephalitis/myelitis. Ten persons died. As the virus spread nationwide, the most affected areas were, as expected, those with the lowest vaccine coverage, mainly in southern France. However, even districts with >90% MMR1 coverage in toddlers were affected, confirming that a very high level of immunity is required for measles elimination (*7*). Our data confirm that a shift in age at infection has occurred compared with that in the prevaccination era and that the risk for complications increases with age: half of the patients were >15 years of age, and among them, one third were hospitalized. In addition, incidence was highest among infants too young to be vaccinated.

Underreporting was estimated to be in excess of 50%, probably changing over time and with patient age. Local outbreak investigations found that <50% of cases had been notified, mainly because of secondary cases in households; these patients were less likely to seek medical advice once a first case had been diagnosed. Among the data collected from the main laboratories testing for measles IgM antibodies, the number of patients with measles-positive results was 1.5′ higher than the number of notified cases. Cases diagnosed in hospitals were probably more often notified than those diagnosed in private practices (*8*); this may explain the higher proportions of hospitalized cases we observed, especially in adults with pneumonia, compared with those in the literature (*9*,*10*).

The absence of a vaccination registry in France precludes comprehensive documentation of patient vaccination status for most of the notified cases. For the majority of cases occurring in older children or young adults, patients did not bring along any vaccination documentation when seeking medical care for measles. Furthermore, these patients may have consulted a different practitioner than the one who followed them during their childhood. However, lack of documentation does not appear to have induced a substantial bias in the description of the vaccination status of the patients; vaccination status measured for patients who had written vaccination documentation (20.1%) was close to that measured in those without such documentation (17.4%).

The proportion of vaccinated patients varied according to patient age, probably reflecting the disparity of vaccine coverage between age groups. Overall, 80% of notified cases occurred in unvaccinated persons. The geographic heterogeneity of vaccine coverage and virus exposure precluded the use of the screening method to estimate vaccine effectiveness (*11*). Nevertheless, the ease that the virus had in spreading could by no means be explained by lower than expected vaccine effectiveness. In 2011, the high number of 1-dose–vaccinated 20- to 29-year-old patients prompted the MOH to extend the 2-dose MMR schedule to everyone born since 1980 (*2*).

The NRC identified a D4 genotype variant as the predominant circulating strain during this outbreak. During the third wave, genotype G3 was identified; this genotype is known to have emerged in several other European countries and likely was imported from Southeast Asia (*12*).

Several European countries were affected by measles outbreaks during the same 4-year period as the outbreak

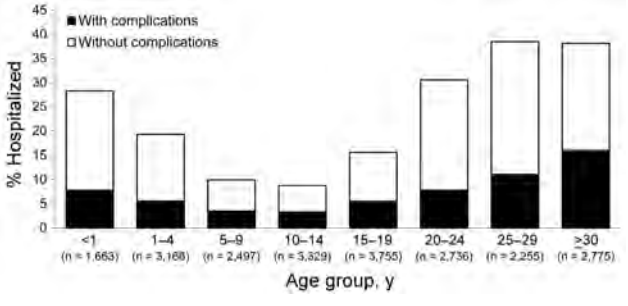

Figure 7. Percentage of measles patients hospitalized, with and without reported complications, by age group, France, January 2008–December 2011.

Table. Measles-related complications among 4,968 hospitalized patients, by age group, France, January 2008–December 2011*

Complications	No. (%) patients, by age group, y					p value†
	<1, n = 470	1–14, n = 1,150	15–29, n = 2,290	≥30, n = 1,058	Total, n = 4,968	
All complications	130 (27.7)	373 (32.4)	669 (29.2)	441 (41.7)	1,613 (32.5)	<0.001
Pulmonary						
Pneumonia	75 (15.9)	227 (19.7)	416 (18.2)	305 (28.8)	1,023 (20.6)	<0.001
Other‡	1 (0.2)	21 (1.8)	32 (1.4)	15 (1.4)	69 (1.4)	NS
Ear, nose, throat						
Otitis media	24 (5.1)	27 (2.3)	11 (0.5)	2 (0.2)	64 (1.3)	<0.001
Other‡	6 (1.3)	12 (1.0)	11 (0.5)	5 (0.5)	34 (0.7)	NS
Digestive tract						
Diarrhea/dehydration	3 (0.6)	26 (2.3)	30 (1.3)	19 (1.8)	78 (1.5)	NS
Liver or pancreas disorder	1 (0.2)	5 (0.4)	105 (4.6)	70 (6.6)	181 (3.6)	<0.001
Other‡	11 (2.3)	18 (1.6)	19 (0.8)	5 (0.5)	53 (1.1)	NS
Neurologic						
Encephalitis or myelitis	0	10 (1.1)	13 (0.6)	4 (0.4)	27 (0.5)	NS
Other‡	2 (0.4)	10 (0.9)	8 (0.3)	1 (0.1)	21 (0.4)	NT
Keratitis	0	1 (0.1)	9 (0.4)	6 (0.6)	16 (0.3)	
Other‡	10 (0.2)	21 (1.8)	32 (1.4)	17 (1.6)	80 (1.6)	NS
Death	0	2 (0.2)	6 (0.3)	2 (0.2)	10 (0.2)	NT

*Total excludes 12 patients with unknown date of birth. NS, not significant; NT, not tested (expected value <5).
†By χ^2 test.
‡Other complications (numbers in brackets indicate no. cases of that complication identified): pulmonary (e.g., bronchitis, pleurisy); ear, nose, throat (e.g., tonsillitis, sinusitis); digestive tract (e.g., vomiting, dysphagia, abdominal ache); neurologic (e.g., Guillain-Barré syndrome [1], meningitis); other (e.g., miscarriage [5], premature delivery [3], neonatal infection [4], myocarditis/pericarditis [8], general state impairment [42], thrombocytopenia [18]).

period in France. The number of cases reported to the European Center for Disease Prevention and Control by the 29 participating countries increased 4-fold, from 7,817 in 2008 to 30,567 in 2011. Five countries (France, Italy, Romania, Spain, and Germany) accounted for >90% of all measles cases reported in 2011; France alone accounted for 50% (*13*–*15*). France also exported cases, not only to other European countries (*16*) but also to areas currently in the measles elimination certification process, such as the Americas (*17*), including the French districts of Martinique, Guadeloupe, and French-Guiana.

Our findings indicate that the measles epidemiologic profile observed in France in 2006–2007 was only a honeymoon period before reemergence, rather than an indication of imminent elimination. This reemergence was the consequence of persistent suboptimal vaccine coverage in toddlers and insufficient catch-up vaccination in older cohorts, leading to the growth of a large reservoir of susceptible persons.

A large outbreak had, in fact, been anticipated in France through modeling (*18*), analysis of coverage data (*19*), and a serosurveillance survey performed in 1998 (*20*). These findings led to initiatives to try to increase measles vaccination coverage. MMR mass media campaigns conducted from 1985 on were reinforced with promotional materials targeting vaccinators and the general public that were designed and distributed through various channels. In 1999, MMR vaccines became 100% free for children. Several studies aiming to identify barriers to MMR vaccination were conducted, and specific interventions to increase vaccine coverage were undertaken, particularly in low-performing districts. The studies consistently showed that the absence of MMR vaccination was the result of explicit parent and/or health care professional choice and not a lack of access to vaccination for geographic, financial, or sociocultural reasons (*21*,*22*). Consequently, tailor-made interventions were implemented, but their effect was disappointing (*21*); MMR1 coverage in children at 2 years of age did not increase above 90%.

To combat the reemergence of measles in France, more drastic control measures around sporadic cases and clusters were implemented in 2008–2009 by local health authorities. The main recommendations were to update the MMR vaccination status when needed and to propose postexposure vaccination or immunoglobulin injection (*1*). Media coverage of the epidemic emphasized the high likelihood of measles exposure associated with a risk for severe measles in young adults and in infants too young to be vaccinated, but these efforts were nevertheless unsuccessful in increasing coverage. Large catch-up vaccination campaigns in schools recommended by experts to the MOH were not implemented, primarily because of lingering effects from a hepatitis B vaccination scare that followed large school-based catch-up vaccination campaigns conducted in the 1990s (*23*). Furthermore, the controversy surrounding the large-scale influenza A(H1N1)pdm09 vaccination campaign conducted during 2009 likely further contributed to the MOH's decision not to undertake any mass vaccination campaigns. In 2010, however, free measles vaccination was extended for children up to 17 years of age.

Although vaccine coverage improved over time during the outbreak, our experience confirms that high coverage in children is insufficient to avoid the spread of measles virus, especially when catch-up vaccination in older cohorts remains insufficient. Vaccine coverage figures at 2 years of

age for children born in 2008 were 89.1% and 60.9% for MMR1 and MMR2, respectively, well below the respective national targets of 95% and 80%. Even if catch-up vaccination has resulted in vaccine coverage for adolescents reaching 95% and 84% for MMR1 and MMR2, respectively, the immunity level in young adults was still too low; results of a national seroprevalence survey conducted in 2009–2010 showed 9% of those 20–29 years of age were susceptible to measles (*24*).

These conclusions raise questions about the possibility of reaching elimination of highly communicable diseases for which levels of immunity >95% at an age as young as 2 years are required for elimination in societies in which a substantial proportion of the population is reluctant to vaccinate. Furthermore, it is necessary to reach and maintain these levels in each birth cohort to avoid new reservoirs of susceptible children. Regulation and social context in France do not currently favor mandatory immunization in the general population. Even more problematic, the very low levels of residual illness and death associated with this disease make effective communication about the serious risks involved difficult.

In the first wave of this epidemic, attempts to convince health professionals and the general public about the urgent need to update the measles vaccination status of the target population were unsuccessful. Specific documents were prepared and widely disseminated, especially during European Immunization Week in 2009, 2010, and 2011, for which measles was chosen by the MOH as the national priority topic. Only in 2011, when the epidemic started to peak and when many hospitalizations, complications, and even deaths were highlighted, did sales data for MMR vaccines show an increase. At the same time, communication about the serious effects of the epidemic was reinforced and greater media coverage garnered. A mandatory check of measles immunization status, with reminders sent to parents of children who were not fully immunized, was also implemented in schools. Provisional results showed MMR1 coverage of 97%–98% in adolescents, which may have contributed to the absence of a notable fourth wave in 2012 (<500 cases reported through the end of June).

Will France be in a situation to meet the 2015 measles elimination target? A reservoir of susceptible persons certainly remains, but levels of susceptibility in those <20 years of age should now be close to the age-specific WHO elimination thresholds. The likelihood of future resurgence depends on several parameters that are difficult to document. The postepidemic level of seroprotection depends on the actual size of the 2008–2011 epidemic and the magnitude of the recent MMR vaccination catch-up for each dose in the various age groups. Clustering of the remaining susceptible persons still needs to be examined; several studies are planned or underway to document those parameters. Estimation of measles vaccination coverage at subdistrict level through a newly available exhaustive national vaccines reimbursement database will help to identify pockets of unvaccinated persons.

As useful as these studies might be, however, they will not solve the underlying issue of improved vaccination coverage through communication strategies targeting persons still reluctant to undertake MMR vaccination, either for themselves or for their children. It is likely that catch-up vaccination campaigns would have helped increase vaccine coverage, and although these are considered inappropriate in France at this time, such campaigns should be considered a primary tool in countries facing similar measles epidemic profiles.

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Nontuberculous Mycobacterial Infection after Fractionated CO_2 Laser Resurfacing

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Nontuberculous mycobacteria are increasingly associated with cutaneous infections after cosmetic procedures. Fractionated CO_2 resurfacing, a widely used technique for photorejuvenation, has been associated with a more favorable side effect profile than alternative procedures. We describe 2 cases of nontuberculous mycobacterial infection after treatment with a fractionated CO_2 laser at a private clinic. Densely distributed erythematous papules and pustules developed within the treated area within 2 weeks of the laser procedure. Diagnosis was confirmed by histologic analysis and culture. Both infections responded to a 4-month course of a multidrug regimen. An environmental investigation of the clinic was performed, but no source of infection was found. The case isolates differed from each other and from isolates obtained from the clinic, suggesting that the infection was acquired by postprocedure exposure. Papules and pustules after fractionated CO_2 resurfacing should raise the suspicion of nontuberculous mycobacterial infection.

Nontuberculous mycobacteria (NTM) are increasingly associated with cutaneous and soft tissue infections after cosmetic and spa procedures, such as liposuction, mammoplasty, blepharoplasty, mesotherapy, and whirlpool footbaths during pedicures (*1*–*5*). These infections are often difficult to diagnose, resulting in major treatment delays (*4*,*6*). Fractionated CO_2 laser resurfacing is a widely used cosmetic procedure that minimizes the appearance of rhytides (skin wrinkles) and acne scars, and compared with older laser procedures, fractionated CO_2 resurfacing is associated with less downtime and a lower rate of infectious and noninfectious complications (*7*–*9*). Although fractionated CO_2 laser therapy is associated with decreased rates of postprocedure infection, infections such as herpes simplex virus, bacterial, and candidal infections have been reported (*8*–*10*). Palm et al. recently reported the first case of NTM infection caused by *Mycobacterium chelonae* after treatment with a fractionated CO_2 laser for facial resurfacing (*11*). Given the length of time from the procedure to the diagnosis (≈2 months), a source of NTM infection was not sought.

We report 2 additional cases of NTM infection after treatment with fractionated CO_2 resurfacing at the same private clinic and an extensive environmental investigation to identify a source of infection. This study received formal exemptions from review by the Institutional Review Boards of the University of North Carolina and Duke University Medical Center.

Case-Patient 1

A 53-year-old woman had multiple erythematous papules and pustules densely distributed over her face, neck, and chest (Figure 1, panel A) 2 weeks after receiving fractionated CO_2 laser resurfacing (Solta Medical Inc., Hayward, CA, USA). Before laser resurfacing, the patient began a prophylactic 7-day course of valacyclovir because of a history of recurrent herpes labialis. Immediately before the procedure, the patient's skin was cleansed with 70% isopropanol. Topical lidocaine/tetracaine ointment was applied to the skin for topical anesthesia, followed by intraoral nerve block and tumescent anesthesia for the face only. The neck and chest were treated at 40 mJ (treatment level 7 mJ) and 25% coverage, the forehead at 60 mJ (treatment level 9 mJ) and 35% coverage, and the nose and cheeks at 70 mJ (treatment level 9 mJ) and 35% coverage (total 10.46 kJ). Immediately after the procedure, the patient's skin was cleansed with sterile saline, and emollient was applied. Postprocedure home wound care consisted of vinegar solution (vinegar diluted with bottled water) applications once a day and avoidance of showering, scrubbing, and cosmetics for 72 h.

Ten days post–laser treatment, erythematous papules and pustules developed over the face, neck, and chest of the patient. Outpatient treatment was initiated with oral

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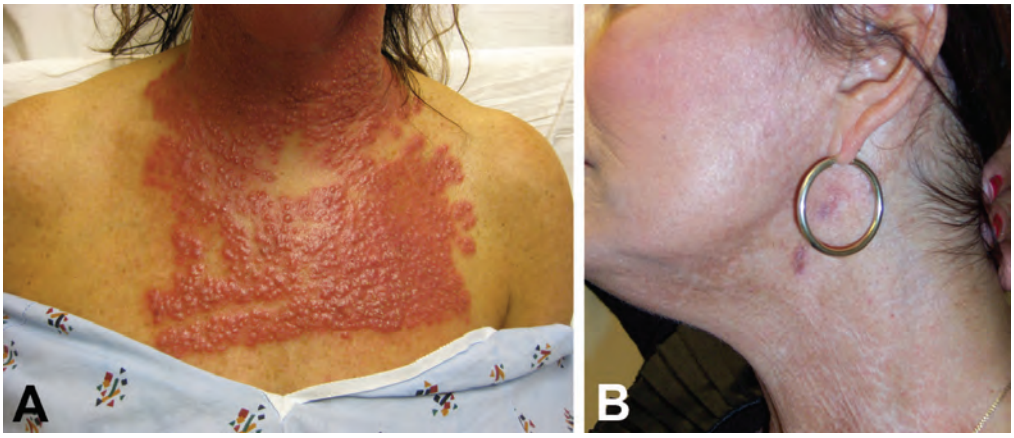

Figure 1. A) Neck and chest of a 53-year-old woman (case-patient 1) 14 days after fractionated CO_2 laser resurfacing. B) Neck of the patient after 5 months of multidrug therapy and pulsed dye laser treatment.

fluconazole, doxycycline, and valacyclovir for presumed fungal, staphylococcal, or disseminated herpes simplex virus infection. Because of extensive pruritus, the patient was given locoid lipocin (0.1% hydrocortisone butyrate) and a tapered dose of prednisone for possible allergic contact dermatitis. She reported adherence to instructions to avoid showering and washing her face with tap water for 72 h after the procedure. However, she was exposed to dust from sanding she did at home during the week after the procedure. She did not show improvement over the next 2 days and, after a low-grade fever developed, was hospitalized and received intravenous acyclovir therapy for presumed disseminated herpes simplex virus infection.

When the patient was hospitalized, lesions were nearly confluent over her neck and chest and scattered over her face but limited to areas treated with the CO_2 laser. PCR results for herpes simplex virus, varicella zoster virus, and fungal cultures were negative. Gram staining showed polymorphonuclear leukocytes and gram-variable rods. Two skin biopsy specimens demonstrated multiple, tiny foci of suppurative granulomatous dermatitis with elastophagocytosis (Figure 2, panel A) and numerous long acid-fast rods that were gram positive (Figure 2, panel B).

The patient was given empiric treatment for nontuberculous mycobacterial infection with intravenous tigecycline combined with oral moxifloxacin and azithromycin. Two weeks later, tissue culture of her lesions grew *M. abscessus*. Drug susceptibility testing showed resistance to moxifloxacin, amoxicillin/clavulanate, tobramycin, trimethoprim/sulfamethoxazole, ciprofloxacin, doripenem, linezolid, and doxycycline, and susceptibility to azithromycin, amikacin, kanamycin, imipenem, cefoxitin, and tigecycline.

One month after initiation of the multidrug regimen, repeat culture of a persistent pustule on her face again grew *M. abscessus*. Treatment with tigecycline and moxifloxacin was stopped after 2 months and treatment with azithromycin was stopped after 5 months because the patient showed clinical improvement. Scarring and dyspigmentation were observed. Thus, for cosmesis, she subsequently underwent a series of procedures with a pulsed dye laser (Figure 1, panel B).

Case-Patient 2

A 52-year-old woman underwent fractionated CO_2 laser resurfacing of the neck at the same private clinic as case-patient 1 (66 days after case-patient 1 was treated). After case-patient 1 was treated, major changes were made in the treatment protocol to make the procedure sterile. Treatment was performed at 30 mJ (treatment level 7) with 25% coverage (total 2.96 kJ) but otherwise as for case-patient 1. Nine days after the procedure, painful pustular lesions developed within the treated area but primarily on the right neck (Figure 3, panel A). The patient reported adherence with instructions to avoid washing with tap water for 72 hours after the procedure and denied any other exposures. Treatment was initiated with valacyclovir, cephalexin, and topical antimicrobial drugs.

After this patient did not show improvement, a biopsy specimen from a lesion showed suppurative and granulomatous dermatitis, which suggested NTM infection. Empiric treatment for NTM infection was initiated with azithromycin and moxifloxacin; some improvement in the lesions was subsequently observed. The organism was identified as *M. chelonae*. Drug susceptibility testing showed resistance to cefoxitin and trimethoprim/sulfamethoxazole; intermediate susceptibility to ciprofloxacin; and susceptibility to amikacin, clarithromycin, linezolid, and tobramycin. Treatment was continued with azithromycin and moxifloxacin for 4 months and clinical improvement was observed (Figure 3, panel B).

Epidemiologic Investigation

After case 1 was detected, an epidemiologic investigation was requested by the physician (dermatologist) who had performed the laser resurfacing to investigate possible sources of the infection within the clinic. The investigation was initiated 27 days after the procedure was performed.

We interviewed the dermatologist and reviewed all steps of the procedure. Several items were obtained and cultured for nontuberculous mycobacteria: a multiuse jar of lidocaine/tetracaine ointment, a multiuse jar of emollient, a multiuse vial of 1% lidocaine used for nerve blocking, a nonsterile package of gauze used to apply ointments to patients' skin, and a multiuse vial of sodium bicarbonate. Small amounts of samples were swabbed onto 7H10 Middlebrook medium supplemented with an additional 5 μg/mL of malachite green by using a sterile swab. A gauze square was immersed in ≈250 mL sterile water and agitated vigorously for ≈1 min. The water was then filtered through a 0.4-m sterile filter, and the filter was plated directly onto the 7H10 Middlebrook medium. Approximately 0.1 mL of each of the other samples was placed directly onto a 7H10 Middlebrook medium plate and spread over the plate by using a sterile loop. Plates were incubated at 30°C, and no growth was observed on any plates after 3 months of incubation.

Although no obvious source of infection was identified, several changes to the routine fractionated CO_2 resurfacing procedure were made after case 1 was detected. These changes included use of sterile gloves; sterile gauze; sterile tongue depressors for application of topical lidocaine/tetracaine ointment; conversion to single-use vials of lidocaine, epinephrine, and sterile saline used for tumescent anesthesia; and single-use postprocedure emollient. Chloroxylenol (3%) was also added in addition to 70% isopropanol for preprocedure cleansing. Postprocedure wound care was not changed.

After case 2 was detected, a second site visit was arranged 46 days after the procedure was performed. The dermatologist was interviewed again, and a complete sham procedure was performed while the investigators observed. Samples were collected from the 3% chloroxylenol, hand scrub, multiuse sodium bicarbonate vial, and single-use lidocaine/tetracaine ointment vial. The suction canister, connection tubing, and smoke filter were removed from the machine and cultured. Copious amounts of skin debris were identified in the long and short tubes of the connection tubing, along with an »2 to 3–cm layer of skin debris on top of the smoke filter. Environmental swabs of the countertops and walls in the procedure room were also collected. Water specimens (≈250 mL) were collected from the taps in the staff and patient bathrooms in the clinic (there was no water source in the procedure room or any other nearby procedure rooms) and from a fountain in the hallway. Patients were routinely instructed to wash the area with soap and water before coming to the office (i.e., they did not wash in the patient sink in the office).

The ointments were plated directly onto Lowenstein-Jensen (LJ) and modified 7H10 medium. The environmental swab was plated directly onto LJ medium only. Two 1 × 3–cm pieces of the paper filter from the filter canister were plated directly onto LJ medium. In addition, the multiuse lidocaine/tetracaine ointment from the first site visit was plated directly onto LJ medium by using a sterile swab. Approximately 100 mL of sterile water were passed through the canister/short tube and the long tube from the apparatus and collected in sterile bottles. The resultant suspensions were brown and contained large quantities of skin debris. Approximately 40 mL of each water sample and 10 mL of the sodium bicarbonate were passed through 0.4-m filters; each specimen was processed in duplicate. One filter from each specimen was then plated directly onto LJ medium, and the other filter was plated onto malachite green–supplemented 7H10 medium. Medium plates were incubated at 30°C. After 1 week, mycobacterial colonies were identified on the medium containing the filtrate from the connector tubing and several of the tap water specimens (staff bathroom and patient bathroom).

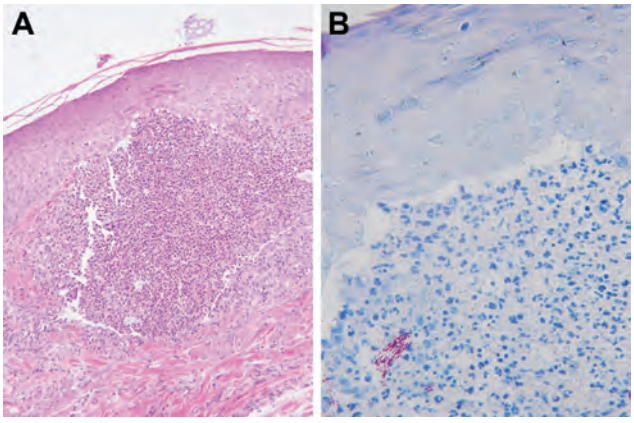

Figure 2. Skin biopsy specimens of a 53-year-old woman (case-patient 1) after fractionated CO_2 laser resurfacing. A) Hematoxylin and eosin–stained and B) Ziehl-Neelsen acid-fast–stained sections show a tiny superficial microabscess surrounded by sparse granulomatous inflammation. Several groups of acid-fast organisms can be seen at the lower left of panel B. Original magnifications ×400 in (A) and ×600 in (B).

Species identification of all isolates was performed at the Microbiology Laboratory of the University of North Carolina by using 16S rRNA and heat shock protein 65 gene sequencing. Results of sequence analysis showed that the 2 patient isolates did not match. They were identical by sequencing of part of the 16S rRNA gene but differed by heat shock protein 65 sequencing; the organism isolated from case-patient 1 was *M. abscessus* and that from case-patient 2 was *M. chelonae*. Analysis of clinic water isolates showed several different mycobacterial organisms. Four colony morphologies were isolated from the tap water in the patient bathroom, 2 of which were identified as *M. mucogenicum*, 1 as *M. obuense/aurum*, and 1 as *M. chelonae*. Three colonies isolated from the tap in the staff bathroom were identified as *M. mucogenicum*. The isolate from the

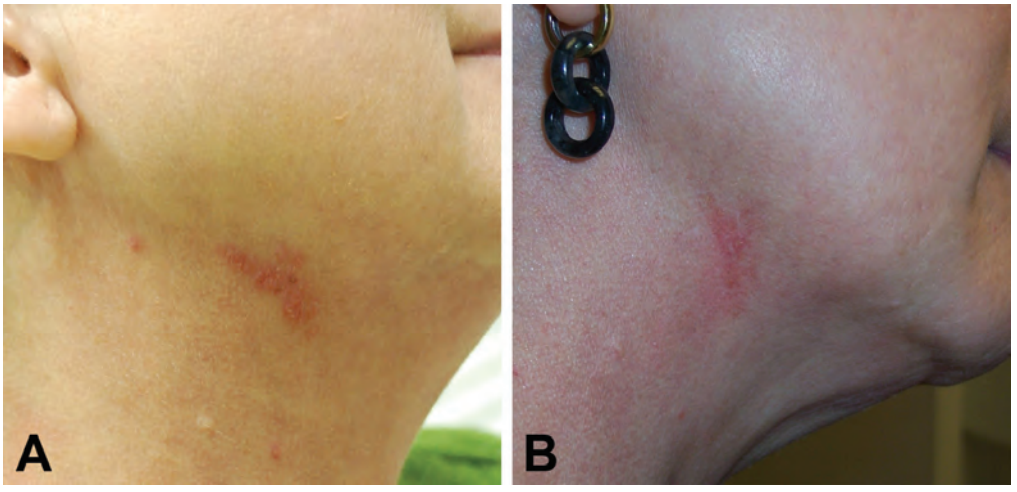

Figure 3. A) Right lateral neck of a 52-year-old woman (case-patient 2) 9 days after fractionated CO_2 laser resurfacing. B) Neck of the patient after 4 months of multidrug therapy.

large tubing leading to the smoke filter was *M. smegmatis*, which was not a match with either patient isolate.

Pulsed-field gel electrophoresis was performed at the University of Texas at Tyler to compare the *M. chelonae* isolate from the case-patient 2 with the isolate from the patient bathroom in the dermatology clinic. The 2 isolates did not match.

Environmental investigation of the homes of the 2 patients was not conducted because case-patient 1 refused and sampling of the home of case-patient 2 was not attempted. The bottles of vinegar used for postprocedure cleansing were not available for either patient. Neither patient had undergone fractionated CO_2 laser resurfacing before the procedures described.

Conclusions

NTM are environmental organisms that are increasingly associated with systemic and cutaneous disease in humans. NTM-induced cutaneous disease typically occurs after injections, such as tattoos, botulinum toxin, and mesotherapy, or after minor surgical procedures in which breaks in the skin barrier occur (*1*,*3*,*5*,*12*–*15*). These organisms also have been associated with whirlpool footbaths before pedicures (*2*,*4*,*16*). In these cases, shaving before a whirlpool footbath was associated with increased rates of infection, presumably caused by microbreaks in the skin, which enable easy inoculation (*2*,*16*,*17*). NTM are ubiquitous in soil and water and have been detected in municipal water sources throughout the United States (*16*,*18*,*19*). They are also found in biofilms and, in the whirlpool footbath associated cases, seem to thrive in nutrient-rich water contaminated by skin debris, which accumulates on bath filters (*2*,*4*,*16*,*17*).

Eradication can be difficult because these organisms are resistant to most disinfectants (*4*,*20*,*21*). Cutaneous infection with NTM is most often caused by *M. marinum* and rapidly growing mycobacteria that belong to 1 of 3 species groups: *M. abscessus*, *M. chelonae*, and *M. fortuitum* (*22*–*24*). Diagnosis is difficult and often requires histologic evaluation and tissue samples for culture. Delays in diagnosis are common and can lead to delays in treatment (*6*). Species identification can be difficult and requires sequencing of multiple genes because of homology between *Mycobacterium* spp. family members (*25*–*27*). More than 120 NTM species have been identified, including ≈30 isolates in the past decade whose names might be unfamiliar to many clinicians (*28*). These organisms are also resistant to many antimicrobial drugs, a factor that complicates treatment.

In the past 5 years, fractionated CO_2 laser resurfacing has become the preferred procedure for rhytides, photodamage, and acne scars (*9*). This procedure combines the efficacy of ablative laser resurfacing with a more favorable side effect profile than traditional ablative therapy. Studies have shown a high degree of safety and efficacy and lower rates of hypopigmentation, scarring, and infectious complications (*7*–*10*,*29*). This technology is based on the principle of creating narrow columns of tissue damage known as microthermal treatment zones, which are evenly distributed over the treated area. Localized epidermal necrosis and collagen denaturation occur in each column but the stratum corneum remains intact. Decreased disruption of the epidermal barrier and areas of viable tissue around each microthermal treatment zone enable more rapid healing and reduce the risk of infection.

Although infections with fractionated CO_2 laser therapy are less common than with traditional ablative laser, they do occur (*10*). Infection with herpes simplex virus was reported in 1.7% of all cases and in 4.6% of cases in which the patient had a history of oral herpes but no antivirus prophylaxis was given (*8*). Bacterial complications, including *Staphylococcus* spp. and *Pseudomonas* spp. infections, and *Candida* spp. infections have also been reported, although at low rates (*10*).

Palm et al. reported the first case of NTM infection after fractionated laser resurfacing (*11*). The causative agent

was identified as *M. chelonae* 3 months after the onset of acneiform eruptions. The patient received multidrug treatment and showed some clinical improvement. She eventually underwent therapy with a pulsed dye laser and showed a decrease in erythema and scarring. A possible source of NTM infection was not sought in this case.

We report 2 additional cases of NTM infection with *M. abscessus* and *M. chelonae* after fractionated CO_2 laser resurfacing. Both patients showed development of erythematous papules and pustules »10–14 days after the procedure, but the extent of skin involvement varied between the 2 patients. For both patients, a diagnosis was made within 1–2 weeks by histologic examination and tissue culture. Early treatment with multidrug therapy specific for the most likely mycobacterial pathogens was initiated while susceptibility testing was performed. In both patients, treatment was continued for ≥4 months.

Results of a thorough epidemiologic investigation showed no evidence that transmission of the NTM infections occurred during the fractionated resurfacing procedure. The 2 patient isolates belonged to 2 different species, and neither matched the isolate obtained from the fractionated laser apparatus. Furthermore, none of the isolates from environmental water at the clinic matched either patient isolate. Although the absence of evidence does not definitively rule out common source transmission during the procedure, it does make it more likely that infection occurred elsewhere after the procedure.

The source of these infections remains unclear. It is possible that the causative NTM isolates were transiently present in the environment but were not detected because of lag times between procedures and environmental investigations. Furthermore, limitations of environmental sampling and culture for mycobacteria did not enable us to rule out a common source of infection at the time of the procedure. Detailed environmental sampling of the home was not permitted by the first patient and was not sought for the second patient. Several alternative environmental sources for infection are possible (aerosols from sinks, toilets, water fountains, and sanding dust for case-patient 1). Although there was no evidence to support exposure during the fractionated laser procedure, an NTM species was isolated from the tubing of the machine. Therefore, the tubing leading to the smoke filter is a potential reservoir for NTM because it is changed infrequently and can contain skin debris within the corrugated tubing.

Patients should be explicitly advised of the risk for NTM infection after fractionated laser resurfacing, and physicians should be highly suspicious of such infections during the postprocedure period. Although incubation periods reported for postprocedure NTM have been reported as 9–10 days, other cutaneous NTM infections may be found ≤3 months after the presumed exposure (*16*). Thus, late manifestations might be possible. Biopsy specimens for histologic evaluation and tissue culture are critical for making an accurate diagnosis. Suppurative neutrophilic and granulomatous dermatitis should raise suspicion for NTM infection, even if results of staining for acid-fast bacilli are negative. As shown by these cases and the case described by Palm et al. (*11*), identification of gram-positive rods during routine histologic examination might suggest NTM infection because these organisms can be weakly gram positive.

Empiric therapy specific for NTM should be considered while awaiting biopsy and culture results for patients with suspicious lesions. However, prophylactic therapy before or after the procedure with active agents against NTM is not recommended. The efficacy of such treatment in preventing infection remains unknown, and the risk for antimicrobial drug–associated side effects likely outweighs any theoretical benefit. Although there is no standard treatment for cutaneous NTM infections, multidrug therapy is usually necessary to minimize the development of drug resistance. Antimicrobial drug susceptibility testing should be conducted to tailor therapy, and treatment should be continued for 4–6 months.

When these infections occur, systematic observation of the procedure should be performed. Specifically, attention should be paid to any liquids or ointments that may contact the skin of a patient during or just after the procedure (particularly multiuse vials or containers) and the proximity of the procedure room to potentially aerosol-generating water sources. Environmental sampling with mycobacterial culture of such liquids seems to be a reasonable first step in identifying a source (although it did not identify a source in this study).

Furthermore, strict postprocedure wound care is critical to minimize risk for NTM infection. It is prudent to advise patients to avoid any municipal water sources for the first 72 h after the procedure (although this time interval is arbitrary). Bottled water, which may not be sterile, could harbor small amounts of NTM. Use of sterile water or sterile saline for postprocedure cleansing is recommended. First and foremost, physicians must remain aware of this potential complication of fractionated laser resurfacing and be highly suspicious even if initial histologic and culture results do not identify microbial pathogens.

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Human Leptospirosis Trends, the Netherlands, 1925–2008

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To increase knowledge of leptospirosis in the Netherlands and identify changing trends of this disease over time, we analyzed historical passive surveillance reports for an 84-year period (1925–2008). We found that 2,553 mainly severe leptospirosis cases were diagnosed (average annual incidence rate 0.25 cases/100,000 population). The overall case-fatality rate for patients with reported leptospirosis was 6.5% but decreased over the period, probably because of improved treatment. Ninety percent of reported leptospirosis cases were in male patients. Most autochthonous leptospirosis infections were associated with recreational exposures, but 15.5% of the cases were attributed to accidents that resulted in injury and to concomitant water contact. Since the end of the 1950s, the proportion of imported infections gradually increased, reaching 53.1% of the total during 2005–2008. Most (80.1%) imported infections were associated with sporting and adventurous vacation activities.

Leptospirosis is a zoonotic disease caused by infection with *Leptospira* spp. bacteria (*1*). Pathogenic leptospires live in the kidneys of many mammalian hosts, including rodents, insectivores, and livestock. Leptospires are shed into the environment, where they can survive for several months in favorable (warm and wet) conditions. Thus, leptospirosis is particularly endemic to warm and humid tropical and subtropical regions (*2*). Humans are infected by direct contact with infected animals or indirectly by contact with a contaminated environment.

Leptospirosis is an emerging public health problem globally (*3*–*6*). However, this disease is often overlooked because it is difficult to clinically diagnose and because laboratory-based diagnosis is cumbersome. Because mild leptospirosis frequently goes unrecognized and notification systems are mostly absent, the global incidence of leptospirosis is underestimated. An international survey conducted by the International Leptospirosis Society reported ≥350,000 cases of severe leptospirosis annually (*7*). This estimate is supported by data from an assessment of the global incidence of leptospirosis (*8*), which indicated a mean global incidence rate for leptospirosis of 5 cases/100,000 population.

In Europe, leptospirosis has been studied and diagnosed since the 1920s. Historical reviews from Germany (*9*) and France (*10*) have contributed to a better understanding of the epidemiology of leptospirosis. In the Netherlands, passive surveillance of human leptospirosis began in 1924. Reporting of cases of this disease is mandatory, and laboratory diagnosis has been centralized in 1 institution. To increase knowledge of leptospirosis, we analyzed historical passive surveillance reports in the Netherlands for 84 years (1925–2008) to determine changing trends of this disease over time.

Passive Surveillance

The Royal Tropical Institute (KIT) in Amsterdam is associated with the World Health Organization/Food and Agricultural Organization/World Organisation for Animal Health and the National Collaborating Centre for Reference and Research on Leptospirosis (NRL), which confirms ≈99% of the suspected cases of leptospirosis in the Netherlands. Detailed records on serologic, clinical, and epidemiologic features are archived at the NRL. Since 1928, leptospirosis has been a mandatory reportable disease in the Netherlands (*11*). A case of leptospirosis is defined as laboratory confirmation of infection as described in this report and by Hartskeerl (*12*) and fever or 2 of the following signs and symptoms: shivering, headache, muscle pain, conjunctival injection, bleeding in skin and mucosa, rash, jaundice, myocarditis, meningitis, renal failure, pulmonary hemorrhage with respiratory failure.

Study Population

Reportable disease data for leptospirosis are compiled from passive surveillance reports received for the entire

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population of the Netherlands. General practitioners and consulting clinicians suspecting leptospirosis send clinical specimens to the NRL for laboratory evaluation. During the period covered by this review, the total number of samples submitted for testing was estimated to be ≈50,000.

Changes in Laboratory Diagnosis

Laboratory tests have changed over time. However, all diagnoses in the Netherlands have been based on identification of leptospires by culture or antibodies against *Leptospira* spp. by agglutination tests (*12*). During 1924–1963, culture of patient specimens was routinely performed by inoculation of blood or urine into guinea pigs or hamsters. In 1964, in vitro culturing was introduced and has been used exclusively since 1972.

Beginning in 1924, the agglutination test was used for diagnosis of leptospirosis; the test was performed as described by Martin and Pettit (*13*) using serovars Pyrogenes and Rachmat and unidentified isolates from patients. Serovar Copenhageni was included in 1927, serovars Icterohaemorrhagiae and Canicola in 1934, serovar Grippotyphosa in 1941, serovars Pomona and Bataviae in 1942, and serovar Ballum in 1945. During 1960–1990, the panel was increased by the addition of serovars Tarassovi (1961), Poi (1963), Bratislava (1964), Saxkoebing (1964), Hardjoprajitno (1980), Hebdomadis (1981), Hardjo-bovis (1983), Proechimys (1987), and Sejroe (1987). This panel was later supplemented with serovars Ballico, Celledoni, Cynopteri, Mini, Panama, and Shermani to include the representative serovars that that cause leptospirosis worldwide (*1*).

The agglutination test was modified in 1954 in accordance with recommendations of Wolff (*14*) and in 1978 in accordance with recommendations of Cole et al. (*15*). An in-house IgM ELISA was introduced in 1984 (*16*). Laboratory diagnosis is currently based on a positive culture, a microscopic agglutination test titer ≥160 and IgM ELISA titer ≥80, or seroconversion (*12*). Most presumptive infecting serogroups are deduced from the highest titers against ≥1 serovars in the microscopic agglutination test. Such titers are only indicative for serogroups (*17*). Therefore, we report data on infecting serogroups. In cases in which a leptospiral isolate has been typed to serovar level, the corresponding serogroup has been used.

We also assessed differences between autochthonous and imported leptospirosis infections. Autochthonous infections are those most likely acquired in the Netherlands. Imported infections are those most likely acquired during a visit to another country ≤1 month before the day of symptom onset.

Data Collection

During 1924–1964, when a case of leptospirosis was confirmed in the Netherlands, demographic, epidemiologic, and clinical data were collected by using a standardized questionnaire sent to the consulting physician by the NRL. When persons with confirmed leptospirosis were hospitalized, the physician was requested to send a copy of the patient's discharge letter to the NRL. Because of changes in privacy laws, during 1964–1999 questionnaires were sent directly to the patients, but since 1999, questionnaires and informed consent forms have been sent to consulting physicians.

Data Analysis

Archived information was entered into a database by using SPSS version 15.0 software (IBM, Armonk, NY, USA). Data were analyzed for trends over time, differences between autochthonous and imported infections, and differences related to sex of the patients.

Total annual incidence for male and female patients was calculated by using the population of the Netherlands as obtained from the Dutch Central Bureau for Statistics (The Hague, the Netherlands). Annual case-fatality rates (CFRs) were calculated by dividing the number of deaths by the total number of confirmed cases per year. Patient data on hospitalization, antimicrobial drug treatment (available since 1950), hemodialysis (available since 1961), and treatment in an intensive care unit (ICU) (available since 1955) were used to give an overview of clinical management of leptospirosis in the Netherlands. Missing data were recoded as "no" for the analysis.

To assess the severity of disease, data were analyzed for changes over time for 10-year intervals during 1925–2008 by using the χ^2 test for trend. Severe leptospirosis was defined as disease for which hospitalization, admission to an ICU, or dialysis was indicated, or for which death occurred. Severity calculations are limited to the period when such supportive treatment was available. These characteristics were analyzed by using the χ^2 test, Student *t* test, or Fisher exact test when appropriate.

Differences between autochthonous and imported infections regarding patient characteristics, likely source of exposure, treatment course, and infecting serogroups were tested by using the χ^2 test and Fisher exact test for values <5. Likely source of exposure was determined by type of contact (recreational activities, accidental, job-related) and route of infection (water, animal or other source) source and route are mutually exclusive, in contrast to assessment of animal hosts.

We examined whether bacterial exposure and disease severity differed by patient sex. Patient data concerning mean age, imported disease, type of contact, and treatment course are presented by sex of the patient. Differences between male and female patients were analyzed by using the χ^2 test. A p value ≤0.05 was considered significant.

Ethical Issues

This study was exempted from ethical review of human subject research by the Medical Ethical Review Committee of the Academic Medical Centre, University of Amsterdam (protocol W12_075#12.17.0092). All data have been de-identified and were not attributable to individual patients.

Cases

During 1925–2008, the NRL reported 2,588 leptospirosis infections. Thirty-five case-patients were excluded: 22 living outside the Netherlands, 1 whose case was reported more than once, and 12 whose cases were retrospectively reclassified. Thus, the study sample comprised 2,553 confirmed case-patients. An overview of patient characteristics by autochthonous and imported infections is shown in Tables 1 and 2. Male patients accounted for 91.1% of all infections. Deaths were almost exclusively reported in men who had autochthonous infections with serogroup Icterohaemorrhagiae. Most (80.8%) imported leptospirosis cases and a substantial (44.4%) proportion of autochthonous cases were associated with recreational activities. A substantial number of infections (14.4%) were attributed to injury (i.e., traffic accidents and concomitant water exposure).

Trends over Time

The average annual incidence rate of leptospirosis in the Netherlands was 0.25 cases/100,000 population (Figure). For male patients, the average incidence was 0.46 cases/100,000 boys and men, which is >10-fold higher than the rate for female patients (0.04 cases/100,000 girls and women).

The mean (SD) age of patients was 33.8 (17.1) years. There was a gradual increase in the mean (SD) age over time: 29.1 (14.6) years during 1925–1934 to 38.0 (16.1) years during 2005–2008 (p<0.001).

Table 1. Characteristics for case-patients with leptospirosis, the Netherlands, 1925–2008*

Characteristic	Total cases, n = 2,553	Autochthonous cases, n = 2,231†	Imported cases, n = 318†	p value
Male sex‡	2,306 (91.1)	2,025 (91.6)	278 (87.4)	0.014
Mean (SD) age, y‡	33.8 (17.1)	34.0 (17.5)	32.6 (14.4)	0.848
Type of contact				
Recreational activity	1,250 (49.0)	990 (44.4)	257 (80.8)	<0.001
Job-related	685 (26.8)	664 (29.8)	21 (6.6)	<0.001
Accident	367 (14.4)	345 (15.5)	22 (6.9)	<0.001
Unknown	250 (9.8)	232 (10.4)	18 (5.7)	
Likely route of infection				
Water	1,457 (57.1)	1,219 (54.6)	236 (74.2)	<0.001
Water and animals	500 (19.6)	446 (20.0)	53 (16.7)	0.162
Animals	351 (13.7)	346 (15.5)	4 (1.3)	<0.001
Other§	16 (0.6)	14 (0.6)	2 (0.6)	1.000
Unknown	229 (9.0)	206 (9.2)	23 (7.2)	
Host exposure¶	(n = 851)	(n = 792)	(n = 57)	
Rats	443 (52.1)	411 (51.9)	32 (56.1)	<0.535
Mice	123 (14.6)	120 (15.2)	3 (5.3)	0.004
Other rodents	31 (3.6)	28 (3.5)	3 (5.3)	0.789
Cows	231 (27.1)	224 (28.3)	6 (10.5)	<0.001
Dogs	172 (20.2)	158 (19.9)	13 (22.8)	0.603
Other animals	185 (21.7)	168 (21.2)	16 (28.1)	0.088
Serogroup				
Icterohaemorrhagiae	1,702 (66.7)	1,588 (71.2)	111 (34.9)	<0.001
Grippotyphosa	196 (7.7)	174 (7.8)	22 (6.9)	0.595
Sejroe	128 (5.0)	116 (5.2)	12 (3.8)	0.771
Canicola	93 (3.6)	87 (3.9)	6 (1.9)	0.230
Pomona	54 (2.1)	45 (2.0)	9 (2.8)	0.107
Autumnalis	16 (0.6)	1 (0.1)	15 (4.7)	<0.001
Bataviae	11 (0.4)	0	11 (3.5)	<0.001
Other#	54 (2.1)	12 (0.5)	42 (13.2)	<0.001
Unknown	299 (11.7)	208 (9.3)	90 (28.3)	<0.001
Cultures performed	1,335 (52.3)	1,151 (51.6)	182 (57.2)	0.060
Positive result	306 (22.9)	256 (22.2)	49 (26.9)	0.162

*Values are no. (%) unless otherwise indicated.
†Data for autochthonous and imported cases could not be obtained for 4 patients.
‡Sex was recorded for 2,532 patients: 2,210 patients with autochthonous cases and 318 patients with imported cases. Age was recorded 2,427 patients; 2,105 patients with autochthonous cases and 381 patients with imported cases.
§Laboratory accidents or contact with mud/soil.
¶Animal specified is not mutually exclusive; 1 patient could have been in contact with >1 animal.
#For total infections, Australis (n = 12), Celledoni (n = 7), Sejroe/Hebdomadis/Mini complex (n = 7), Javanica (n = 6), Pyrogenes (n = 6), Hebdomadis (n = 4), Shermani (n = 4), Ballum (n = 2), Cynopteri (n = 2), Tarassovi (n = 2), Celledoni/Javanica complex (n = 1), Mini (n = 1). For autochthonous infections, Australis (n = 6), Ballum (n = 2), Tarassovi (n = 2), Javanica (n = 1), Sejroe/Hebdomadis/Mini complex (n = 1). For imported infections, Celledoni (n = 7), Australis (n = 6), Pyrogenes (n = 6), Sejroe/Hebdomadis/Mini complex (n = 6), Javanica (n = 5), Hebdomadis (n = 4), Shermani (n = 4), Cynopteri (n = 2), Celledoni/Javanica complex (n = 1), Mini (n = 1).

Table 2. Treatment parameters for and deaths among case-patients with leptospirosis, the Netherlands, 1925–2008*

Characteristic	No. (%) total cases	No. (%) autochthonous cases	No. (%) imported cases	No. patients	p value
Deaths					
Total	166 (6.5)	162 (7.3)	4 (1.3)	2,553	<0.001
Male patients	160 (97.0)	156 (96.9)	4 (100.0)	2,306	0.861
Treatment course†					
Hospitalization	1,851 (72.5)	1,612 (72.3)	235 (73.9)	2,553	0.539
Antimicrobial drugs	1,216 (68.5)	994 (68.2)	219 (69.5)	1,597‡	0.652
Dialysis	119 (8.8)	103 (9.8)	15 (5.0)	796§	0.009
ICU	106 (6.5)	83 (6.3)	23 (7.3)	626¶	0.485

*ICU, intensive care unit.
†Percentages were calculated from available data (all missing values were regarded as no). It was assumed that analyzing data that contained large amounts of missing values would result in findings that could not be generalized and lead to substantial overestimated values.
‡Antimicrobial drug data were available for 1,597 of 1,776 infected patients, 1,306 of 1,457 patients with autochthonous cases, and 288 of 315 patients with imported cases (January 1950–December 2008). Before 1950, treatment with antimicrobial drugs was not expected to be used.
§Dialysis treatment data were available for 796 of 1,360 infected patients, 559 of 1,053 patients with autochthonous cases, and 234 of 303 patients with imported cases (January 1961–December 2008). Before 1961, dialysis treatment was not expected to be used.
¶ICU data were available for 626 of 1,641 infected patients, 441 of 1,324 patients with autochthonous cases, and 183 of 313 patients with imported cases (April 1955–December 2008). Before 1955, treatment in an ICU was not expected to be used.

Although leptospirosis is endemic to most of the Netherlands, there have not been any large outbreaks. Peak periods of incidence rate increases were seen in 1932 (1.08 cases/100,000 population), 1941 (0.64 cases/100,000 population), and 1961 (0.64 cases/100,000 population), and to a lesser extent in 1967 (0.37 cases/100,000 population) and 1988 (0.42 cases/100,000 population) (Figure).

During 1925–2008, a total of 166 persons with leptospirosis died (CFR 6.5%) (Table 2). The annual number of deaths decreased over time, probably as a result of the introduction of dialysis treatment in 1961; there were 67 deaths (10.5%) in the first 20 years and 21 (3.9%) during 1975–1994 ($p<0.001$). Since 1995, the NRL has only recorded 5 (1.2%) deaths. During 1925–2008, the mean (SD) age of patients who died was 49.2 (15.6) years, and the mean (SD) age of patients who survived was 32.8 (16.9) years ($p<0.001$). The mean (SD) age of patients who died increased from 43.6 (16.0) years during 1925–1934 to 64.2 (12.7) years over the last 4 years of the study ($p<0.05$).

A total of 1,851 (72.5%) of the 2,553 patients were hospitalized. The proportion of patients hospitalized increased from 37.4% during 1925–1934 to 92.1% during 1955–1964 ($p<0.001$) and then decreased to 74.4% during 2005–2008 ($p<0.001$). Overall, 6.5% of patients were treated in an ICU and 8.8% received dialysis treatment.

The first ICU admissions for leptospirosis were reported in 1978. During 1975–1984, a total of 7.5% of patients were reported as being admitted to an ICU. The percentage of ICU admissions increased to 17.8% during 2005–2008. Dialysis treatment was introduced and recorded for the first time in 1961; its use increased markedly from 3 (2.0%) patients with leptospirosis during 1955–1964 to 16 (12.4%) patients during 2005–2008. Widespread use of penicillin to treat leptospirosis first began during World War II. Treatment with antimicrobial drugs increased from 53.3% of patients during 1945–1954 to 83.7% of patients during 2005–2008.

During 1925–2008, the main infecting serogroup in autochthonous cases was Icterohaemorrhagiae (1,588 identifications; 71.2%). Other common serogroups were Grippotyphosa (174 identifications; 7.8%), Sejroe (116 identifications; 5.2%), Canicola (87 identifications; 3.9%), and Pomona (45 identifications; 2.0%). All infections with serogroup Canicola occurred during the first 50 years of the study, and there was a peak during 1945–1954. Serogroup Icterohaemorrhagiae appeared to be the major cause of fatal leptospirosis, followed by serogroup Canicola (Table 3). Within the group of patients infected with serogroup Icterohaemorrhagiae, 90 patients died (CFR 5.3%). This number represents 94.7% of the patients who died for whom the infecting serogroup is known.

The CFR for patients infected with serogroup Canicola was 3.2%. None of the patients infected with serogroup Pomona died, although the percentages of hospitalization, ICU admission, and dialysis treatment were higher among these patients. Because all patients infected with serogroup Canicola were observed before 1967, dialysis and ICU treatment were not available for these patients, but absence of these treatments does not indicate milder clinical illness. Serogroups Grippotyphosa and Sejroe appeared to cause less severe disease, although 1 patient infected with serogroup Sejroe died (Table 3). Fatal leptospirosis can have a rapid, fulminate course, which often makes identification of infecting serogroups impossible. Information for the causative serogroup was available for only 95 of the 166 patients who died. The average CFR was 4.2% for patients in whom the infecting serogroup was determined and 23.7% for patients in whom the infecting serogroup was not identified.

Autochthonous versus Imported Leptospirosis

In the Netherlands, the total number of leptospirosis patients infected outside the country through 2008 was 318 (12.5% of all reported patients). The annual proportion of imported leptospirosis cases has gradually increased over

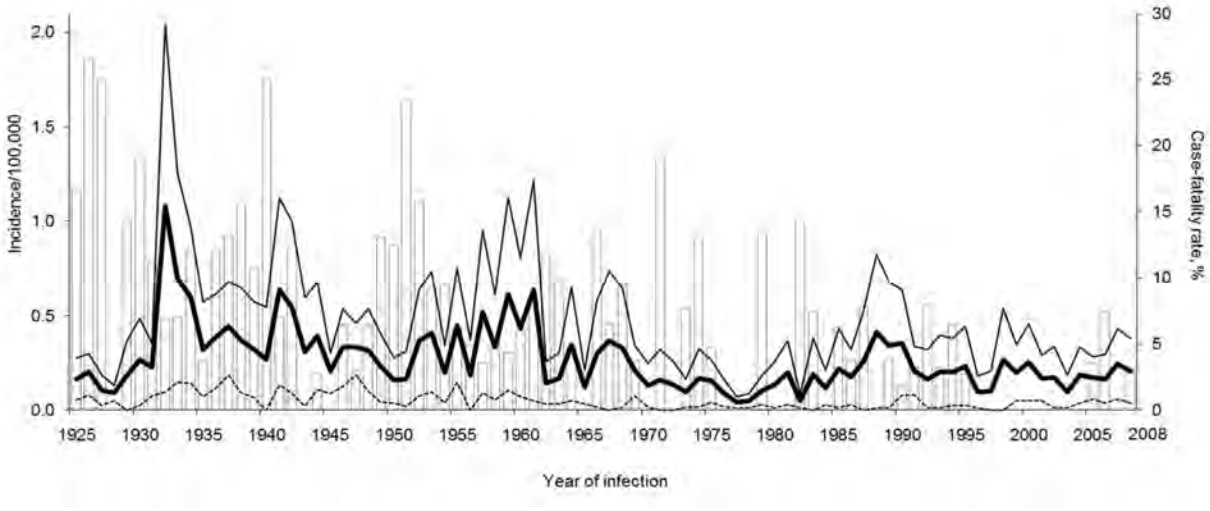

Figure. Incidence rates of leptospirosis, the Netherlands, 1925–2008. White bars indicate case-fatality rate (percentage of deaths/no. of confirmed cases), thick black line indicates total incidence rate (no. cases/100,000 population), thin black line indicates incidence rate among male patients (no. cases in male patients/100,000 male population), and dashed line indicates incidence rate among female patients (no. cases in female patients/100,000 female population). The total population of the Netherlands was 7.3 million in 1925, 8.4 million in 1935, 9.2 million in 1945, 10.7 million in 1955, 12.2 million in 1965, 13.6 million in 1975, 14.5 million in 1985, 15.4 million in 1995, and 16.3 million in 2005.

time; >50% of all infections during 2005–2008 were acquired outside the Netherlands (Table 4). In the early years of the study, a substantial proportion of imported infections occurred after exposure in other countries in Europe, mainly during vacations. Since the mid-1970s, the number of leptospirosis infections acquired outside Europe has increased markedly, mostly from exposures in Asia (134 cases; 42.1%), notably Thailand (Table 4). More than 80% of the imported leptospirosis infections were associated with water-related sport and adventure activities, such as white-water rafting. In contrast, 44.4% of the autochthonous infections were attributed to recreational activities (Table 1), 29.8% were attributed to occupational exposures, and 15.5% were attributed to accidents. Except during 1985–1994, the ratio of infections related to recreational activities, occupations, and accidents has remained similar over time.

Sex Differences

Of 2,532 patients, 2,306 (91.1%) were male patients and 226 (8.9%) were female patients (Table 5). On the basis of CFR data, male patients were more likely to have had a more severe leptospirosis infection; 160 male patients died (CFR 6.9%) and 6 female patients died (CFR 2.7%) (Table 2). In addition, a higher percentage of male patients were treated with dialysis (Table 5). The greater severity of leptospirosis among male patients does not appear to be attributable to infections caused by more virulent serovars: 67.2% of male patients were infected with serogroup Icterohaemorrhagiae compared with 61.1% of female patients (p = 0.06). However, male patients were older than female patients: mean (SD) was 34.5 (17.0) years for male patients and 26.4 (16.5) years for female patients (p<0.001).

Table 3. Treatment course and deaths, by infecting *Leptospira* spp. serogroups, for case-patients with leptospirosis, the Netherlands, 1925–2008*

Serogroup	No. patients	No. (%) hospitalized	No. (%) treated with dialysis	No. (%) treated in ICU	No (%) died
Icterohaemorrhagiae	1,702	1,311 (77.0)	90 (11.9)	68 (7.0)	90 (5.3)
Grippotyphosa	196	124 (63.3)	3 (2.2)	2 (1.2)	0
Sejroe	128	48 (37.5)	3 (2.4)	2 (1.6)	1 (0.8)
Canicola	93	60 (64.5)	0	0 (0.0)	3 (3.2)
Pomona	54	40 (74.1)	3 (5.6)	7 (13.0)	0
Other	81	55 (67.9)	3 (3.8)	6 (7.6)	1 (1.9)
Unknown	299	213 (71.2)	17 (8.6)	21 (9.3)	71 (23.7)

*ICU, intensive care unit. Data were available for 2,553 patients who were hospitalized or died, 1,360 patients treated with dialysis, and 1,641 patients treated in an ICU.

Conclusions

Leptospirosis is endemic to the Netherlands. During the study period, the average incidence was 0.25 cases/100,000 population. The reported incidence probably reflects the more severe end of the clinical spectrum for leptospirosis because mild forms of this disease are more likely to go unrecognized (*1*,*8*,*18*,*19*). In the 84 years covered by this study, there were 5 years with notably increased annual incidences. The peaks in 1932 and 1941 coincided with the global economic depression and compulsory reporting of leptospirosis (1932) (*20*) and with World War II (1941). The increase in 1988 was associated with a dairy fever outbreak among farmers who were infected while handling leptospirosis-infected cattle. (*18*,*21*). No specific situations or events are known to be associated with the peaks in 1961 and 1968.

The overall CFR of 6.5% was high and exceeded CFRs reported in some countries with a higher prevalence of leptospirosis (*3*,*4*,*22*,*23*). The CFR decreased over the analysis period, probably because of improved treatments. No deaths were recorded during 1995–2005. However, this finding was probably caused by underreporting because transient stringent privacy regulations hampered identification of deaths. Thus, the actual average CFR might be higher than calculated for years after 1999.

An explanation for the high CFR might be that most (71.2%) autochthonous leptospirosis cases identified in the Netherlands were caused by more virulent serovars of serogroup Icterohaemorrhagiae; infections with this serogroup are less common among imported cases. Another reason might be that clinicians in the Netherlands are more proactive in treating severe leptospirosis in travelers than in persons with locally acquired infections. Potentially fulminant leptospirosis leading to rapid death, in combination with limited diagnostic potential in countries to which this disease is endemic, often prevents confirmation of leptospirosis cases (*24*) and might lead to underestimation of the CFR (*3*,*4*). Serogroups Grippotyphosa, Sejroe, Canicola, and Pomona frequently cause leptospirosis in the Netherlands. Infections with serovar Hardjobovis in serogroup Sejroe were found mainly in dairy fever cases among farmers from 1985 until early 1990. Infections with serogroup Pomona were more recent and mostly caused by the newly identified serovar Mozdok (*25*).

Infections with serogroup Canicola were not found after 1966. This finding was probably caused by introduction of bivalent Copenhageni/Canicola canine vaccines. Dogs are the reservoir of serovar Canicola, and vaccination interfered with the transmission cycle, resulting in elimination of serovar Canicola infections in the dog population in the Netherlands (*26*). Lack of dialysis and ICU treatments in patients infected with serogroup Canicola was mainly caused by absence of these treatments and does not indicate less severe or underestimated symptoms.

Table 4. Characteristics for case-patients with leptospirosis, the Netherlands and other regions, 1925–2008*

	Period								
Characteristic	1925–1934	1935–1944	1945–1954	1955–1964	1965–1974	1975–1984	1985–1994	1995–2004	2005–2008
No. case-patients	289	352	271	434	259	159	379	281	129
Hospitalized, %	37.4	68.8	78.6	92.1	83.8	84.3	62.5	72.6	74.4
Antimicrobial drug treatment, %	NA	NA	53.3	63.8	61.8	57.9	72.3	82.9	83.7
Dialysis, %	NA	NA	0	2.0	5.0	17.6	8.2	10.0	12.4
ICU, %	NA	NA	0	0.0	0.0	7.5	6.6	16.4	17.8
CFR, %	11.1	9.9	9.6	6.0	8.1	3.8	4.0	0.4	3.1
Imported infections	0	2 (0.6)	3 (1.1)	20 (4.6)	22 (8.5)	17 (0.7)	92 (24.3)	94 (33.5)	68 (53.1)
Europe	0	2	2	19	20	9	40	27	13
Asia	0	0	1	0	1	4	38	48	42
Sub-Saharan Africa	0	0	0	0	0	0	2	4	1
South America	0	0	0	0	1	4	6	7	7
Central and North America	0	0	0	0	0	0	2	6	5
Middle East	0	0	0	1	0	0	0	1	0
Australia	0	0	0	0	0	0	0	1	0
Likely route of infection									
Water									
Autochthonous	222 (92.8)	251 (82.3)	194 (78.7)	333 (87.4)	189 (88.3)	111 (79.9)	157 (60.4)	162 (88.5)	46 (79.3)
Imported	NA	2 (100)	2 (66.7)	18 (94.7)	19 (95.0)	15 (93.8)	84 (98.8)	89 (100.0)	60 (98.4)
Animal									
Autochthonous	15 (6.3)	52 (17.0)	48 (19.5)	44 (11.5)	25 (11.7)	27 (19.4)	103 (39.6)	21 (11.5)	11 (19.0)
Imported	NA	0	0	1 (5.3)	1 (5.0)	1 (6.2)	1 (1.2)	0	0
Other									
Autochthonous	2 (0.8)	2 (0.7)	4 (1.6)	4 (1.0)	0	1 (0.7)	0	0	1 (1.7)
Imported	NA	NA	1 (33.3)	0	0	0	0	0	1 (1.6)

*Values are no. (%) except as indicated. ICU, intensive care unit; CFR, case-fatality rate; NA, not available.

Table 5. Characteristics for 2,532 case-patients with leptospirosis, by sex, the Netherlands, 1925–2008*

Characteristic	Male patients, n = 2,306	Female patients, n = 226	p value
Mean age, y (SD)	34.5 (17.0)	26.4 (16.5)	<0.001
Patients with imported cases	278 (12.1)	40 (17.8)	0.014
Type of contact			
Recreational activity	1,086 (47.1)	162 (71.7)	0.001
Job-related	666 (28.9)	18 (8.0)	<0.001
Accident	339 (14.7)	27 (11.9)	0.235
Unknown	215 (9.3)	19 (8.4)	
Treatment course†			
Hospitalization	1,683 (73.0)	163 (72.1)	0.781
Dialysis‡	118 (9.3)	1 (1.1)	0.007
ICU§	102 (6.7)	4 (3.4)	0.063
Death†	160 (6.9)	6 (2.6)	0.013

*Values are no. (%) unless otherwise indicated. Data for age and sex were available for 2,422 patients, and data for autochthonous or imported infections and sex were available for 2,528 patients. ICU, intensive care unit.
†Percentages were calculated from available data (missing values were regarded as no). It was assumed that analyzing data that contained large amounts of missing values would result in findings that could not be generalized and lead to substantial overestimated values.
‡Dialysis data were available for 795 of 1,360 patients (732 of 1,263 male patients and 63 of 93 female patients) (January 1961–December 2008). Before 1961, dialysis was not expected to be used.
§ICU data were available for 625 of 1,641 patients (573 of 1,518 male patients and 52 of 119 female patients) (April 1955–December 2008). Before 1955, ICU was not expected to be used.

Recreational activities accounted for most (44.4%) autochthonous leptospirosis cases in this analysis, and ≈80% of imported infections were acquired during recreational water contact. Our data indicate that infections acquired during holidays in tropical countries are increasing. An increase in the incidence of leptospirosis related to exposures in tropical countries has been reported (*9*). Therefore, clinicians should consider leptospirosis in the differential diagnosis for patients with a febrile illness and a history of travel abroad.

A total of 15.5% of autochthonous and 6.9% of imported cases were reportedly caused by accidents with water exposure, indicating a need to further study this issue (*22*). In Germany accidental exposure has been reported and represented 3% of all reported cases during 1997–2000 (*9*).

The total incidence of leptospirosis in the Netherlands showed a small decrease over the 84-year study period. A decreasing trend in incidence has also been observed in France by Baranton and Postic (*10*), albeit, more pronounced. These authors attribute the decrease to changes in lifestyle and the rural environment. In the Netherlands, overall incidence is not decreasing as rapidly because of the increase in imported cases. The decrease in autochthonous infections in the Netherlands may reflect the success of the surveillance system and associated dairy control measures and vaccination of dogs, as shown by elimination of infections with serogroup Canicola since 1974 (*12*,*18*,*21*).

During the study period, most leptospirosis cases in the Netherlands were in male patients. However, surveillance systems based on passive reporting are biased toward including more severe cases, which are found more often in male patients (*27*). Therefore, if milder symptoms develop in female patients, these cases might also be more likely to be underdiagnosed. Certain occupations are more likely to be performed by men, and work-related exposure differences might contribute to the disproportional number of male patients given a diagnosis of leptospirosis. However, similar exposure risks during travel do not necessarily indicate similar rates of disease for persons of both sexes (*5*,*9*). In the Netherlands, risky vacation activities are found equally among men and women. However, these activities do not indicate an equal distribution of leptospirosis in men and women.

In addition, the disparity in the incidence of leptospirosis by sex of the patient was unlikely to be caused by differences in infecting serogroups because similar proportions of male and female patients were infected with serogroup Icterohaemorrhagiae. Therefore, we propose that sex of the patient might play a role in disease progression, which might influence the likelihood of diagnosis and reporting. Differences in health care–seeking behavior between male and female patients might also play a major role (*28*), However, genetic and physiologic differences that may affect disease manifestations in men should also be considered (*29*–*31*). Further research is needed to substantiate this hypothesis.

Our analysis has several major limitations. Use of passive surveillance data probably underestimates the total number of infections because mild cases of leptospirosis are less likely to be diagnosed. Furthermore, nationwide access to laboratory confirmation of leptospirosis has changed over time. In the early years of the study period, many features of the surveillance system were different, including diagnostic methods available, knowledge of existing serovars, collation of data, and clinician awareness of leptospirosis. However, the early data have contributed to our understanding of changes in leptospirosis over time because this dataset is comprehensive and includes an entire national cohort for >80 years.

We conclude that the effective surveillance system in the Netherlands, combined with adequate control measures,

has reduced the incidence of leptospirosis in this country. Efforts to prevent imported infections should include providing better information on risks to travelers and greater awareness by clinicians about development of leptospirosis in persons with a history of travel abroad.

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Parallels in Amphibian and Bat Declines from Pathogenic Fungi

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Pathogenic fungi have substantial effects on global biodiversity, and 2 emerging pathogenic species—the chytridiomycete *Batrachochytrium dendrobatidis*, which causes chytridiomycosis in amphibians, and the ascomycete *Geomyces destructans*, which causes white-nose syndrome in hibernating bats—are implicated in the widespread decline of their vertebrate hosts. We synthesized current knowledge for chytridiomycosis and white-nose syndrome regarding disease emergence, environmental reservoirs, life history characteristics of the host, and host–pathogen interactions. We found striking similarities between these aspects of chytridiomycosis and white-nose syndrome, and the research that we review and propose should help guide management of future emerging fungal diseases.

Fungi and fungus-like organisms have been recognized historically as prominent plant pathogens that can have detrimental effects on agricultural crops and wild flora (*1*,*2*). Fisher et al. (*3*) recently reviewed the increasing role and recognition of pathogenic fungi that affect global biodiversity. Their analyses showed that most (91%) recent extinctions and extirpations caused by fungal disease have affected animals rather than plants. In particular, 2 emerging pathogenic fungi—the chytridiomycete *Batrachochytrium dendrobatidis*, which causes chytridiomycosis in amphibians (*4*,*5*), and the ascomycete *Geomyces destructans*, which causes white-nose syndrome (WNS) in hibernating bats (*6*)—are implicated in the widespread decline of their vertebrate hosts. In general, increased global biosecurity and monitoring are recommended to prevent and manage emerging fungal diseases (*3*), but there are also pressing research needs that can help specifically address these 2 devastating pathogenic fungi. We call attention to parallels between chytridiomycosis and WNS and highlight areas where urgent research is required (Table). Comparison of these diseases also illustrates broader themes and questions that can be used to direct research on future emerging fungal diseases.

Disease Emergence

Effective control of any disease requires understanding of the processes that have resulted in its emergence. The study of a pathogen's geographic origin and phylogenetic history often provides critical insight in this regard. In general, infectious diseases can emerge from 2 distinct scenarios: 1) the geographic spread of a novel pathogen into a new area with naive hosts or 2) a shift in pathogenicity or host specificity of an endemic pathogen as a result of environmental changes that alter host–pathogen interactions (*25*). Current evidence predominantly suggests that chytridiomycosis and WNS outbreaks are driven by anthropogenic transport of novel pathogenic fungi into new geographic regions.

As chytridiomycosis began emerging globally, it was initially unclear whether *B. dendrobatidis* was newly introduced in regions affected by chytridiomycosis or whether it was globally endemic and had recently increased in pathogenicity as a result of environmental degradation or climate change (*4*,*5*,*25*). Spatiotemporal patterns of declines in amphibian populations from chytridiomycosis and population genetics data on *B. dendrobatidis* provide increasing evidence that the pathogen has been recently introduced in many areas (*4*,*5*). Wave-like patterns of amphibian deaths from chytridiomycosis have been documented at multiple sites globally, and these spatiotemporal dynamics suggest that *B. dendrobatidis* behaves as a novel pathogen spreading geographically through naive host populations (*4*). In addition, *B. dendrobatidis* strains isolated from across the world have low levels of genetic diversity, consistent with a pathogen that has recently undergone rapid range expansion (*4*,*5*).

In early research, Africa was proposed as the place of origin for *B. dendrobatidis*, and the African clawed frog (*Xenopus laevis*), which is not susceptible to chytridiomycosis, was thought to be a primary carrier responsible for global dispersal of *B. dendrobatidis* out of Africa (*26*). This amphibian species was used in human pregnancy assays beginning in the mid-1930s and later became a widely used biological model. On the basis of museum evidence that some African clawed frogs were infected with *B. dendrobatidis* in the late 1930s, Weldon et al. (*26*) hypothesized that global demand for the species resulted in the spread of *B. dendrobatidis* out of Africa to other areas where it subsequently became established in native, naive

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Table. Current knowledge and unresolved research questions regarding the pathogenic fungi *Batrachochytrium dendrobatidis* and *Geomyces destructans*, the causative agents of chytridiomycosis and WNS, respectively*

Area of knowledge	*B. dendrobatidis*		*G. destructans*	
	Current knowledge	Unresolved research questions	Current knowledge	Unresolved research questions
Disease emergence	Multiple regions of endemism and 1 widely introduced hypervirulent lineage (*7*–*9*)	How and from where did the hypervirulent lineage emerge?	Limited genetic differentiation in North America (*10*) Possibly endemic to Europe and introduced to North America (*6,11,12*)	How do strains from North America and Europe compare genetically, and is genetic variation greater in Europe, suggesting historic endemism?
Abiotic reservoirs	Can survive in water and soil (*13,14*)	Can *B. dendrobatidis* form desiccation-resistant resting spores? Can *B. dendrobatidis* survive and reproduce as a saprophytic, nonparasitic form?	Apparent persistence in soils and on cave walls (*12,15*)	How widespread is *G. destructans* in the environment? Can *G. destructans* survive and reproduce as a saprophytic, nonparasitic form?
Biotic reservoirs	Host generalist pathogen of amphibians (*4,5*) Can also infect reptiles, nematodes, and waterfowl (*16*–*18*)	Can *B. dendrobatidis* complete its life cycle on other vertebrate hosts?	Host generalist pathogen of bats (*6*)	Can *G. destructans* infect or persist on other vertebrates?
Life history and infection risk of the host	Aquatic, biphasic, tropical amphibian species at greatest risk for chytridiomycosis (*19*)	To what extent can life history characteristics of the host predict global patterns of disease-related population decline among amphibian species?	Bat species that hibernate experience most deaths from WNS (*20*)	Are only those species that hibernate susceptible to population decline from WNS? What role does life history of the host play in predicting species declines and extinctions from WNS?
Host–pathogen interactions	Antimicrobial peptides and antifungal metabolites from skin-associated bacteria contribute to *B. dendrobatidis* resistance (*21*) Susceptible species appear to show little innate or adaptive immune response to *B. dendrobatidis* infection (*22,23*)	What is the immune response of *B. dendrobatidis*–tolerant hosts to infection? Does *B. dendrobatidis* evade the amphibian immune system through activity of secreted proteases?	Host immune down-regulation during hibernation probably important to WNS progression (*24*)	What is the host immune response to *G. destructans* infection? How does host immunity vary seasonally? What role does immune function play in the observed winter season/hibernation mortality from WNS? Do proteases contribute to pathogenicity of *G. destructans*?

*WNS, white-nose syndrome.

wild populations. Amphibian species in pet stores, zoos, museums, and the food market are now known to carry *B. dendrobatidis* infections (*4*,*7*,*8*), and the international trade of amphibians is thought to be a major factor driving the spread of *B. dendrobatidis*, with other key carrier species, such as the North American bullfrog (*Lithobates catesbeianus*), contributing to the pathogen's global invasion (*4*,*5*,*9*,*26*,*27*).

The leading hypothesis, based on population genomic evidence, posits that a single hypervirulent *B. dendrobatidis* lineage is largely responsible for global chytridiomycosis-related amphibian declines and that the emergence of this lineage likely resulted from anthropogenic pathogen transport (*9*). Anthropogenic activity might have helped generate this hypervirulent strain by promoting fungal lineage mixing. Lineage mixing enables distantly related pathogen strains to outbreed, and this process can result in the emergence of novel, virulent genotypes that spread rapidly through susceptible host populations, as appears to have occurred with chytridiomycosis (*9*).

Studies of population genetics have contributed substantially to our understanding of *B. dendrobatidis* as a novel pathogen in regions affected by chytridiomycosis; however, wider global sampling and phylogenetic analysis of the fungus is needed (Table). Africa remains a critical region for the study of endemic *B. dendrobatidis* diversity, but research on strains isolated worldwide suggests that other strains also are endemic to Eurasia (*7*–*9*). Although these endemic strains might play a relatively small role in the global decline of amphibian populations, further study and broader sampling of *B. dendrobatidis* can improve our understanding of its phylogenetic history and shed light on

the possible contributions of lineage mixing and genetic variation to pathogenicity.

Our ability to accurately reconstruct *B. dendrobatidis* phylogeny is hampered by the limited number of *B. dendrobatidis* samples collected from species other than those susceptible to chytridiomycosis. As a result, additional cryptic, endemic lineages may remain overlooked or undersampled because they are associated with *B. dendrobatidis*–tolerant hosts (*7*,*8*), and environmental reservoirs could represent another source of undiscovered pathogen diversity. The global distribution of *B. dendrobatidis* before chytridiomycosis emergence and the geographic origin of the hypervirulent lineage remain uncertain (*27*), but both African (*26*) and Japanese (*7*) amphibian species appear to have been asymptomatic *B. dendrobatidis* hosts decades before amphibian declines were reported in other regions.

The origin of *G. destructans* and processes resulting in WNS emergence are unresolved. Bats in Europe are known to carry *G. destructans* asymptomatically, which has led to speculation that *G. destructans* was introduced from the Old World to susceptible North American bats, probably through human transport (*6*,*11*). Genetic markers show no differentiation among North American *G. destructans* samples collected across the expanding area of WNS, suggesting that *G. destructans* was recently introduced and is spreading rapidly through North America (*10*). Furthermore, the first observation of WNS in North America occurred in a cave that had high levels of tourism, providing circumstantial evidence for a link between human activity and *G. destructans* introduction (*11*). Although no global population genomic study has been conducted for *G. destructans*, the possibility that lineage mixing and recombination among fungal strains has contributed to WNS outbreaks is intriguing. In a laboratory study, North American bats died when infected with North American or European strains of *G. destructans* (*28*). These results were interpreted as further indirect evidence that *G. destructans* is novel to North America. However, recent sampling suggests that bat hibernacula host a diversity of previously uncharacterized *Geomyces* spp (*15*), and given our limited understanding of the global distribution of *G. destructans* strains and their phylogenetic relationships (*6*), it remains possible that *G. destructans* is endemic to North America. The distribution of *G. destructans* lineages in North America, similar to *B. dendrobatidis* distribution in some parts of Asia (*7*,*8*), might therefore include endemic, avirulent fungal strains in addition to recently introduced, potentially more virulent lineages. Currently, however, the lack of evidence for *G. destructans* presence in North America outside of WNS-affected areas supports the hypothesis that the fungus is indeed novel to this region (*6*). As with *B. dendrobatidis*, firm conclusions regarding the geographic origins of *G. destructans* await a much broader global sampling of *G. destructans* combined with fine-scale population genetic analyses of highly polymorphic markers (Table) (*6*).

Environmental Reservoirs

Theoretical work suggests that if environmental reservoirs (abiotic substrates or alternative biotic hosts that enable pathogen persistence) exist, diseases can have serious population-level effects on host species and even precipitate host extinction (*29*). The existence of multiple hosts can buffer a pathogen against population fluctuations in focal host species, and tolerant species may therefore increase pathogen burden for susceptible species by supporting high pathogen densities. Abiotic reservoirs also enable pathogen persistence when suitable vertebrate hosts are rare or absent, which also may increase pathogen burden for susceptible species. For example, mathematical models show that the risk for host extinction from chytridiomycosis increases when fungal zoospores have long residence time in the environment or if *B. dendrobatidis* can reproduce apart from amphibian hosts (*30*). Because of their effects on disease dynamics, recognition of the full range of environmental reservoirs is essential for effective management of any emerging disease.

There is strong reason to suspect that *B. dendrobatidis* and *G. destructans* have environmental reservoirs. In general, fungi may be unique pathogens because many can persist in the environment apart from animal hosts, yet environmental pressures can select for fungal traits that contribute to virulence during infection of certain host organisms (*31*). Fungi in the phylum Chytridiomycota have a nearly global distribution and occupy roles as heterotrophs and saprobes in water and soil (*32*). Similarly, multiple species of *Geomyces* are saprophytic (*20*). Given the ecologic characteristics of their broader taxonomic groups, it is perhaps unsurprising that environmental sampling is now revealing a variety of reservoirs for *B. dendrobatidis* and *G. destructans*.

Further work is needed to fully characterize the abiotic reservoirs of both pathogens (Table). As an aquatic fungus, *B. dendrobatidis* can survive in various aqueous media for several weeks (*13*) and may grow in moist soil (*14*). Although these laboratory studies demonstrate the range of conditions under which *B. dendrobatidis* might occur, patterns of fungal persistence in natural environments are yet to be studied intensively. In addition, Di Rosa et al. (*33*) have suggested that *B. dendrobatidis* may exist as a stress-tolerant resting spore, representing another abiotic reservoir for the fungus. However, whether the structure in question is in fact an alternate life stage of *B. dendrobatidis* remains unclear (*5*). Environmental distribution of *G. destructans* also is poorly understood,

but the pathogen has been detected in soil samples (*15*), and viable spores have been collected from cave walls in bat hibernacula (*12*), both factors that suggest the fungus has abiotic reservoirs.

Chytridiomycosis and WNS are caused by host-generalist pathogens that have multiple biotic reservoirs. *B. dendrobatidis* infects hundreds of amphibian species globally (*4*,*5*). In North America, WNS has spread among 9 bat species (*6*). Although research has predominately focused on the prevalence of *B. dendrobatidis* and *G. destructans* in taxa negatively affected by those pathogens (amphibians and bats, respectively), wider sampling of these fungi is warranted (Table). For example, despite more than a decade of *B. dendrobatidis*–related research, it was only recently discovered that the fungus may infect reptiles without causing disease (*16*). Furthermore, experimental *B. dendrobatidis* infections can lead to death of nematodes, although whether these organisms frequently serve as hosts for *B. dendrobatidis* in the wild is unclear (*17*). *B. dendrobatidis* has also recently been found on migratory waterfowl and may be carried among bodies of water by the infected keratinized feet and webbings of such birds (*18*). Whether bats are the only vertebrates capable of hosting *G. destructans* remains to be seen. Raccoons, bears, rodents, or other mammals that frequent cave systems where *G. destructans* is present are the most likely candidates to serve as alternative hosts (Table). Mammalian species that experience lowered body temperatures during winter torpor or hibernation may warrant particular attention, given *G. destructans*'s preference for psychrophilic growth (*6*,*34*).

Life History Characteristics and Infection Risk of the Host

Host life history characteristics can play a critical role in determining infection risk, and some amphibians and bats appear to be susceptible to pathogenic fungi in part because of their life histories. Amphibians often reach high population densities and have increased contact rates when breeding at aquatic sites; both factors promote pathogen transmission (*35*). Amphibians also may have intraspecific host reservoirs because of their complex life histories (i.e., aquatic larvae experience persistent sublethal infections and expose terrestrial adults that visit aquatic sites), enabling pathogen persistence within amphibian populations (*35*). Finally, many amphibians undergo immune suppression during metamorphosis, increasing their infection risk during this life stage (*35*). These factors probably explain, in part, the results of a study from Central America that found amphibian species that were highly aquatic were those most likely to suffer *B. dendrobatidis*–related population declines or extirpations (*19*). Analyses of amphibian communities in other regions are needed to determine whether life history characteristics of the host are broadly useful in predicting global chytridiomycosis-associated declines in amphibian populations (Table).

Just as some amphibian species are particularly susceptible to chytridiomycosis because of their life histories, the species of North American bats that are affected by WNS have life history characteristics that similarly predispose them to disease outbreaks. Bat populations in North America that are declining from WNS are known to breed, roost, and hibernate communally in large aggregations in cave systems where increased densities and contact between bats can facilitate pathogen transmission (*6*,*20*). There is speculation that if host density during hibernation influences WNS severity, this life history attribute may help explain the lack of WNS in Europe, where bat species typically form smaller hibernation aggregations than in North America (*11*). Bats in warm Mediterranean regions of Europe that have short hibernation periods appear to be free of *G. destructans* (*12*), which further supports a role for host life history in mediating pathogen transmission. In addition, the down-regulation of immune function in bats during hibernation (*24*) is roughly analogous to the immune suppression that occurs during amphibian metamorphosis, a trait that seems to be linked to increased disease susceptibility (*35*). WNS emerged in the northeastern United States but is rapidly spreading southwestward toward regions of high bat species richness where many species do not hibernate. Whether those species in the southwestern United States that do not hibernate will be at risk for WNS is unclear (*20*). To best predict infection risk and effectively manage amphibian and bat diversity, the degree to which life history and ecologic variation among hosts influence disease susceptibility needs to be better understood (Table). In this regard, broad epidemiologic studies and analyses of incoming reports and publications could yield key insights.

Host–Pathogen Interactions

The pathologies of chytridiomycosis and WNS are broadly similar in that both diseases result from infection by dermatophyte fungi that can severely disrupt host physiology (*5*,*6*,*24*,*27*). Amphibian skin plays a major role in physiologic regulation, and *B. dendrobatidis* infection of this tissue causes electrolyte imbalance in host individuals that ultimately leads to death from cardiac arrest (*27*). In bats, WNS results in death by increasing arousal frequency during hibernation, which depletes bat energy reserves (*28*). These behavioral changes in bats may ultimately be a reaction to the disruption of physiologic processes, including water balance, gas exchange, and thermoregulation caused by *G. destructans* infection of the wing membrane structure (*24*). However, in amphibians and bats, considerable interspecific variation

exists in disease-related illness and death from these fungal infections (*4*–*6*). One unresolved question for both chytridiomycosis and WNS is the degree to which interspecific differences in host–pathogen interactions underlie variation in disease outcome.

Some evidence indicates that host defenses mediate species-specific responses to chytridiomycosis and WNS. In amphibians, basic physiologic and anatomic traits that vary among species, such as skin sloughing rate and skin thickness, could provide innate defenses against the keratinophilic activity of *B. dendrobatidis* (*5*,*27*). Research also has shown that species-specific assemblages of skin-associated bacteria and suites of antimicrobial peptides affect amphibian susceptibility to chytridiomycosis (*21*). Not surprisingly, innate and adaptive amphibian immune function probably plays a critical role in determining chytridiomycosis disease outcome (*27*). The few studies that have investigated host immune response to chytridiomycosis suggest that susceptible frog species have a weak adaptive immune response to *B. dendrobatidis* infection (*22*,*23*). In contrast, work on the disease-resistant frog *Xenopus laevis* suggests that innate and adaptive immune components constitute host response to *B. dendrobatidis* infection in this species (*36*). Examining host immune response to *B. dendrobatidis* among closely related amphibians that differ in disease susceptibility would be of tremendous value to more fully elucidate the immune genes, pathways, and responses that contribute to *B. dendrobatidis* tolerance (Table). Bat immune response to *G. destructans* infection is poorly characterized (*6*), but species may be susceptible to WNS partly because of natural immune system down-regulation during hibernation (*24*). This current gap in knowledge deserves particular attention given that differences in host immune function between bat species in North America and Europe could account for their differential susceptibility to WNS (*6*,*11*). Immunogenetic studies are urgently needed to better understand the immunologic mechanisms driving WNS-associated population declines among bat species in North America. Comparisons between susceptible and nonsusceptible species, species in North America and Europe, and active and hibernating bats will be particularly important in this regard (Table).

Alternatively, disease processes in chytridiomycosis and WNS may largely reflect activity of the pathogenic fungi themselves, and further work is needed to understand the factors that drive pathogenicity in these fungal species (Table). Researchers have hypothesized that *B. dendrobatidis* may evade or suppress the amphibian immune system (*21*,*22*), but understanding of molecular and cellular mechanisms for such activity is lacking. Recent genomic comparisons with other fungi suggest that expansions of protease gene families during the recent evolutionary history of *B. dendrobatidis* might account for the pathogen's ability to colonize amphibian skin and evade the amphibian immune system (*37*). Because secreted proteases also appear to contribute to virulence in other dermatophyte fungi (*27*), activity of these genes could help explain pathogenicity of *G. destructans* as well. *G. destructans* is known to produce secretory proteases in culture (*34*), although the role of these proteins during pathogenesis of WNS has yet to be elucidated.

Conclusions

In general, the fungal diseases chytridiomycosis and WNS show striking similarities. Both diseases appear to be driven by novel pathogens introduced to new geographic regions by human transport. *B. dendrobatidis* and *G. destructans* are host-generalist pathogens with abiotic reservoirs that can persist even when the density of host species is low. The life history characteristics of many amphibians and bats result in high host densities, high rates of host contact, and depressed immune function during specific life stages or seasons, all of which are factors that contribute to pathogen persistence and transmission within a host community. Finally, both *B. dendrobatidis* and *G. destructans* are dermatophyte fungi that appear to successfully overcome host defenses in some of their primary host species.

Recognizing the commonalities between chytridiomycosis and WNS can help identify management efforts that may best address future emerging fungal diseases. Because anthropogenic transport of novel pathogens appears to play a major role in fungal disease emergence, increased global biosecurity aimed at minimizing the spread of invasive pathogens may be among the most effective fungal disease mitigation strategies (*3*). Although biosecurity will aid in detecting and preventing transport of many pathogen types, these efforts are particularly critical for controlling pathogenic fungi, given that they may infect a broad range of host species and persist on abiotic substrates long enough to increase their likelihood of successful dispersal to new areas. In addition, disease monitoring might focus on animals with life history characteristics that increase their risk for pathogen exposure or illness following pathogen introduction into a population. Species that exhibit social behavior or other intraspecific interactions might be especially vulnerable to disease outbreaks. For example, an opportunistic pathogenic fungus was recently identified in a declining population of timber rattlesnakes (*38*), and susceptibility to outbreaks of fungal disease might be predicted for this and similar species given their communal denning behavior and potential for lowered immune function during hibernation.

Although pathogen virulence and disease outbreaks are extremely difficult to predict, scientists focusing on wildlife

disease might prioritize research on fungi closely related to currently pathogenic species while remaining vigilant of fungal disease outbreaks in new host species. Pathogenic fungi have primarily been viewed as threats to ectothermic organisms because ectotherms can have low body temperatures that are suitable for the growth of many fungi (*31*,*39*). *B. dendrobatidis* (*4*,*5*) and *G. destructans* (*6*,*20*) are psychrophilic species with growth optima at lower temperatures (17°–25°C for *B. dendrobatidis*, 10°–15°C for *G. destructans*). In contrast, endothermic body temperatures generally exceed the upper thermal tolerance limits of fungi, a factor that might partially explain why WNS affects bats during hibernation when their internal temperature is lowered and immune function is inhibited (*24*,*39*). However, some researchers hypothesize that anthropogenic climate warming will select for increasing heat tolerance in fungi, presumably resulting in a greater number of fungi capable of surviving in the range of endothermic body temperatures (*40*). Regional warming and associated changes in fungal heat tolerance may therefore create new pathogen risks for endothermic vertebrate hosts. In conclusion, we suggest psychrophilic fungal species probably will continue to be major pathogens of ectothermic vertebrates, whereas fungi with thermal tolerances closer to endothermic body temperatures may represent increasing threats to endotherms. Despite the associated challenges, the recent devastating effects of chytridiomycosis and WNS suggest that biologists and epidemiologists should give greater attention to pathogenic fungi if they wish to preserve vertebrate biodiversity in a rapidly changing global environment.

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Increasing *Pneumocystis* Pneumonia, England, UK, 2000–2010

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Learning Objectives

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

- Describe changes in incidence of *Pneumocystis jirovecii* pneumonia in England from 2000–2010, based on findings of a database study
- Describe changes in risk factors associated with *P. jirovecii* pneumonia in England from 2000–2010, based on findings of a database study
- Describe the clinical and public health implications of the study findings.

CME Editor

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After an increase in the number of reported cases of *Pneumocystis jirovecii* pneumonia in England, we investigated data from 2000–2010 to verify the increase. We analyzed national databases for microbiological and clinical diagnoses of *P. jirovecii* pneumonia and associated deaths. We found that laboratory-confirmed cases in England had increased an average of 7% per year and that death certifications and hospital admissions also increased. Hospital admissions indicated increased *P. jirovecii* pneumonia diagnoses among patients not infected with HIV, particularly among those who had received a transplant or had a hematologic malignancy. A new risk was identified: preexisting lung disease. Infection rates among HIV-positive adults decreased. The results confirm that diagnoses of potentially preventable *P. jirovecii* pneumonia among persons outside the known risk group of persons with HIV infection have increased. This finding warrants further characterization of risk groups and a review of *P. jirovecii* pneumonia prevention strategies.

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Anecdotal reports from clinicians suggest that incidence of *Pneumocystis jirovecii* pneumonia, previously referred to as *P. carinii* pneumonia or PCP, among immunosuppressed patients, especially renal transplant recipients, has increased substantially (*1*). To investigate this claim, we analyzed data for January 2000 through December 2010, using several national data sources: Hospital Episode Statistics, routine laboratory reporting, death certificate data, and HIV surveillance data.

P. jirovecii pneumonia gained notoriety during the AIDS pandemic (*2*); however, the reservoirs, modes of transmission, and pathogenesis of this organism remain poorly understood (*3*). Subclinical infection is considered common because studies have shown that anti–*P. jirovecii* antibodies develop during early childhood (*4*). Reactivation of latent infection after immunosuppression of the host was thought to be the main pathogenic mechanism (*3*); however, recent studies indicate that person-to-person spread might cause acute infection in susceptible persons (*5*).

Although not fully characterized, the known risk factors for *P. jirovecii* infection include impaired immunity because of HIV infection, hematologic malignancies, and connective tissue disorders (*6*). Immunosuppressive agents used to treat or prevent graft rejection have been implicated; such agents include corticosteroids, methotrexate, cyclosporine, mycophenolate mofetil, bendamustine, cyclophosphamide (*7–11*), and, recently, novel immunomodulating drugs, such as tumor necrosis factor–α inhibitors (*12*).

Prophylactically administered oral trimethoprim–sulfamethoxazole, dapsone, or atovaquone prevent the clinical manifestation of *P. jirovecii* infection. Also effective for decreasing *P. jirovecii* infection incidence among HIV-positive patients with a $CD4^+$ count <200/μL is routine prophylactic administration of antimicrobial drugs (*13*,*14*).

Given the existence of effective chemoprophylaxis, identification of new risk groups might help prevent future increases in *P. jirovecii* infection incidence. Therefore, we conducted a retrospective analysis of multiple national data sources to examine trends in *P. jirovecii* infection.

The Health Protection Agency has approval from the National Information Governance Board for Health and Social Care for the collation of surveillance data in accordance with section 251 of the National Health Service Act 2006. No additional ethical approval was required for this study.

Materials and Methods

Hospital Episode Statistics

The Hospital Episode Statistics (HES) database contains details of all inpatient admissions to National Health Service hospitals in England. We identified all patients for whom an International Classification of Diseases,10th Revision (ICD-10), code B59, which corresponds with *P. jirovecii* infection, was recorded in any of the first 10 diagnosis fields from January 2000 through December 2010. By using ICD-10 and Operating Procedure Code Supplement 4 codes, we then subdivided cases into non–mutually exclusive, condition-specific categories that are frequently cited in the literature in association with *P. jirovecii* (*7–13*,*15–19*). The categories covered were renal failure, hematologic malignancy, other hematologic disorders, systemic connective tissue disorders, inflammatory diseases (such as rheumatoid or psoriatic arthritis), and receipt of immunosuppressive agents or an organ transplant. Patients with chronic lung conditions, such as pulmonary fibrosis, were categorized as a single group, given the observed frequency in this study of concurrence of this condition with *P. jirovecii* infection. Patients who did not fit into any risk category were also included in the analysis.

We cross-checked for duplicate records and selected the record of first admission for each patient. We examined information about sex, age, and geographic distribution of patients. HIV-infected patients were excluded from analysis because the clinical records for these patients did not contain patient-identifiable information (unlike the other clinical records in the HES database), thereby making identification and exclusion of duplicate records not possible for this group.

Routine Laboratory Reporting

LabBase2 is the Health Protection Agency's national communicable diseases database for England, Wales, and Northern Ireland; it receives semiautomated downloads of results from 99% of microbiology diagnostic laboratories (Health Protection Agency, unpub. data). Laboratory-confirmed cases of *P. jirovecii* infection in England during 2000–2010 were extracted from LabBase2, and duplicate laboratory samples were excluded.

Death Certificate Data

For the study period, deaths in England with an ICD-10 clinical code indicating *P. jirovecii* as the cause or contributory cause of death were extracted from Office for National Statistics data. Deaths from *P. jirovecii* infection linked to a diagnosis of HIV or AIDS were also analyzed.

HIV Surveillance Data

Data from the Health Protection Agency's HIV and AIDS New Diagnoses and Deaths database were analyzed (*20*). Because HIV surveillance data are available for adults only, epidemiologic information in this study was restricted to patients ≥15 years of age. *P. jirovecii* infections were reported as co-infections at the time of HIV diagnosis, as subsequent AIDS diagnoses, or as the cause of death.

Statistical Analyses

We used the statistical software STATA/SE 11.2 (*21*) for all analyses. Poisson regression with an offset for resident population, which used Office for National Statistics midyear estimates, was used to calculate the annual incidence rate ratio with 95% CIs. The Pearson χ^2 test was used to examine changes in the proportion of cases by risk category over time (2000–2005 vs. 2006–2010).

Results

The absolute numbers of cases of *P. jirovecii* pneumonia in England during 2000–2010, reported by each national surveillance system, are shown in Figure 1 and Table 1. We describe data from each system separately.

Hospital Episode Statistics

During the study period, HES recorded 2,258 cases of *P. jirovecii* pneumonia. The number of cases increased from 157 in 2000 to 352 in 2010, an average annual increase of 9% ($p<0.001$).

Cases reported to HES were not restricted to a particular geographic area, and the data showed no obvious seasonal trends. Because the increase in cases began in the latter half of the decade (Figure 1), we compared data from 2000–2005 with that from 2006–2010. This comparison showed a marked change in the age distribution of patients hospitalized for *P. jirovecii* infection during 2006–2010; relatively more patients were 60–69 years of age (Figure 2). Among all age groups, there was a higher proportion of male than female patients with *P. jirovecii* infection.

During the study period, 81% of patients within the HES database who had a diagnosis of *P. jirovecii* pneumonia could be classified according to a defined risk category (Table 2). Most (40.6%) had a hematologic malignancy, and 17.5% had preexisting lung disease. Relative distribution of risk groups differed significantly between 2000–2005 and 2006–2010 for all risk categories (χ^2 28.2, 7 degrees of freedom, $p<0.001$). The numbers of patients with *P. jirovecii* pneumonia increased significantly in all risk groups, but the difference in rates between the 2 periods was most marked among patients who had undergone transplantation, 47% of whom had undergone kidney transplantation during 2000–2010. The number of patients who were not in any of the risk groups described above dropped by 19% between the 2 periods. This test was conservative because there was some overlap between the risk categories.

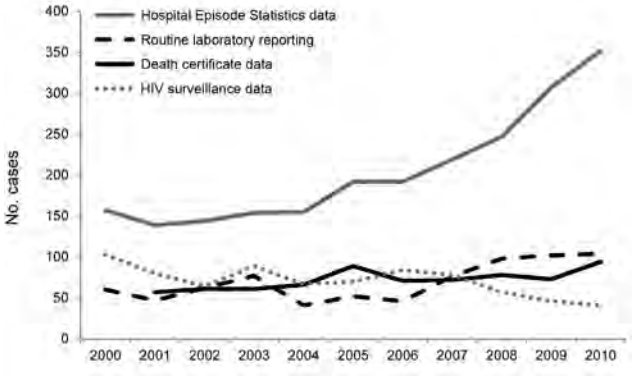

Figure 1. *Pneumocystis jirovecii* infections reported by national data collection systems, England, UK, 2000–2010. Hospital admissions exclude patients with HIV diagnoses.

Routine Laboratory Reporting

During the study period, LabBase2 recorded 765 laboratory-confirmed cases. Reported cases of *P. jirovecii* pneumonia remained relatively unchanged during 2000–2006 (range 41–77 cases/year, mean 55 cases/year) but increased from 76 cases in 2007 to 98–104 cases during 2008–2010 (Figure 1), particularly in older patients. The male-to-female ratio of *P. jirovecii* pneumonia patients during 2000–2010 was 2.5 to 1.0.

Death Certificate Data

Deaths for which *P. jirovecii* pneumonia was recorded as a cause or contributing factor rose from 57 in 2001 to 94 in 2010 ($p<0.001$). For several years, the numbers of *P. jirovecii* infections reported on death certificates as a contributory cause of death were greater than those captured by laboratory reports (Figure 1).

HIV Surveillance Data

The numbers of patients with *P. jirovecii* pneumonia and HIV infection decreased 7% per year during 2000–2010 ($p<0.001$) (Figure 3). Most *P. jirovecii* infection diagnoses were made at the time of HIV diagnosis. Within this group of HIV-infected patients, death from *P. jirovecii* infection remained relatively stable over this period.

Discussion

In this study, we found an increasing trend in rates for clinical cases recorded in HES and microbiologically confirmed and reported cases in England during 2000–2010. This finding suggests a real increase in the numbers of cases of *P. jirovecii* pneumonia diagnosed. We also found an association between *P. jirovecii* infection and a variety of chronic lung diseases not described in the literature as being associated with *P. jirovecii* infection. On the basis of these data, we propose preexisting lung disease as a new *P. jirovecii* pneumonia risk category.

The HES database yielded 2,258 cases of *P. jirovecii* pneumonia during 2000–2010, but LabBase2 found only 765. The differences in number of cases suggests substantial underreporting by laboratories, although most cases might be diagnosed on the basis of clinical or radiologic

Table 1. Annual change in incidence rate of *Pneumocystis jirovecii* cases, England, UK, 2000–2010*

Surveillance system	Total no. cases	Annual incidence rate ratio (95% CI)†
Laboratory reporting	765	1.07 (1.05–1.09)
Hospital admissions‡	2,258	1.09 (1.08–1.11)
HIV surveillance data	779	0.94 (0.92–0.96)
Death registrations	722	1.04 (1.01–1.06)

*Midyear population estimates used.
†p<0.001 for all.
‡Excludes *P. jirovecii* diagnoses for patients with diagnosed HIV infection.

findings or by immunofluorescence in the cytology department without being microbiologically confirmed.

An analysis of the Health Protection Agency database of HIV-infected persons shows clear evidence of a substantial reduction in *P. jirovecii* infections during 2000–2010, consistent with an earlier diagnosis of HIV and receipt of effective antiretroviral therapy (*14*). *P. jirovecii* infections among HIV-infected persons declined, whereas *P. jirovecii* infections among non–HIV-infected persons increased, suggesting that other risk factors must be responsible for the increased numbers of cases.

Given the substantial illness and death associated with *P. jirovecii* infection and the resources needed to manage these cases, the increase in cases is of serious concern. Many patients need treatment in intensive care units. However, prophylactic use of antimicrobial drugs is highly effective for preventing the disease. A study in the United States suggested that almost $5 million a year could be saved in the state of Maryland alone if prophylaxis were instituted for all HIV-positive patients at risk for *P. jirovecii* infection (*22*).

Potential Causes of the Observed Increase

The increased number of cases might reflect changes in ascertainment of cases and increased infections in immunosuppressed patients who have received chemotherapy. It is possible that ascertainment increased over the study period because of improved diagnostic methods; immunofluorescence staining is being replaced by more sensitive PCR methods (*23*). We were not able to test the hypothesis that the increased number of cases is the result of increased testing for *P. jirovecii* because the laboratory surveillance system captures positive samples only, not the total number of samples submitted. However, the change in age distribution of patients toward a much older age group suggests that increased testing is not the main reason for increased case detection.

With regard to immunosuppression, an area that has seen an increase in the use of potent immunosuppressant agents is transplant surgery. Recipients who are not well matched to donor human leukocyte antigens now receive more powerful drugs. That said, the proportion of patients receiving renal transplants with a moderate degree of human leukocyte antigen mismatch has remained stable, represented by 43.9% of patients during financial year 2009–10 (National Health Service Blood and Transplant Authority, pers. comm.). Similarly, data from the National Health Service Blood and Transplant Authority indicate that the number of renal transplantations increased by 25% during 2006–2010. Again, this increase was not proportional to that observed for *P. jirovecii* infections reported for renal transplant recipients, which was ≈388% over the same period (National Health Service Blood and Transplant Authority, pers. comm.), so the increase cannot be explained simply by an increase in the number of patients in this risk group.

The largest group of persons affected by *P. jirovecii* pneumonia is those with hematologic malignancies. This finding might reflect the 30% increase in diagnoses of these malignancies during 2000–2010 (*24*). However, the increase in patients in this risk group with *P. jirovecii* pneumonia was 209% over the same period.

A possible explanation for the increase in *P. jirovecii* pneumonia cases is an increase in the number of potentially vulnerable patients who did not receive appropriate prophylactic therapy. Guidelines recommend the use of antimicrobial drug prophylaxis for kidney transplant recipients and for patients with hematologic malignancies who are receiving certain chemotherapy (*25–28*). A Cochrane review recommends prophylaxis for patients with hematologic malignancies and for recipients of bone marrow and solid organ transplants (*29*). Our study identified a new group at risk for *P. jirovecii* infection: patients with

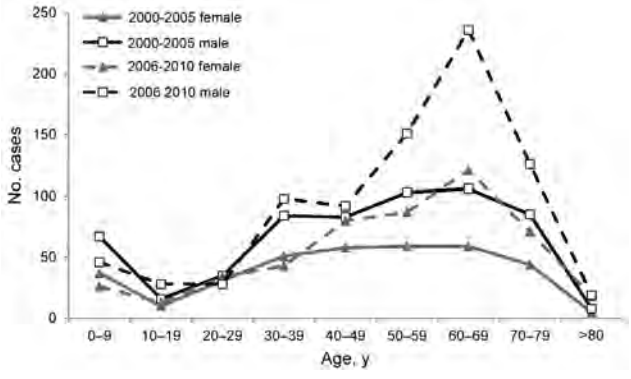

Figure 2. Age and sex distribution of patients with *Pneumocystis jirovecii* infections (excluding HIV-infected patients) among hospital admissions, England, UK, 2000–2010.

Table 2. Proportion of all *Pneumocystis jirovecii*–associated hospital admissions and change in population rates over time, England, UK, 2000–2010

Risk category*	No. admissions (% all cases)		Annual rate/million population		Rate ratio between periods (95% CI)
	2000–2005	2006–2010	2000–2005	2006–2010	
Any transplant†	59 (6.3)	193 (14.7)	0.20	0.75	3.80 (2.84–5.09)
Other lung disease‡	120 (12.8)	276 (21.0)	0.24	0.47	1.97 (1.47–2.64)
Hematologic disorders	217 (23.1)	354 (26.9)	0.32	0.81	2.55 (2.00–3.25)
Hematologic malignancy	349 (37.1)	568 (43.1)	1.17	2.21	1.89 (1.66–2.16)
Connective tissue/inflammatory disease§	71 (7.6)	120 (9.1)	0.31	0.62	2.02 (1.56–2.61)
Renal failure and dialysis	95 (10.1)	208 (15.8)	0.16	0.35	2.23 (1.56–3.17)
Immunosuppressive/ chemotherapeutic drugs	47 (5.0)	90 (6.8)	0.73	1.38	1.90 (1.60–2.25)
Malignancy other than hematologic	92 (9.8)	160 (12.2)	0.40	1.07	2.67 (2.16–3.31)
Not in the above risk categories	255 (27.1)	177 (13.4)	0.85	0.69	0.81 (0.67–0.98)
Total no. cases¶	941	1,317	3.15	5.13	1.62 (1.50–1.77)

*Excludes HIV infection.
†includes liver, heart, lung, kidney and bone transplants.
‡Includes tuberculosis, chronic obstructive pulmonary disease, cystic fibrosis, bronchiectasis, asthma, and interstitial lung disease.
§Includes systemic connective tissue disorder, psoriatic arthropathy, rheumatoid arthritis, and inflammatory bowel disease.
¶Because some patients belong to >1 risk category, numbers do not add up to the total number of cases.

preexisting lung disease. To determine whether any preventative measures would be advisable for these patients will require further detailed characterization and quantification of risk within this group.

Another possible explanation for the increase in *P. jirovecii* pneumonia cases is increased transmission of the *P. jirovecii* organism between susceptible persons. Levels of exposure of susceptible persons to infectious persons might be increased as a result of changes in the delivery of health care. New, more transmissible strains could be emerging and leading to increased spread in the health care environment. Further investigation into the contribution of outbreaks—and, thus, increased person-to-person transmission—to the increase is warranted.

As a result of increased awareness of *P. jirovecii* infection, other infections might be clinically misdiagnosed as *P. jirovecii* infection. In the HES database, some patients might have been incorrectly coded as having *P. jirovecii* pneumonia, thereby resulting in a misclassification bias, but we have no reason to suspect that this coding would have changed over time. The death statistics should also be interpreted with caution because the cause of death and contributory causes are probably not recorded consistently. The analyses did not differentiate between outbreaks and sporadic cases of disease because this information could not be reliably determined from the data sources used. Although the most recent data might be subject to reporting delays, such delays would result in underestimation rather than overestimation of recent cases.

Next Steps

Incidence of *P. jirovecii* pneumonia has increased across all groups of immunosuppressed patients known to be at risk for this infection (excluding HIV patients) and in new groups not previously known to be at risk. To determine whether current indications for prophylaxis need to be widened, enhanced surveillance should be introduced to help characterize any additional groups of patients for whom prophylaxis is not currently recommended but who might be at risk. Particular focus should be given to patients with chronic lung disease, systemic inflammatory diseases, and solid tumors and to transplant recipients who do not currently fulfill the criteria for prophylaxis. When introducing new immunosuppressive agents and regimens, consideration should be given as to whether these agents might increase the patients' risk for *P. jirovecii* pneumonia.

More studies involving sequencing of *P. jirovecii* clinical isolates identified by PCR, coupled with national surveillance, should be used to better understand transmission dynamics and thereby inform infection control policies and clarify the role of any environmental factors (*1*,*30*–*32*). More basic knowledge of the biology, pathogenesis, virulence factors, and the contribution of different strains will be crucial for explaining observed changes in *P. jirovecii* epidemiology.

To ensure adherence to current guidelines and to ensure that preventive prophylaxis is optimal for all groups at risk for this potentially life-threatening infection, auditing of prescribing practices for patients known to be at risk is warranted. Raising awareness among clinicians could also help ensure that prophylaxis is correctly used.

In conclusion, data from a variety of national sources demonstrate an increase in the number of cases of *P. jirovecii* in non–HIV-infected persons. *P. jirovecii* infections are largely preventable by use of inexpensive drugs. The current case numbers are taking a substantial toll on health care costs and human health. Further investigation leading to improved preventive strategies for this largely preventable infection is warranted.

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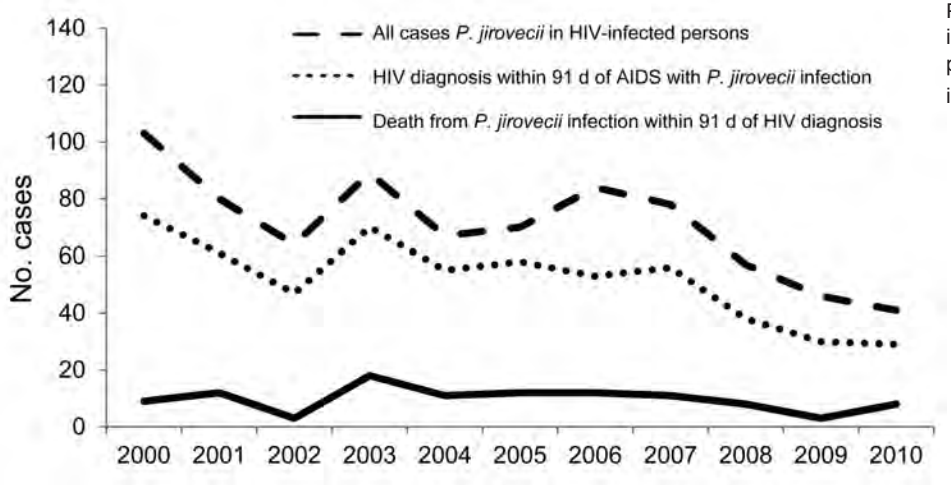

Figure 3. *Pneumocystis jirovecii* infections and deaths among persons with diagnosed HIV infection, England, UK, 2000–2010.

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etymologia

Leptospira [lep′to-spi′rə]

From the Greek *leptos* (slender) and *speira* (coil), a genus of bacteria consisting of single, finely coiled, motile, aerobic cells. In 1886, German physician Adolf Weil described a clinical syndrome characterized by splenomegaly, jaundice, and nephritis, although the disease was likely recognized in ancient China as an occupational hazard of rice farming. The organism was first described in 1907 by Arthur Stimson, who observed spirochetes with curved ends in the kidneys of a patient thought to have died of yellow fever. He named it *Spirochaeta interrogans* because it looked like a question mark.

The cause of Weil's disease was isolated independently in 1915 in Japan and Germany. In Japan, Inada et al. detected spirochetes, which they named *Spirochaeta icterohaemorrhagiae*, in the blood of coal miners with infectious jaundice. In Germany, 2 groups of physicians (Uhlenhuth et al. and Hubener et al.) studied soldiers afflicted with "French disease" in the trenches of northeastern France. The Germans were arguing over priority, however, and overlooked the publications by Inada's group, which predated their own by 8 months. The genus *Leptospira* was suggested in 1917 by Hideyo Noguchi "on account of its fine and minute windings."

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Clinical and Therapeutic Features of Pulmonary Nontuberculous Mycobacterial Disease, Brazil, 1993–2011

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Learning Objectives

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

- Analyze the epidemiology of pulmonary nontuberculous mycobacterial (PNTM) disease
- Evaluate the clinical presentation of PNTM disease
- Distinguish mycobacteria associated with the most cases of PNTM disease in the current study
- Distinguish the mycobacterium species associated with the lowest cure rates of PNTM in the current study.

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To identify clinical and therapeutic features of pulmonary nontuberculous mycobacterial (PNTM) disease, we conducted a retrospective analysis of patients referred to the Brazilian reference center, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil, who received a diagnosis of PNTM during 1993–2011 with at least 1 respiratory culture positive for NTM. Associated conditions included bronchiectasis (21.8%), chronic obstructive pulmonary disease (20.7%), cardiovascular disease (15.5%), AIDS (9.8%), diabetes

(9.8%), and hepatitis C (4.6%).Two patients had Hansen disease; 1 had Marfan syndrome. Four mycobacterial species comprised 85.6% of NTM infections: *Mycobacterium kansasii,* 59 cases (33.9%); *M. avium* complex, 53 (30.4%); *M. abscessus*, 23 (13.2%); and *M. fortuitum,* 14 (8.0%). A total of 42 (24.1%) cases were associated with rapidly growing mycobacteria. In countries with a high prevalence of tuberculosis, PNTM is likely misdiagnosed as tuberculosis, thus showing the need for improved capacity to diagnose mycobacterial disease as well as greater awareness of PNTM disease prevalence.

Pulmonary disease caused by nontuberculous mycobacteria (PNTM) frequently causes sickness and death. These bacteria are found in water sources and soil and are particularly concentrated in biofilms (*1*,*2*). Certain clinical conditions are known to be associated with an increased risk of PNTM, particularly immunosuppressive conditions and structural changes in the lung, such as those associated with chronic obstructive pulmonary disease (COPD), bronchiectasis, sequelae from prior pulmonary tuberculosis (TB), and cystic fibrosis (*3*).

Recent studies have documented the emergence of NTM lung diseases in industrialized countries, such as the United States (*4*,*5*). The current prevalence estimated for PNTM is ≈6 cases/100,000 population, with the highest prevalence in persons >50 years of age (*4*–*6*). In many developing countries with a high prevalence of TB, the prevalence of PNTM among immunocompetent persons remains unknown, largely because of the lack of routine culture and species identification from samples of persons with suspected cases. In Brazil in 2010, 70,601 cases of TB were reported, indicating a prevalence of 38 cases/100,000 population. In the same year, 4,500 deaths from TB were reported. In 2011, TB was the third leading cause of death (from infectious diseases) and the first among AIDS patients, according to surveillance data from Brazil's National TB Control Program (*7*). However, because TB is routinely diagnosed presumptively, solely on the basis of identifying acid-fast bacilli (AFB) from sputum samples, an unknown proportion of patients may in fact be infected with NTM. Therefore, the true prevalence of NTM in Brazil remains unknown.

In Brazil, the Professsor Helio Fraga Reference Center (CRPHF) has served as a reference center for multidrug-resistant TB (MDR TB) and NTM since 1993. From 1993 to 2011, 5,638 cases of MDR TB were reported in Brazil, 1,894 of them in Rio de Janeiro; 1,595 of these patients were treated at CRPHF (*8*).

Prior studies have described some of the features of clinical isolates from PNTM patients (*9*–*14*), and others have described the clinical features for small populations infected with NTM clinical isolates in Brazil (*15*,*16*). To more fully describe the emerging prevalence and associated conditions of PNTM in a large urban population in Brazil, we present results of a large and detailed review of PNTM case-patients with PNTM who were treated at CRPHF.

Materials and Methods

Referral Population

CRPHF is a national reference center for the diagnosis and treatment of MDR TB, and it also functions as a local reference center for treatment of PNTM case-patients from Rio de Janeiro through an outpatient unit. In Brazil, mycobacterial cultures are only performed for specific groups: 1) patients newly diagnosed with TB who remain positive for AFB positive in the second month of treatment; 2) patients who have a history of prior treatment for TB and are newly AFB positive; 3) patients who are contacts of persons with drug-resistant TB; and 4) patients who are part of specific population groups, including health professionals, the homeless, prisoners, indigenous populations, and HIV-positive persons (*7*). Thus, patients are referred to CRPHF from public and private healthcare facilities for mycobacterial species identification when NTM or MDR TB is suspected, typically because the patients remain AFB positive and do not improve clinically while receiving TB treatment, or when 1 sample culture is positive for NTM with no species identified. Radiographic evaluations are performed at baseline and at follow-up visits through x-ray films or, more recently, computed tomographic scans.

Study Population and Data Collection

The study population comprised patients from the state of Rio de Janeiro who were referred to CRPHF for further evaluation, for either PNTM or MDR TB. Patients included in this analysis had at least 1 respiratory isolate identified as NTM, were residents in the state of Rio de Janeiro, and were referred to CRPHF during January 1993–January 2011. Only patients with NTM isolated from respiratory specimens were included. Demographic, clinical, and epidemiologic information was collected from patient clinical records. Information included co-existing conditions, smoking history, and results of radiographic imaging. With respect to microbiologic information, data included treatment duration for NTM disease, month of the first negative sputum culture, and treatment outcome. Additional information included the number of prior episodes of TB and prior treatment for TB, including information about those who had been treated for TB for at least 6 months before the diagnosis of NTM disease. Microbiologic confirmation with sputum culture and species identification were not able to be carried out for samples from patients' prior TB episodes.

Table 1. Demographic and clinical features for patients with PNTM, Rio de Janeiro, Brazil, 1993– 2011*

Characteristic	No. (%) patients
Male sex	108 (62.1)
Age, median y (range)	55 (24–86)†
Past or current smoker‡	95 (62.1)
Clinical findings	
Respiratory symptoms only	106 (60.9)
Fever and weight loss	15 (8.6)
Respiratory and systemic symptoms (fever, weight loss)	53 (30.5)
Previous TB treatment	
Treated previously for TB	101 (58)
Referred while being treated for TB	80 (79)
Radiology results (lesion type)	
Cavitary disease	118 (67.8)
Fibronodular	56 (32.2)
Median duration of treatment, mo (range)	19 (2–36)†
ATS microbiologic criteria	
1 positive sputum culture	47 (27)
≥2 positive sputum cultures	101 1 (58)
Bronchoalveolar lavage	24 (13.8)
Lung biopsy	2 (1.1))

*PNTM, pulmonary nontuberculous mycobacterial disease; TB, tuberculosis; ATS, American Thoracic Society.
†Data are as indicated in the left column.
‡Smoking data were missing for 21 patients.

Treatment and Assessment of Microbiologic Response

After a patient's diagnosis at CRPHF, sputum samples are collected monthly or bimonthly until treatment is completed. Subsequently, sputum samples are collected every 3 months in the first 12 months following treatment, and then every 6 months for at least 2 years following treatment. Recommended treatment depends on species, and generally follows American Thoracic Society (ATS) guidelines (*3*), with a course of 12–18 months for slow-growing mycobacteria, such as *M. kansasii* or *M. avium* complex (MAC), and longer courses for rapidly growing mycobacteria. The definitions for classification of treatment response were as follows: 1) cure was indicated by at least 3 consecutive respiratory specimens negative for NTM during 12 consecutive months; 2) treatment failure was indicated by at least 2 positive cultures at the end of 12 months of treatment; 3) relapse was indicated by cultures positive for NTM ≥30 days after a prior cure; and 4) death meant death from any cause during treatment, regardless of whether death was confirmed as associated with NTM disease.

Laboratory Methods

Before 2004, species identification for *M. kansasii*, MAC, *M. abscessus*, and *M. fortuitum* was done through biochemical tests. Subsequently, the *hsp65* PCR restriction analysis (PRA) method was used for species identification

Data Analysis

To assess association of species and cavitary disease with culture conversion, we used Epi Info version 3.5.3 wwwn.cdc.gov/epiinfo/ and SAS version 9.2 (SAS Institute Inc., Cary, NC, USA). The significance of associations was assessed by using χ^2 with $p<0.05$. We limited this analysis to those patients who met the ATS criteria (>1 NTM-positive sputum sample or 1 sample from bronchoalveolar lavage or lung biopsy specimens).

Results

Demographic and Clinical Features of Patients with NTM Lung Disease

We identified a total of 174 patients in our study population; 108 (62.1%) were male. The median age was 55 years (range 24–86 years). Smoking history was available for 153 patients, of whom 95 (62.1%) reported past or current smoking. The most frequent symptoms were respiratory (60.9%), but 30.5% of the total patients had both

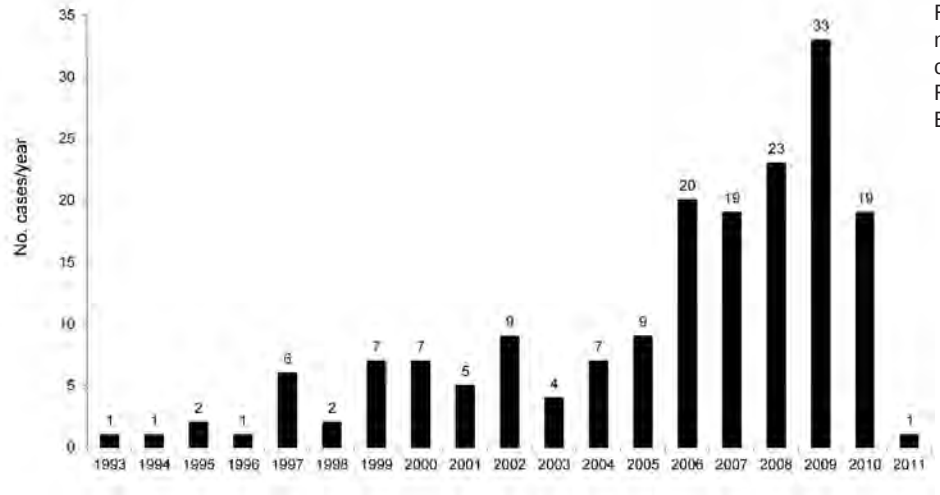

Figure. Number of nontuberculous mycobacterial pulmonary disease cases per year, Professor Helio Fraga Reference Center, Rio de Janeiro, Brazil, January 1993–January 2011.

Table 2. Coexisting medical conditions for174 patients with PNTM, Brazil, 1993–2011*

Coexisting condition†	No. (%) patients
None	59 (33.9)
Bronchiectasis	38 (21.8)
Chronic obstructive pulmonary disease	36 (20.7)
Cardiovascular disease	27 (15.5)
AIDS	17 (9.8)
Diabetes	17 (9.8)
Asthma	8 (4.6)
Hepatitis C	8 (4.6)
Cancer, excluding lung cancer	6 (3.4)
Gastroesophageal reflux	6 (3.4)
Rheumatioid arthritis	3 (1.7)
Nonviral cirrhosis	2 (1.1)
Hansen disease	2 (1.1)
Lupus	2 (1.1)
Anemia falciforme	1 (0.57)
Silicosis	1 (0.57)
Marfan syndrome	1 (0.57)
Kidney transplantation and immunosupression	1 (0.57)
Mitral valve prolapse	1 (0.57)

*PNTM, pulmonary nontuberculous mycobacterial disease.
†Patients may have exhibited >1 coexisting condition.

respiratory and systemic symptoms, including cough, dyspnea, hemoptysis, thoracic pain, fever, weight loss, and night sweats (Table 1). Overall, 101 (58.0%) patients reported prior treatment for TB, based only on a positive AFB smear (without microbiologic confirmation for *M. tuberculosis*), of whom 80 (79%) were referred while being treated empirically for TB for up to 6 months before the diagnosis of NTM infection. Overall, 127 (72.9%) patients met the ATS criteria for NTM disease (Table 1). The number of PNTM cases identified from 1993 to 2005 ranged from 5 to 7 cases per year. However, beginning in 2006, the number of identified cases reached ≈20, and it has remained at 20–40 cases per year since then (Figure).

With respect to coexisting conditions, 59 (33.9%) patients had no identified coexisting conditions. The most frequently identified conditions were bronchiectasis (21.8%), COPD (20.7%), and cardiovascular disease (15.5%). A lower proportion of patients were identified with AIDS (9.8%), diabetes (9.8%), and hepatitis C (4.6%). All patients with bronchiectasis had this condition identified through computed tomographic scans. Two patients had Hansen disease, 1 of whom also had hepatitis C. One patient had Marfan syndrome and no other reported coexisting conditions (Table 2). Patients may have had >1 coexisting condition.

Patients without prior treatment for TB were not significantly different from those with prior treatment with respect to age, sex, infecting species, or clinical features. Among the 73 patients with no prior TB treatment, 47 (64.4%) were male with a mean age of 54 years. In this group, 43 (58.9%) showed cavitary lesions, of which 29 (67.4%) were bilateral.

Species Identification and Response to Treatment

Four species comprised 85.6% of all species identified: *M. kansasii*, 33.9%, MAC, 30.4%, *M. abscessus*, 13.2%, and *M. fortuitum*, 8.0%. Overall, 42 (24.1%) patients had an isolate identified as a rapidly growing mycobacterium (*M. fortuitum, M. abscessus, M. peregrinum, M. massiliense*) (Table 3). The proportion of species identified through PRA was as follows: *M. kansasii*, 69.5%; MAC, 62.3%; *M. abscessus*, 55.5%; *M. fortuitum*, 57.1%; all other species, 100%. The species distribution in the group of patients with prior treatment for TB was similar to the overall distribution, with 37.3% infected with *M. kansasii* and 26.6% infected with MAC. The species distribution in the group of patients with bronchiectasis also showed a similar distribution: *M. kansasii* was the most frequent (31.5%), followed by MAC (31.5%), *M. abscessus* (10.5%), and *M. massiliense* (5.2%). Infection with uncommon species, such as *M. simiae, M. lentiflavum*, *M. celatum,* and *M. szulgai,* all occurred in this group.

Treatment outcome varied significantly by infecting species, with the highest cure rate (71.4%) observed among patients infected with *M. kansasii*, followed by those infected with MAC (57.8%), and the lowest cure rate (25.0%) was observed in patients infected with *M. abscessus* (Table 4). Although the majority of patients had cavitary disease, among those infected with *M. kansasii*, the outcome of disease was similar for those with cavitary and noncavitary lesions (72.7% and 67.0%, respectively); among those infected with MAC or *M. abscessus*, the cure rates appeared somewhat higher for those with noncavitary disease, although this effect was not significant (Table 5).

Treatment regimens by infecting species are shown in Table 6. Of the patients with MAC infection, 54.7% received combination antimicrobial drug therapy, consisting of rifampin, ethambutol, and clarithromycin; 52.0% of patients infected with *M. kansasii* underwent regimens containing rifampin, ethambutol, and isoniazid. Infections

Table 3. *Mycobacterium* species infecting 174 patients with PNTM, Brazil, 1993–2011

Species	No. (%) patients infected
M. kansasii	59 (33.9)
Mycobacterium avium complex	53 (30.4)
M. abscessus	23 (13.2)
M. fortuitum	14 (8.0)
M. massiliense	3 (1,7)
M. peregrinum	2 (1.1)
M. asiaticun	1 (0.57)
M. simae	1 (0.57)
M. lentiflavum	1 (0.57)
M. szulgai	1 (0.57)
M. celatum	1 (0.57)
M. terrae	1 (0.57)
Not identified	14 (8.0)

*PNTM, pulmonary nontuberculous mycobacterial disease.

Table 4. Cure rates by species infecting patients with ATS-defined PNTM, Brazil, 1993–2011*

Species†	No. (%) patients cured	Total no.
Mycobacterium kansasii	30 (71.4)	42
M. avian complex	26 (57.8)	45
M. abscessus	6 (25.0)	24
M. fortuitum	5 (45.4)	11
Other	2 (40.0)	5
Total	69 (54.3)	127

*ATS, American Thoracic Society; PNTM, pulmonary nontuberculous mycobacterial disease. Patients without identified species were excluded from this analysis.

of patients with rapidly growing mycobacteria were treated with clarithromycin in combination with amikacin.

Discussion

We report the emergence of PNTM among patients receiving care at a referral center for MDR TB. Although we were unable to determine the true prevalence of disease caused by these organisms in the state of Rio de Janeiro, the current study confirmed an increasing frequency of patients referred with NTM lung disease. In some industrialized countries, the prevalence of NTM has been found to be increasing (*5*,*17*), although it remains unclear whether this increase is related to increasing awareness of physicians that leads to more frequent diagnosis, to improved laboratory capacity, or to a combination of both factors.

Brazil has a high prevalence of TB, so initial the treatment for TB is based on smear results; culturing is not done and species is not identified before treatment. Thus, patients infected with slowly growing mycobacteria (e.g., *M. kansasii*,) which have a pulmonary manifestation similar to that of *M. tuberculosis* and might respond to empirical treatment with anti-TB drugs, may not have received an appropriate diagnosis. A notable limitation of this study is that we could not accurately determine whether these patients were truly infected with *M. tuberculosis* and had NTM disease as a sequela of their TB disease, or if they were initially infected with NTM. Because empirical treatment is not appropriate for those with *M. tuberculosis* infections, but is done in many parts of the world due to lack of resources, strengthened laboratory capacity is needed to correctly identify the prevalence of emerging NTM disease (*18*). Given the lack of capacity for culture confirmation of all cases of TB, the current disease effects of NTM are likely underestimated in Brazil as well as in other countries with similar resource limitations for mycobacterial diagnostics. The introduction of more affordable rapid diagnostic tools to improve diagnostic capacity should reduce potential misdiagnosis to better estimate the true extent of PNTM.

One third of the NTM patients in this study population were infected with *M. kansasii*, a proportion substantially higher than that seen in the United States (*5*,*19*)and in other areas of Brazil (*10*,*14*) and Australia (*20*). Global geographic variability in *M. kansasii* has been noted; a high prevalence of *M. kansasii* lung disease occurs in Western Europe, Switzerland, and the United Kingdom (*17*,*21*–*23*). In Brazil, available data suggest regional differences in species distribution, although distinct study populations and methods limit comparability of estimates. A prior report from CRPHF, analyzing samples sent from throughout Brazil during 1994–1999, found that of 433 pulmonary isolates, 203 (46.9%) were MAC and 61 (14.0%) were *M. kansasii*. In that study, some regional variability in the relative proportions of *M. kansasii* and MAC was evident, with a somewhat lower proportion of *M. kansasii* in the Southeast (18%) and South regions (13.7%) relative to the North (24.3%) *(24*). Other studies conducted in different areas of Brazil have found that *M. kansasii* infections range from 16.0% in Bahia (*15*) to 2.2% in São Paulo (*12*) and 0% in the northern Amazonian region (*9*). For *M. kansasii*, municipal water systems are the primary source of *M. kansasii*; thus, the degree of urbanization is likely to affect isolation rates. Climatic and ecologic factors as well as the prevalence of cofactors, such as HIV infection, are also likely to influence the prevalence of NTM species. Brazil encompasses a wide geographic area with large variations in climatic conditions and urbanization. We cannot sort out these factors in our analysis, and future research is needed to address these issues.

M. kansasii infection is manifested as a lung disease that is nearly identical to TB with a high prevalence of cavities and a predominance of fibrocavitary lesions in the

Table 5. Association of cavitary disease with cure rates of patients with ATS-defined PNTM, Brazil, 1993–2011*

Species	No. (%) patients		Relative risk (95% CI)
	With noncavitary disease	With cavitary disease	
Mycobacterium kansasii			
Cure	6 (67)	24 (72.7)	0.92 (0.55–1.5)
Total	9	33	
M. avium complex			
Cure	12 (85.7)	14 (45.2)	1.9 (1.2–3.0)
Total	14	31	
M. abscessus			
Cure	3 (42.9)	3 (17.7)	2.4 (0.64–9.2)
Total	7	14	

*ATS, American Thoracic Society; PNTM, pulmonary nontuberculous mycobacterial disease.

Table 6. Treatment regimens for all patients with ATS-defined PNTM, per infecting species, Brazil, 1993–2011*

Species	Treatment regimen (%)†
Mycobacterium avium complex	Clarithromycin, amikacin,ethambutol, rifampin (54.7)
	Clarithromycin, ethambutol,quinolone, terizidon (28.3)
	Clarithromycin, amikacin, ethambutol, quinolone‡ (17.0)
Mycobacterium kansasii	Rifampin, ethambutol, isoniazid (52.5)
	Rifampin, ethambutol, isoniazid, clarithromycin, amikacin (47.5)
M. abscessus	Clarithromycin, amikacin (68.4)
	Clarihromycin, amikacin, doxycycline (10.5)
	Clarithromycin, amikacin, terizidon (21)
M. massiliense	Clarithromycin, amikacin (33.3)
	Clarithromycin, amikacin, terizidon (33.3)
	Clarithromycin, amikacin, imipenem, tigeciclin (33.3)
M. fortuitum	Clarithromycin, amikacin, quinolone (64.2)
	Clarithromycin, amikacin, terizidon (28.5)
	Clarithromycin, amikacin, quinolone, doxycycline (7.3)

*ATS, AmericanThoracic Society; PNTM, pulmonary nontuberculous mycobacterial disease.
†Median duration of treatment, 19 mo.
‡Quinolones: levofloxacin, ciprofloxacin, moxifloxacin, ofloxacin.

upper lobe (*3*). However, more recent studies have found a lower frequency of cavitary lesions associated with *M. kansasii* infection in Korea (32%) (*25*) and Israel (57%) (*26*). A culture conversion rate of 95% was found from the study in Korea, with conversion defined as 3 negative samples within a 6-month period (*25*).

In our study, approximately one quarter of patients had no coexisting condition, and 40% had no prior history of TB treatment. NTM have historically been associated with disease in male smokers with COPD (*27*). However, over the last 20 years, the epidemiology has been changing in some industrialized countries, particularly the United States, with most patients identified without known risk factors or with bronchiectasis, all in the context of declining rates of TB (*5*,*6*).

The finding from this study of 1 patient with Marfan syndrome and 1 patient with mitral valve prolapse is consistent with the association of predisposing genetic disorders of the connective tissue with a certain morphotype, as has been found in in the United States (*28*). In our cohort, 8 patients had hepatitis C (from different genotypes and with various degrees of hepatic fibrosis). Of the 8 case-patients with hepatitis C, 5 had *M. kansasii* infection, and the 2 other patients with nonviral cirrhosis of the liver were also infected with *M. kansasii*. Another study found chronic liver disease was an underlying medical problem in patients infected with *M. kansasii* (*26*).

In summary, in Brazil, a country with a high prevalence of TB, the misdiagnosis of NTM disease may lead to inaccurate and inappropriate treatment. This study suggests that despite the high level of TB in Brazil, subpopulations of patients with NTM exist, and these infections are not being adequately detected and treated. Continued monitoring of PNTM is needed in Brazil, and more detailed information should be obtained on pulmonary disease resulting from these infections, including their regional distribution, species, associated coexisting conditions, and treatment response. In addition, adequate laboratory infrastructure and affordable testing are needed at the local level to ensure accurate diagnosis and proper treatment for all.

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Dr Couto de Mello is a physician at the Reference Center Professor Helio Fraga in Rio de Janeiro and completed this work as part of her master's thesis at the Federal University of Rio de Janeiro. Her research interests include the epidemiology and clinical characteristics of nontuberculous pulmonary diseases.

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Tuberculosis and HIV Co-infection, California, USA, 1993–2008

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Learning Objectives

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

- Describe rates of HIV co-infection among persons with tuberculosis (TB) in California, and changes in TB incidence among persons with HIV from 1993 to 2008 in California, based on an epidemiological study using registry and surveillance system data
- Compare characteristics of patients with TB/HIV co-infection in the modern era with those in the pre-HAART era, based on an epidemiological study using registry and surveillance system data
- Describe mortality and other characteristics of TB/HIV in California, based on an epidemiological study using registry and surveillance system data.

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To understand the epidemiology of tuberculosis (TB) and HIV co-infection in California, we cross-matched incident TB cases reported to state surveillance systems during 1993–2008 with cases in the state HIV/AIDS registry. Of 57,527 TB case-patients, 3,904 (7%) had known HIV infection. TB rates for persons with HIV declined from 437 to 126 cases/100,000 persons during 1993–2008; rates were highest for Hispanics (225/100,000) and Blacks (148/100,000). Patients co-infected with TB–HIV during 2001–2008 were significantly more likely than those infected before highly active antiretroviral therapy became available to be foreign born, Hispanic, or Asian/Pacific Islander and to have pyrazinamide-monoresistant TB. Death rates decreased after highly active antiretroviral therapy became available but remained twice that for TB patients without HIV infection

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and higher for women. In California, HIV-associated TB has concentrated among persons from low- and middle-income countries who often acquire HIV infection in the peri-immigration period.

The modern resurgence of tuberculosis (TB) in conjunction with the HIV pandemic remains a major public health dilemma. In 2011, nine percent of all newly reported TB cases in the United Sates for which HIV status was known (*1*) and 13% (1.1 million cases) of cases reported worldwide (*2*) were associated with HIV co-infection. Despite compelling declines in TB incidence and associated deaths with use of highly active antiretroviral therapy (HAART), TB remains the leading cause of death among persons with HIV/AIDS (*3*,*4*).

California, the most populous state in the United States (38 million persons [12% of the US population]), reports the highest annual number of persons with TB (22.1% of total) and the second highest number of HIV-infected persons (103,073 [12.4%] cases) (*5*). Yet, because of prior restrictions on HIV reporting and limited systematic linking of state TB and HIV surveillance systems, California has not been included in key national surveillance reports of HIV incidence (*6*) or death in persons with TB–HIV (*7*).

Effective control of TB–HIV requires an understanding of the changing epidemiology of these diseases. To provide information for disease-reduction efforts and to improve survival among persons with TB–HIV, we retrospectively reviewed all incident TB–HIV cases in a 16-year period in California during which dynamic changes occurred in the HIV epidemic as a consequence of the introduction of HAART.

Methods

Study Population

We analyzed all TB cases reported to the California TB registry during January 1, 1993–December 31, 2008. California state law requires reporting of all verified cases of TB and HIV/AIDS (California Health and Safety Code Title 17§2505, and Section 121022 [2006]) to their respective programs. TB–HIV patients were identified through a statewide registry match with the California Office of AIDS by using Registry Plus Link Plus software (online Technical Appendix, wwwnc.cdc.gov/EID/article/19/3/12-1521-Techapp1.pdf). Annual state HIV prevalence was estimated through nonparametric back-calculation based on racial/ethnic group–specific counts of reported AIDS cases and reported AIDS-related deaths during 1981–2008 (online Technical Appendix Table 1) (*8*). Demographic, behavioral, and clinical information, including deaths, was abstracted from state surveillance forms (Report of a Verified Case of Tuberculosis and Adult HIV/AIDS Confidential Case Report). Surveillance data for both diseases have demonstrated high validity (*9*,*10*).

Definitions

HIV cases were classified in accordance with current Centers for Disease Control and Prevention (CDC) surveillance case definitions (*11*). For this analysis, patients not identified in the California HIV/AIDS registry were considered HIV-negative. TB diagnoses were grouped into 3 periods on the basis of HAART availability and approximately equal distribution of TB–HIV cases: pre-HAART (1993–1995), early HAART (1996–2000), and late HAART (2001–2008). Late diagnosis of HIV infection was defined as an AIDS diagnosis made <12 months after an initial diagnosis of HIV infection. Advanced immunosuppression was defined as a CD4+ T-lymphocyte count <50 cells/mm^3; valid CD4+ T-lymphocyte counts were those collected within 6 months of TB diagnosis. Drug susceptibility testing was performed at local laboratories or at the California Microbial Diseases Laboratory (Richmond, CA, USA) by using the BACTEC 460TB System (Becton Dickinson Diagnostic Instruments, Sparks, MD, USA), BACTEC MGIT 960 MycoBacterial Detection System (Becton Dickinson), or the agar proportion method.

Statistical Analysis

Stratum-specific TB incidence per 100,000 population was calculated by dividing the number of incident cases by total (*12*) and HIV-infected population denominators. Clinical trends and demographic characteristics were described in 2 ways. First, we calculated prevalence ratios (PRs) and 95% CIs for comparison of characteristics associated with TB and TB–HIV cases (*13*). Second, we compared rates of annual percentage change (1993–2008) in prevalence of binary covariates by using logistic regression with robust SEs. This model included main effects for year (as a categorical variable) and HIV infection status, as well as the interaction between them.

Multivariate associations with death among TB–HIV patients were examined by using a generalized linear model with a log link and robust SEs to generate relative risk (RR) estimates (*14*); the model was a priori specified to include time period, age, sex, race/ethnicity, foreign birth, HIV risk factor, CD4+ T-lymphocyte count, sputum smear positivity, and interval between the diagnoses of HIV infection and TB. Death at diagnosis or during treatment was calculated from patients for whom outcome was known (3,754/3,904 [96.2%]); clinical outcomes during 2007 were excluded because of incomplete reporting. Multiple imputation was used to impute missing values for CD4+ T-lymphocyte count and viral load (*15*). The results obtained after multiple imputation were compared with those from an unimputed complete-case analysis (online Technical Appendix Table 2).

Proportions were compared by using χ^2 tests, and continuous variables were compared by using the Wilcoxon rank-sum test. All p values were 2-sided with $\alpha = 0.05$ as the significance level. Data were analyzed by using Stata 12.1 (Stata Corporation, College Station, TX, USA) and R, version 2.13.2 (Foundation for Statistical Computing, Vienna, Austria).

Results

During 1993–2008, a total of 57,527 TB cases occurred in California, 3,904 (6.8%) of which were identified in a registry match with the California Office of AIDS. Overall, the proportion of TB–HIV cases decreased from 1,343 (9.2%) of 14,640 in the pre-HAART era to 1,254 (5.3%) of 23,812 in late HAART era. TB incidence among patients with HIV/AIDS declined from 437 cases per 100,000 persons to 126 per 100,000 (71% decrease); TB incidence rates for persons without known HIV co-infection declined from 16.5 cases per 100,000 persons to 7.0 per 100,000 (58% decrease) (Figure 1). Throughout the study period, incidence declined markedly in all strata of race/ethnicity but remained highest among Hispanics (225/100,000) and Blacks (148/100,000) with HIV/AIDS (Figure 2).

Demographic Trends

The median age of patients with known TB–HIV increased throughout the study period (Table 1, Appendix, wwwnc.cdc.gov/EID/article/19/3/12-1521-T1.htm); the number of persons >50 years of age with TB–HIV increased from 12% in the pre-HAART era to 21% in the late HAART era, out of proportion to the increase (from 40% to 46%) for TB cases alone (p<0.001 for difference in slopes). Likewise, female patients with TB–HIV increased from 11% to 17%, out of proportion to the increase (from 40% to 41%) for TB cases alone (p<0.001 for difference in slopes).

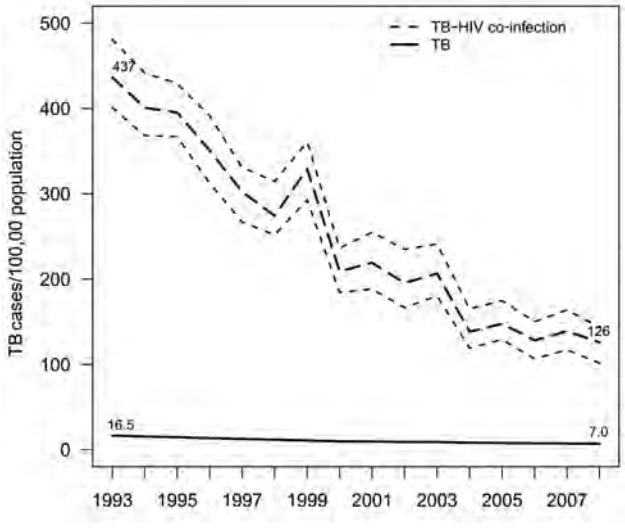

Figure 1. Rates of tuberculosis (TB) and TB–HIV, California, USA, 1993–2008. Area between dashed lines represents 95% bootstrap percentile CIs for TB–HIV rates. Annual state HIV prevalence was estimated through nonparametric back-calculation based on racial/ethnic group–specific counts of reported AIDS cases and reported AIDS-related deaths during 1981–2008 (online Technical Appendix, wwwnc.cdc.gov/EID/article/19/3/12-1521-Techapp1.pdf).

Foreign-born Persons

The proportion of TB–HIV patients who were foreign born increased from 37% in the pre-HAART era to 63% in the late HAART era, a greater increase than for TB patients without known HIV co-infection (from 67% to 77%; p<0.001 for difference in slopes). Seventy-six percent of foreign-born patients with TB–HIV (and 37% of all patients with TB–HIV) immigrated from Mexico or Central America. Among persons with HIV infection, TB was diagnosed a median of 11 years (interquartile range [IQR] 4–19 years) after entry into the United States, significantly longer than for persons without known HIV infection (8 years [IQR 2–18 years], p<0.001 by Wilcoxon rank-sum test). In contrast, TB–HIV patients were consistently younger (25 years [IQR 19–33 years]) than TB patients (31 years [IQR 20–49 years]; p<0.001 by Wilcoxon rank-sum test) at time of US entry. Immigrants from Mexico or Central America who had TB–HIV were younger (23 years [IQR 17–35 years]) than those from Southeast Asia or sub-Saharan Africa (36 years [IQR 24–55 years]; p<0.001 by Wilcoxon rank-sum test). In HIV-infected persons from sub-Saharan Africa, active TB developed sooner after immigration (2.6 years [IQR 0.2–5.7 years]) than in persons from all other regions (10.8 years [IQR 4.5–19.3 years]; p<0.001 by Wilcoxon rank-sum test). Compared with the pre-HAART era, patients with TB–HIV in the late HAART era were more likely to originate from Southeast Asia (PR 2.4; 95% CI 1.2–4.8) or sub-Saharan Africa (PR 4.6; 95% CI 2.1–10.1).

Race/Ethnicity

Hispanics accounted for 57% of all TB–HIV cases in the late HAART era (a 20% increase from the pre-HAART era); in contrast, the proportion of TB patients without known HIV co-infection who were Hispanic (38%) did not change. Asian/Pacific Islanders also represented an increasing proportion of TB–HIV patients in the late from the early HAART eras (8% vs. 4%; PR 2.2 [95% CI 1.6–3.0]). Among US-born persons, TB–HIV declined among Blacks (pre-HAART vs. late HAART; 31% vs. 17%) and Whites (23% vs. 12%) but not Hispanics (8% vs. 8%; p<0.001 for difference in slopes).

HIV Risk Group

From the pre-HAART era to the late HAART era among TB–HIV patients, the HIV transmission risk factors of men who have sex with men (MSM) (47% vs. 41%),

injection drug use (IDU) (22% vs. 15%), and MSM/IDU (16% vs. 8%) decreased, whereas presumed heterosexual transmission increased (6% vs. 16%; $p<0.01$ by χ^2). MSM was the most commonly reported HIV risk factor for all racial/ethnic groups. Presumed heterosexual transmission increased over time and was more common for TB–HIV cases diagnosed in the late HAART era among Hispanics (12%; 95% CI 10%–13%) and Blacks (11%; 95% CI 9%–13%) than among Whites (4%; 95% CI 3%–5%). Similarly, unknown or unreported HIV transmission risk factors were more common among Hispanics (16%; 95% CI 15%–18%) and Blacks (10%; 95% CI 9%–12%) than among Whites (5%; 95% CI 4%–7%).

Clinical Trends

HIV Characteristics

Overall, AIDS developed within 12 months after HIV diagnosis in 72% of patients, a percentage that did not substantially change throughout the study. Median CD4+ T-lymphocyte count was 114 cells/mm^3 (IQR 60–179 cells/mm^3) during the pre-HAART era and 100 cells/mm^3 (IQR 55–150; $p<0.01$ by Wilcoxon rank-sum test) during the late HAART era (Table 2, Appendix, wwwnc.cdc.gov/EID/article/19/3/12-1521-T2.htm). Approximately 20% of patients had advanced immunosuppression (CD4+ T-lymphocyte count <50 cells/mm^3) during both the pre-HAART and late HAART eras.

TB Characteristics

Patients with TB–HIV were more likely to be sputum smear positive (PR 1.11; 95% CI 1.05–1.17) and less likely to have culture-negative pulmonary TB (PR 0.58; 95% CI 0.49–0.68) than patients without known HIV co-infection during the pre-HAART era. These differences diminished in the late HAART era (PR 1.01; 95% CI 0.96–1.07 and PR 0.81; 95% CI 0.70–0.93, respectively).

Among initial isolates, pyrazinamide-monoresistant TB (PR 2.21; 95% CI 1.90–2.57) was more common, and isoniazid-resistant (PR 0.69; 95% CI 0.60–0.79) and multidrug-resistant TB (PR 0.58; 95% CI 0.39–0.85) less common among TB–HIV patients than among patients without known HIV co-infection. Pyrazinamide-monoresistant TB among TB–HIV patients increased from 2% during the pre-HAART era to 8% during the late HAART era, which was out of proportion to the increase (from 1% to 3%) among patients with TB alone ($p<0.05$ for difference in slopes).

Clinical Outcomes

Compared with the pre-HAART era, death during treatment decreased in the late HAART era among TB–HIV patients (30% vs. 14%) but not among TB patients without known HIV co-infection (6.7% vs. 6.7%; $p<0.001$ for difference). In multivariate analysis, older age (RR 1.3 per 10 years; 95% CI 1.2–1.4), lower CD4+ T-lymphocyte count (RR for reference value <50 cells/mm^3 vs. >350–500 cells/mm^3, 6.5; 95% CI 2.7–15.6), pre-HAART characteristics (RR 2.2; 95% CI 1.9–2.6), sputum smear positivity (RR 1.2; 95% CI 1.1–1.4), and female sex (RR 1.4; 95% CI 1.1–1.7) were associated with increased risk for death, whereas the heterosexual HIV risk group (relative to MSM, RR 0.6; 95% CI 0.4–0.8) was protective (Table 3). Results were similar in a sensitivity analysis excluding rather than imputing missing CD4+ T-lymphocyte data (online Technical Appendix).

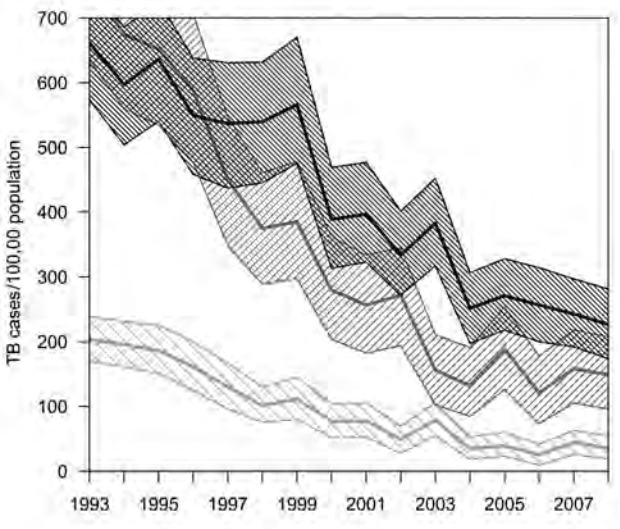

Figure 2. Rates of tuberculosis (TB) for persons with HIV/AIDS, California, USA, 1993–2008. Shaded areas represent 95% bootstrap percentile CIs, by race. TB–HIV rates for Asians/Pacific Islanders could not be calculated because of small numbers of cases during some years. Annual state HIV prevalence was estimated through nonparametric back-calculation on the basis of racial/ethnic group–specific counts of reported AIDS cases and reported AIDS-related deaths during 1981–2008 (online Technical Appendix, wwwnc.cdc.gov/EID/article/19/3/12-1521-Techapp1.pdf). Light gray, Whites; medium gray, Blacks; dark gray, Hispanics.

Discussion

In California, a dramatic decline in TB–HIV rates coincided with the introduction of HAART and improvements in TB control. The TB–HIV intersection has evolved from one in which active TB and AIDS progressed in a marginalized, US-born population to an intersection increasingly comprising persons from areas outside the United States with elevated TB incidence who acquire HIV infection in the peri-immigration period. TB–HIV-associated death has decreased substantially but remains approximately twice that associated with TB alone.

Population declines in TB–HIV after introduction of HAART are well documented in low-income countries

Table 3. Multivariate analysis of factors associated with deaths among HIV-infected TB patients, California, USA, 1993–2008*

Characteristic	Adjusted relative risk (95% CI)
Time period	
2001–2008	Referent
1996–2000	1.18 (0.98–1.41)
1993–1995	2.21 (1.88–2.60)
Age†	1.29 (1.22–1.36)
Female Sex	1.36 (1.12–1.65)
Race/ethnicity	
White non-Hispanic	Referent
Black non-Hispanic	0.86 (0.73–1.01)
Hispanic	0.86 (0.67–1.09)
Asian/Pacific Islander	0.70 (0.11–4.52)
Foreign birth	0.65 (0.36–1.17)
HIV risk group‡	
MSM	Referent
IDU	1.02 (0.88–1.19)
Heterosexual contact	0.58 (0.43–0.78)
Unknown	1.24 (1.04–1.48)
Sputum smear positivity	1.23 (1.07–1.40)
CD4+ T-lymphocyte count, cells/mm^3§	
<50	6.45 (2.67–15.58)
50–99	5.57 (2.40–13.90)
100–199	3.09 (1.28–7.46)
200–349	1.47 (0.58–3.72)
350–499	Referent
>500	1.99 (0.66–6.08)
TB as AIDS-defining diagnosis¶	1.22 (1.07–1.38)

*TB, tuberculosis; MSM, men who have sex with men; IDU, injection drug user.
†Per 10-year increase in age.
‡Categories are mutually exclusive; any IDU was included in the IDU category.
§CD4+ T-cell counts were imputed (online Technical Appendix, wwwnc.cdc.gov/EID/article/12-1521-Techapp1.pdf).
¶TB was considered the AIDS-defining event if TB and AIDS were reported within 6 months of each other.

(*16*,*17*). Despite the resurgence of TB in the United States during 1985–1992 (*18*), overall case rates unexpectedly decreased in some metropolitan areas before the availability of HAART because of improvements in TB control (*19*); in California, specific declines in TB–HIV began before the widespread use of HAART in 1996 (Figure 1). Improvements in programmatic TB control and declines in new annual HIV infections through the 1980s resulting from HIV awareness and prevention programs might have contributed to TB–HIV declines independent of HAART availability.

As in other high-income settings (*20*,*21*) and consistent with TB and TB–HIV (*22*) trends in the United States, immigrants from low-income countries increasingly represent the face of TB–HIV in California. Increased risk for TB–HIV among newcomers from sub-Saharan Africa reflects the hyperendemic nature of TB and HIV in this region. Yet, the demographic transformation of TB–HIV in California has been characterized largely by immigrats from Mexico or Central America and, to a lesser extent Southeast Asia, regions that have concentrated rather than generalized HIV epidemics. The finding that the median time in-country for foreign-born persons with TB–HIV is 11 years has specific TB control implications. This finding suggests that HIV transmission commonly occurs during the peri- or post-immigration period (*23*), and screening for HIV and latent TB infection restricted to new immigrants will not address the large number of co-infected residents who might benefit from treatment. Furthermore, immigrants who arrive without documentation lack systematic opportunities for HIV screening. These testing gaps will translate to undetected infection and progression to AIDS unless post-entry screening for immigrants is intensified. Finally, the substantial increase in pyrazinamide monoresistance, a surrogate marker for *Mycobacterium bovis* (an organism inherently resistant to pyrazinamide) further corroborates this demographic shift because TB in the United States caused by *M. bovis* primarily occurs among Mexican immigrants exposed to unpasteurized milk products (*24*). This finding has implications for transmission, epidemiologic surveillance, and preventive interventions, as well as improvements in pyrazinamide susceptibility testing (*25*).

In the United States, new HIV infections (*26*) and TB–HIV (*22*,*27*) remain concentrated among Blacks. However, Hispanics have the highest TB–HIV rates in California, a finding corroborated by reports from municipalities along the US–Mexico border (*28*). Current rates of TB among HIV-infected Hispanics in California exceed rates in many World Health Organization–defined high-prevalence TB countries but are modest compared with the high rates in southern African countries (*29*) or marginalized HIV-infected populations in pre-HAART–era United States (*30*).

The dramatic decline in TB among HIV-infected persons in California has not been accompanied by a concurrent decrease in the proportion of patients who have advanced AIDS or late diagnosis of HIV infection, and TB rates continue to far exceed background TB incidence in the state. The distribution of CD4+ T-lymphocyte counts at TB diagnosis in California, even in the late HAART era, is not dissimilar to that found in studies from sub-Saharan Africa (*31*) or Southeast Asia (*32*). The opportunities missed are costly and underscore the need for early and innovative approaches to reach immigrants who are at particular risk for both infections. Implementation research that improves understanding of barriers to HAART as provided through key existing programs, such as the AIDS Drug Assistance Program and the Ryan White HIV/AIDS Program, is needed.

Although contrary to findings in much of the published literature from regions of high TB incidence, the higher prevalence of sputum smear positivity among HIV-infected patients is consistent with that found in prior studies from the United States (*33*). The prevalence of smear positivity is a function of multiple factors that affect the denominator (e.g., completeness of case reporting, reference standard testing) and numerator (e.g., quality of laboratory services, including staff workload and smear microscopy methods),

and a combination of these factors probably contribute to differences in sputum smear positivity noted in areas of high versus low TB incidence.

Deaths associated with TB–HIV have declined remarkably in California since the pre-HAART era, consistent with national trends (*7*). Yet, although postmortem diagnoses of TB decreased significantly among patients with TB and with TB–HIV, patients with TB–HIV remain more than twice as likely to die during anti-TB treatment in the late HAART era as patients without known HIV co-infection. Women with HIV infection in the United States and other high-income areas have lagged behind men with respect to declines in mortality during the HAART era (*34*).

Our study has some potential limitations. First, TB–HIV cases may be underreported because TB patients without matches in the state HIV/AIDS registry were classified as HIV-negative. Persons who did not undergo HIV testing or whose providers did not adhere to the CDC AIDS case definition at TB diagnosis might not have been reported. However, assuming HIV awareness and testing have increased over time (*35*), misclassification of TB–HIV cases has declined in a time-dependent fashion. Moreover, during 1996–2006, the sensitivity of our case match procedure for capturing AIDS cases was ≈98% (95% CI 97.3%–98.7%) (*36*), and since confidential name-based HIV reporting began in 2006, <5% of TB–HIV cases were not also reported as AIDS cases (data not shown). Second, because antiretroviral therapy was unavailable in California Department of Public Health HIV/AIDS surveillance data, risk stratification according to HAART was not possible. Third, HIV prevalence estimates are a function of multiple parameters, some of which (race-stratified HAART-coverage, HIV incubation period, and migration patterns) carry considerable uncertainty. However, our estimates were subjected to multiple sensitivity analyses and are broadly consistent with extended back-calculation procedures undertaken by CDC (*26*). Applying national HIV rates to California would yield slightly higher HIV prevalence estimates. Last, trends in TB–HIV in California may not be generalizable to the United States as a whole.

In California, declines in TB–HIV incidence and death in the HAART era have been accompanied by a demographic shift toward foreign-born persons, particularly from Mexico and Central America. The opportunities for preventing TB and AIDS among foreign-born persons are underappreciated. Documentation of HIV status for TB patients in California (66% in 2008) remains below the national average (80%) (*37*). Screening and treatment completion rates for latent TB infection also are suboptimal despite the national standard and federal benefit (through the Health Resources and Services Administration) covering this practice (*38*). Improvements are hoped for with the recent availability of shorter course latent TB infection regimens (*39*). Progress toward comprehensive TB–HIV surveillance and recent lifting of legal barriers to HIV reporting at TB diagnosis are further cause for encouragement (*40*). TB and HIV programs must collaborate to monitor the confluence and changing epidemiology to ensure early detection of HIV and TB and to avert preventable deaths.

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Attribution of Foodborne Illnesses, Hospitalizations, and Deaths to Food Commodities by using Outbreak Data, United States, 1998–2008

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Each year, >9 million foodborne illnesses are estimated to be caused by major pathogens acquired in the United States. Preventing these illnesses is challenging because resources are limited and linking individual illnesses to a particular food is rarely possible except during an outbreak. We developed a method of attributing illnesses to food commodities that uses data from outbreaks associated with both simple and complex foods. Using data from outbreak-associated illnesses for 1998–2008, we estimated annual US foodborne illnesses, hospitalizations, and deaths attributable to each of 17 food commodities. We attributed 46% of illnesses to produce and found that more deaths were attributed to poultry than to any other commodity. To the extent that these estimates reflect the commodities causing all foodborne illness, they indicate that efforts are particularly needed to prevent contamination of produce and poultry. Methods to incorporate data from other sources are needed to improve attribution estimates for some commodities and agents.

Despite advances in food safety, foodborne illness remains common in the United States; >9 million persons each year have a foodborne illness caused by a major pathogen (*1*). One challenge in preventing foodborne illness is determining how to prioritize limited food safety resources across a large number of foods (*2*). Furthermore, attributing all illnesses to specific foods is challenging because most agents are transmitted through a variety of foods, and linking an illness to a particular food is rarely possible except during an outbreak.

To help determine priorities for food safety efforts, we organized the large number of foods implicated in outbreaks in the United States into 17 mutually exclusive food commodities. Here, we provide estimates of the number of domestically acquired foodborne illnesses, hospitalizations, and deaths attributable to these commodities.

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Methods

Data Sources

State and local health departments report foodborne disease outbreaks to the Centers for Disease Control and Prevention (CDC) through the Foodborne Disease Outbreak Surveillance System (*3*). Reports include, when available, number of persons ill, outbreak etiology, description of the implicated food vehicle(s), lists of ingredients, and identification of the contaminated ingredient(s). We reviewed all outbreaks from 1998, the first year with detailed information on ingredients, through 2008 that were reported to the CDC by October 2010. For this analysis, we included all outbreaks with an implicated food vehicle and a single etiologic agent.

Health officials may report whether an etiologic agent was confirmed or suspected on the basis of published criteria (*4*,*5*) and the method of confirmation. Reports may include ≥1 of 5 reasons for implicating a food vehicle: 1) statistical evidence from an epidemiologic investigation; 2) laboratory evidence identifying the etiologic agent in the implicated food; 3) compelling or other supportive data; 4) previous experience suggesting that the food vehicle is the source; and 5) other data, such as identification of the same etiologic subtype on the farm that supplied the implicated food. We considered an implicated food confirmed when 1 of the first 2 reasons was reported. Other implicated food vehicles were considered suspect.

To determine whether to analyze outbreaks with suspect foods, we reviewed a convenience sample of 117 outbreak reports for which the reason for implication was not reported. Supporting evidence implicated the food vehicle

for 65% of these reports. Some of these outbreaks involved too few persons to conduct an epidemiologic investigation; in most, no food was tested. Outbreaks with suspect vehicles constituted a large proportion of the dataset, but it was not possible to locate and review the documentation for all investigations. However, because a large percentage of documentation reviewed had reasonable evidence to implicate the reported food, we included all outbreaks with suspect foods in the analysis.

During 1998–2008, a total of 13,352 foodborne disease outbreaks, causing 271,974 illnesses, were reported in the United States (online Technical Appendix 1 Table 1, wwwnc.cdc.gov/EID/article/19/3/11-1866-Techapp1.pdf). Of those outbreaks, 4,887 (37%), causing 128,269 (47%) illnesses, had an implicated food vehicle and a single etiology; 298 of those outbreaks were excluded because information about the vehicle was insufficient to categorize the ingredients. We also did not include the 3% of outbreaks that had multiple etiologies reported.

To assess possible bias when including outbreaks with a suspected vehicle or etiology in our estimates, we compared the rank order of each of the 17 food commodities in our model based on the total number of associated illnesses with the rank order when including only those illnesses with a confirmed etiology and vehicle. The order of the top 8 commodities associated with the highest number of illnesses changed only slightly (ranks 5 and 6 switched); therefore, we included all outbreaks to maximize the data available for the lower-ranking commodities.

The estimated number of domestically acquired illnesses, hospitalizations, and deaths for each etiology was obtained from published estimates (*1*) or, when not available, by extrapolating from available data. To highlight differences in sources for nontyphoidal *Salmonella* spp. serotypes, we made estimates for those most frequently isolated from humans (i.e., Enteritidis, Heidelberg, Javiana, Newport, Typhimurium) and, separately, for all others. We estimated the number of illnesses, hospitalization, and deaths by multiplying the numbers for nontyphoidal *Salmonella* spp. (*1*) by the proportion of all serotyped human *Salmonella* isolates reported during 1998–2008 (*6*).

The outbreak dataset included outbreaks with chemical etiologies and those caused by *Anisakis simplex*, for which published illness estimates were not available. For these, the number of illnesses was estimated as the product of the mean annual number of illnesses reported to CDC through outbreak surveillance during 1998–2008 by using the same multipliers for underdiagnosis (×25), underreporting (×30), case-hospitalization rate (×0.006), and case-fatality rate (×0.0004) as for infection with *Clostridium perfringens*, a short-duration illness (*1*).

We attempted to attribute food commodities for an estimated 9,638,301 illnesses, 57,462 hospitalizations, and 1,451 deaths caused by known agents (online Technical Appendix 1 Table 2). We did not attribute illnesses to commodities for illnesses caused by astrovirus, *Mycobacterium bovis*, *Toxoplasma gondii*, and *Vibrio vulnificus* because no outbreaks were reported for these pathogens. These pathogens caused an estimated 1.1% of illnesses, 8.1% of hospitalizations, and 25.2% of deaths (a high number of deaths were estimated to be caused by toxoplasmosis [*1*]).

Food Categorization

We defined 3 commodities for aquatic animals (fish, crustaceans, and mollusks), 6 for land animals (dairy, eggs, beef, game, pork, and poultry), and 8 for plants (grains-beans; oils-sugars [refined plant foods]; fruits-nuts; fungi; and leafy, root, sprout, and vine-stalk vegetables) (*7*). Foods were categorized into ≥1 of 17 mutually exclusive commodities according to ingredients listed in outbreak reports, or, when ingredients were not listed, in recipes found on the Internet (*7*). In some analyses, we grouped commodities (Figure 1).

We defined as simple an implicated food vehicle that contained ingredients from 1 commodity, such as apple juice (fruits-nuts commodity). This category included foods such as fruit salad that were composed of several ingredients from the same commodity. We defined as complex an implicated food vehicle that contained ingredients from ≥1 commodity, such as apple pie (made of ingredients from several commodities: fruits-nuts [apples],

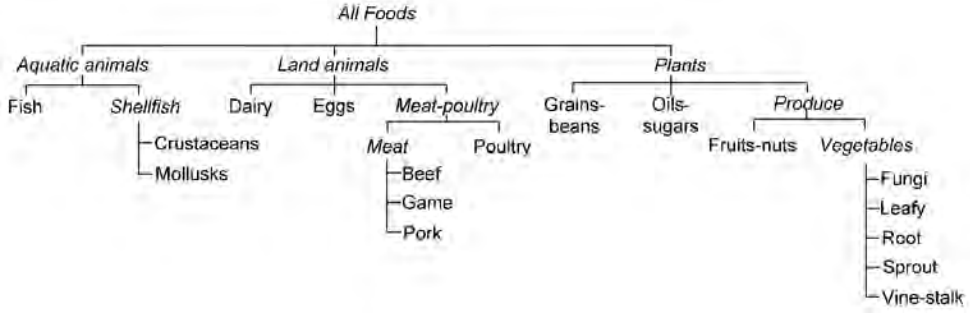

Figure 1. Hierarchy of food commodities. *Italics* indicate commodity groups.

grains-beans [flour], oils-sugars [sugar], and dairy [butter]). We excluded water as an ingredient.

Estimation Method

We calculated for each etiology the proportion of outbreak-associated illnesses transmitted by each commodity. We allocated illnesses from simple food outbreaks of a given etiology to their single implicated commodities. For each complex food outbreak, we partitioned the associated illnesses to the multiple implicated commodities in proportion to the relative numbers of illnesses in all simple food outbreaks that implicated those specific commodities; we then added the results from all outbreaks to obtain commodity illness percentages. We then applied the commodity-specific percentage of ill persons to the total estimated proportion of domestically acquired illnesses, hospitalizations, and deaths for each etiology (*1*). Last, we added the total proportions of commodity-specific illnesses, hospitalizations, and deaths for simple and complex foods for all etiologies. We considered these the most probable estimates for each commodity (online Technical Appendix 2, wwwnc.cdc.gov/EID/article/19/3/11-1866-Techapp2.pdf).

To provide a range for the most probable estimates, we determined a minimum estimate by attributing illnesses to commodities implicated only in outbreaks where illness was transmitted by simple foods and a maximum estimate by including complex food outbreaks and attributing the outbreak illnesses to each ingredient in the implicated food (online Technical Appendix 1 Table 3). Thus, all illnesses in a complex food outbreak with 3 ingredient commodities were included 3 times, once for each commodity. The numbers provided in the Results section are the most probable estimate, unless stated otherwise. Calculations were performed in SAS version 9.3 (SAS Institute, Cary, NC, USA).

Results

The final dataset consisted of 4,589 outbreaks with an implicated food vehicle and a single etiologic agent (online Technical Appendix 3, wwwnc.cdc.gov/EID/article/19/3/11-1866-Techapp3.xlsx; online Technical Appendix 1 Table 1); a total of 120,321 outbreak-associated illnesses were caused by 36 agents (online Technical Appendix 1 Table 2). Norovirus caused the most outbreaks (1,419) and outbreak-associated illnesses (41,257), far above the median for all agents (29 outbreaks, 1,208 illnesses). No outbreaks were caused by *Mycobacterium bovis*, *Vibrio vulnificus,* astrovirus, or *Toxoplasma gondii.* The implicated food vehicle was complex for 2,239 (49%) outbreaks (online Technical Appendix 1 Table 2); the median number of commodities for complex food vehicles was 4 (range 2–13).

We applied percentages derived from outbreak-associated illnesses for each etiology to the 9.6 million estimated annual illnesses assessed and attributed ≈4.9 million (≈51%) to plant commodities, ≈4.0 million (≈42%) to land animal commodities, and ≈600,000 (≈6%) to aquatic animal commodities (Table 1). Produce commodities (fruits-nuts and the 5 vegetable commodities) accounted for 46% of illnesses; meat-poultry commodities (beef, game, pork, and poultry) accounted for 22%. Among the 17 commodities, more illnesses were associated with leafy vegetables (2.2 million [22%]) than any other commodity. The high estimate for illnesses attributable to leafy vegetables was many times higher than the low estimate (Figure 2, panel A), which indicates that leafy vegetables were frequently found in complex foods. After leafy vegetables, the commodities linked to the most illnesses were dairy (1.3 million [14%]), fruits-nuts (1.1 million [12%]), and poultry (900,000 [10%]). Norovirus comprised 57% of all illnesses.

An estimated 26,000 (46%) annual hospitalizations were attributed to land animal commodities, 24,000 (41%) to plant commodities, and 3,000 (6%) to aquatic animal commodities (Table 2). Produce commodities accounted for 38% of hospitalizations and meat-poultry commodities for 22%. Dairy accounted for the most hospitalizations (16%), followed by leafy vegetables (14%), poultry (12%), and vine-stalk vegetables (10%) (Figure 2, panel B). Among the estimated 57,000 hospitalizations, 8% were not attributed to a pathogen, mainly because the dataset did not include data for *Toxoplasma* spp.

An estimated 629 (43%) deaths each year were attributed to land animal, 363 (25%) to plant, and 94 (6%) to aquatic commodities (Table 3). Meat-poultry commodities accounted for 29% of deaths and produce 23%. Among the 17 commodities, poultry accounted for the most deaths (19%), followed by dairy (10%), vine-stalk vegetables (7%), fruits-nuts (6%), and leafy vegetables (6%) (Figure 2, panel C). Of the 278 deaths attributed to poultry, most were attributed to *Listeria monocytogenes* (63%) or *Salmonella* spp. (26%). Among the 1,451 estimated deaths, 25% were not attributed to a pathogen, mainly because the dataset did not include data for *Toxoplasma* spp.

Most bacterial illnesses were attributed to dairy (18%), poultry (18%), and beef (13%) commodities (Table 1). Most chemical illnesses were attributed to fish (60%, most caused by the marine biotoxin ciguatoxin). Most parasitic illnesses were attributed to mollusks (33%) and fruits-nuts (26%); this reflects the fact that 1 simple food outbreak was caused by *Giardia intestinalis* (mollusks) and 1 by *Cryptosporidium* spp. (fruits-nuts). Most viral illnesses were attributed to leafy vegetables (35%), fruits-nuts (15%), and dairy (12%). Of the 20 outbreaks associated with simple foods and caused by norovirus transmitted by dairy, 14 (70%) were transmitted by cheese products.

The plant commodity group accounted for 66% of viral, 32% of bacterial, 25% of chemical, and 30% of

Table 1. Estimates of annual domestically acquired foodborne illnesses attributed to specific food commodities and commodity groups, by pathogen type, United States, 1998–2008*

Commodity or commodity group	No. (%) illnesses				
	All agents	Bacterial	Chemical	Parasitic	Viral
Aquatic animals†	589,310 (6.1)	142,415 (3.9)	153,488 (61.6)	77,795 (33.3)	215,613 (3.9)
Fish	258,314 (2.7)	15,362 (0.4)	148,958 (59.8)	955 (0.4)	93,040 (1.7)
Shellfish†	330,997 (3.4)	127,053 (3.5)	4,531 (1.8)	76,840 (32.9)	122,573 (2.2)
Crustaceans	46,528 (0.5)	32,626 (0.9)	1,247 (0.5)		12,654 (0.2)
Mollusks	284,469 (3.0)	94,427 (2.6)	3,283 (1.3)	76,840 (32.9)	109,919 (2.0)
Land animals†	4,021,839 (41.7)	2,334,000 (64.0)	33,031 (13.3)	156 (0.1)	1,654,651 (30.0)
Dairy	1,330,098 (13.8)	656,951 (18.0)	3,773 (1.5)		669,374 (12.1)
Eggs	574,298 (6.0)	179,421 (4.9)	6,995 (2.8)		387,882 (7.0)
Meat-poultry†	2,117,442 (22.0)	1,497,628 (41.1)	22,263 (8.9)	156 (0.1)	597,394 (10.8)
Meat†	1,174,257 (12.2)	844,006 (23.2)	2,437 (1.0)	156 (0.1)	327,658 (5.9)
Beef	639,640 (6.6)	482,199 (13.2)	661 (0.3)		156,780 (2.8)
Game	9,934 (0.1)	5,111 (0.1)	1,568 (0.6)	156 (0.1)	3,100 (0.1)
Pork	524,684 (5.4)	356,697 (9.8)	209 (0.1)		167,778 (3.0)
Poultry	943,185 (9.8)	653,622 (17.9)	19,826 (8.0)		269,737 (4.9)
Plants†	4,924,877 (51.1)	1,169,202 (32.1)	62,753 (25.2)	69,023 (29.5)	3,623,899 (65.8)
Grains-beans	435,936 (4.5)	183,394 (5.0)	12,995 (5.2)		239,547 (4.3)
Oils-sugars	65,631 (0.7)		2,344 (0.9)		63,287 (1.1)
Produce†	4,423,310 (45.9)	985,807 (27.0)	47,414 (19.0)	69,023 (29.5)	3,321,066 (60.3)
Fruits-nuts	1,123,808 (11.7)	230,636 (6.3)	29,483 (11.8)	60,573 (25.9)	803,116 (14.6)
Vegetables†	3,299,501 (34.2)	755,171 (20.7)	17,931 (7.2)	8,450 (3.6)	2,517,949 (45.7)
Fungi	4,542 (0.0)	686 (0.0)	3,857 (1.5)		
Leafy	2,152,652 (22.3)	188,327 (5.2)	9,113 (3.7)	7,256 (3.1)	1,947,955 (35.4)
Root	349,715 (3.6)	96,910 (2.7)	1,240 (0.5)		251,566 (4.6)
Sprout	32,703 (0.3)	32,703 (0.9)			
Vine-stalk	759,889 (7.9)	436,546 (12.0)	3,721 (1.5)	1,194 (0.5)	318,428 (5.8)
Undetermined	102,275 (1.1)	156 (0.0)		86,686 (37.1)	15,433 (0.3)
Total	9,638,301 (100.0)	3,645,773 (100.0)	249,273 (100.0)	233,660 (100.0)	5,509,596 (100.0)

*Most estimates from (*1*); some were made as described in Methods. Numbers of illnesses are the most probable estimate, as described in Methods. Estimates are rounded; some row and column sums may differ from their totals. Blank cells indicate no data.
†Indicates commodity group.

parasitic illnesses (Table 1). This group accounted for a greater proportion of illnesses than the land or aquatic animal commodity groups for *Bacillus cereus*; *Clostridium botulinum*; enterotoxigenic *Escherichia coli*; Shiga toxin–producing *Escherichia coli* (STEC) O157; non-O157 STEC; *Salmonella enterica* serotypes Javiana, Newport, and other (e.g., serotypes other than Javiana, Newport, Enteritidis, Heidelberg, Typhimurium, and Typhi); *Shigella* spp.; mycotoxins; other chemicals; *Cryptosporidium* spp.; *Cyclospora cayetansesis*; hepatitis A; norovirus; and sapovirus (Table 4, Appendix, wwwnc.cdc.gov/EID/article/19/3/11-1866-T4.htm). The land animal group accounted for the highest proportion of illnesses for *Campylobacter* spp., *Clostridium perfringens*, *Listeria* spp., *Salmonella* serotypes Enteritidis and Heidelberg, *Streptococcus* spp. group A, *Yersinia enterocolitica*, and *Trichinella* spp.

Discussion

We developed a method to attribute domestically acquired foodborne illnesses, hospitalizations, and deaths in the United States to specific commodities by using outbreak data. We found most illnesses were attributed to plant commodities and most deaths to land animal commodities. We attributed 46% of illnesses to produce; the large number of norovirus illnesses was a major driver of this result. More deaths were attributed to poultry than to any other commodity. To the extent that these outbreak-based estimates reflect the commodities associated with all foodborne illness, they indicate that efforts are particularly needed to prevent contamination of produce and poultry.

More illnesses were attributed to leafy vegetables (22%) than to any other commodity; illnesses associated with leafy vegetables were the second most frequent cause of hospitalizations (14%) and the fifth most frequent cause of death (6%). Previous studies have shown that produce-containing foods were the food source for approximately half of norovirus outbreaks with an identified simple food vehicle during 2001–2008 (*8*) and the second most frequent food source for *E. coli* O157 outbreaks during 1982–2002 (*9*). Outbreaks of *E. coli* O157 infections transmitted by spinach (*10*) and lettuce (*11*) and *Salmonella* spp. infections transmitted by tomatoes (*12*,*13*), juice (*14*,*15*), mangoes (*16*), sprouts (*17*,*18*), and peppers (*19*,*20*) underline concerns about contamination of produce consumed raw.

More deaths were attributed to poultry (19%) than to any other commodity, and most poultry-associated deaths were caused by *Listeria* or *Salmonella* spp. From 1998 through 2002, three large listeriosis outbreaks were linked to turkey delicatessen meat contaminated in the processing

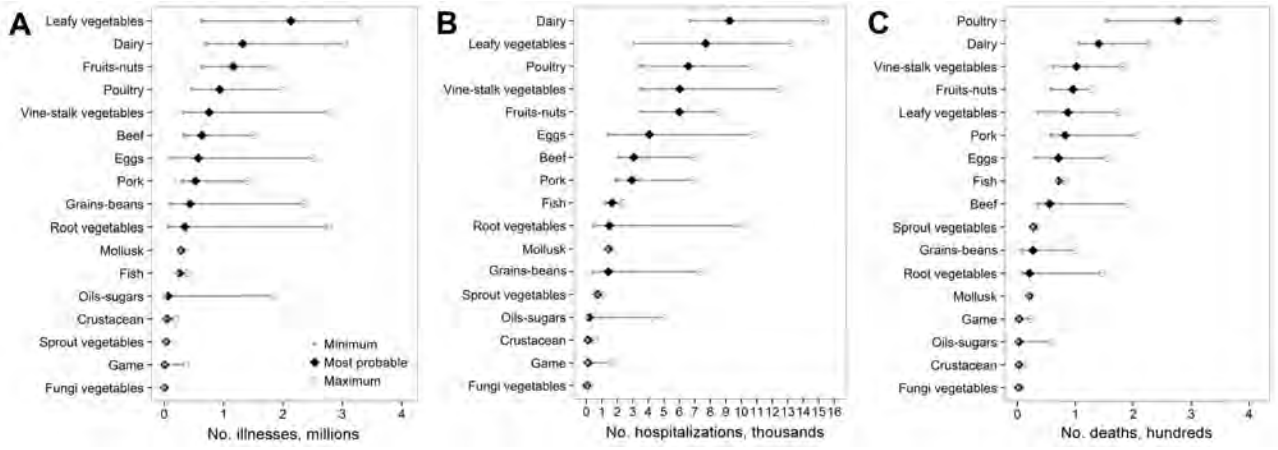

Figure 2. Minimum, most probable, and maximum estimates of the annual number of foodborne illnesses, hospitalizations, and deaths from all etiologies attributed to food commodities, United States, 1998–2008. A) Foodborne illnesses; 102,275 (1.1%) illnesses were not attributed to a commodity and are not shown. B) Foodborne illness–associated hospitalizations; 4,639 (8.1%) hospitalizations were not attributed to a commodity and are not shown. C) Foodborne illness–associated deaths; 366 (25.2%) deaths were not attributed to a commodity and are not shown. Minimum and maximum values represent extreme boundaries for the most probable estimate; they are not the SE of the most probable estimate. For commodities with outbreaks associated with only simple food vehicles, the minimum, maximum, and most probable estimate are the same. For commodities with outbreaks associated with both simple and complex foods, the minimum and maximum estimates reflect the different weighting given to outbreaks associated with complex foods relative to simple. When the most probable estimate for a commodity is close to the minimum estimate, most illnesses from outbreaks associated with complex foods were attributed to another commodity in the food implicated in the outbreak; when the most probable estimate for a commodity is close to the maximum estimate, most illnesses from outbreaks associated with complex foods were attributed to that commodity.

plant after cooking (*21*–*23*). A risk-ranking model for listeriosis among ready-to-eat foods identified delicatessen meat as the highest risk food (*24*).

The dairy commodity was the second most frequent food source for infections causing illnesses (14%) and deaths (10%). Foods in this commodity are typically consumed after pasteurization, which eliminates pathogens, but improper pasteurization and incidents of contamination after pasteurization occur (*25*). In our dataset, norovirus outbreaks associated with cheese illustrate the role of contamination of dairy products after pasteurization by food handlers. Because of the large volume of dairy products consumed, even infrequent contamination of commercially distributed products can result in many illnesses (*26*). The prominence of dairy in our model reflects a relatively high number of reported outbreaks associated with raw milk compared with the quantity of raw milk consumed (*27*) and issues related to *Campylobacter* spp. infection (discussed below); these factors likely resulted in an overestimation of illnesses attributed to dairy. Models that partition raw versus pasteurized milk and that incorporate other data sources for *Campylobacter* spp. infection could improve estimates of illnesses related to dairy.

Our method of attributing illnesses incorporated data from outbreaks associated with complex foods and attributed most of the estimated number of illnesses caused by known pathogens to specific food sources. Other methods for attributing illnesses to food sources may be applied to various stages of the food distribution chain and therefore may yield different but complementary estimates (*2*). A method for *Salmonella* spp. attribution used in Denmark compared isolates from food animal reservoirs with human isolates to attribute infections to the reservoirs, the live animals (*28*). A similar method in a US study attributed *Salmonella* spp.–associated foodborne illnesses to the point of processing (*29*). Risk assessment models have focused primarily on the point of processing; case studies of sporadic illness, expert elicitation, and analysis of outbreak data represent attribution at the point of consumption. Outbreak investigations have been reported for most foodborne etiologies and food commodities and provide the most comprehensive data for attribution.

We made several assumptions. We assumed that using the number of outbreak-associated illnesses rather than number of outbreaks would enable better assignment of illnesses to commodities. Our choice had the potential to bias the results toward large outbreaks. However, large outbreaks often represent system failures that have resulted in smaller, undetected outbreaks; investigation may determine the source for illnesses that otherwise might have been considered sporadic. Small outbreaks may better represent sources of sporadic illnesses, but because many small outbreaks are not detected or investigated, their sources would not be well represented by any method. Similar studies have used outbreak counts (*30*,*31*); either choice (number of outbreak-associated illnesses or number of outbreaks)

Table 2. Estimates of annual hospitalizations for domestically acquired foodborne illnesses attributed to specific food commodities and commodity groups, by pathogen type, United States, 1998–2008*

Commodity or commodity group	No. (%) hospitalizations				
	All agents	Bacterial	Chemical	Parasitic	Viral
Aquatic animals†	3,199 (5.6)	1,158 (3.2)	921 (61.6)	231 (4.7)	889 (5.8)
Fish	1,661 (2.9)	210 (0.6)	894 (59.8)	6 (0.1)	551 (3.6)
Shellfish†	1,538 (2.7)	948 (2.6)	27 (1.8)	225 (4.6)	338 (2.2)
Crustaceans	117 (0.2)	75 (0.2)	7 (0.5)		34 (0.2)
Mollusks	1,421 (2.5)	873 (2.4)	20 (1.3)	225 (4.6)	303 (2.0)
Land animals†	26,118 (45.5)	21,471 (60.0)	198 (13.3)	6 (0.1)	4,443 (29.1)
Dairy	9,284 (16.2)	7,464 (20.9)	23 (1.5)		1,798 (11.8)
Eggs	4,062 (7.1)	2,979 (8.3)	42 (2.8)		1,041 (6.8)
Meat-poultry†	12,772 (22.2)	11,029 (30.8)	134 (8.9)	6 (0.1)	1,604 (10.5)
Meat†	6,138 (10.7)	5,238 (14.6)	15 (1.0)	6 (0.1)	880 (5.8)
Beef	3,075 (5.4)	2,650 (7.4)	4 (0.3)		421 (2.8)
Game	117 (0.2)	94 (0.3)	9 (0.6)	6 (0.1)	8 (0.1)
Pork	2,946 (5.1)	2,494 (7.0)	1 (0.1)		450 (2.9)
Poultry	6,634 (11.5)	5,791 (16.2)	119 (8.0)		724 (4.7)
Plants†	23,506 (40.9)	13,043 (36.4)	377 (25.2)	221 (4.5)	9,865 (64.5)
Grains-beans	1,437 (2.5)	695 (1.9)	78 (5.2)		664 (4.3)
Oils-sugars	184 (0.3)		14 (0.9)		170 (1.1)
Produce†	21,885 (38.1)	12,349 (34.5)	284 (19.0)	221 (4.5)	9,031 (59.1)
Fruits-nuts	5,829 (10.1)	3,279 (9.2)	177 (11.8)	213 (4.4)	2,160 (14.1)
Vegetables†	16,057 (27.9)	9,070 (25.3)	108 (7.2)	8 (0.2)	6,871 (45.0)
Fungi	37 (0.1)	14 (0.0)	23 (1.5)		
Leafy	7,769 (13.5)	2,393 (6.7)	55 (3.7)	7 (0.1)	5,314 (34.8)
Root	1,501 (2.6)	793 (2.2)	7 (0.5)		700 (4.6)
Sprout	713 (1.2)	713 (2.0)			
Vine-stalk	6,038 (10.5)	5,157 (14.4)	22 (1.5)	1 (0.0)	857 (5.6)
Undetermined	4,639 (8.1)	124 (0.3)		4,428 (90.6)	87 (0.6)
Total	57,462 (100.0)	35,797 (100.0)	1,496 (100.0)	4,886 (100.0)	15,284 (100.0)

*Most estimates from (*1*); some were made as described in Methods. Numbers of hospitalizations are the most probable estimate, as described in Methods. Estimates are rounded; some row and column sums may differ from their totals. Blank cells indicate no data.
†Indicates commodity group.

results in biases (*32*). Because of other methodological differences, direct comparison of the results for these studies is difficult. To assess the effect of outbreak size on our estimates, we adjusted our model to give no weight to outbreak size (online Technical Appendix 1 Tables 4, 5); the rank order of commodities by number of attributed illnesses changed by no more than 1 for most commodities. The largest outbreak in our study was 1,644 *Campylobacter* spp.–associated illnesses resulting from the consumption of pasteurized milk; even so, counting outbreaks instead of illnesses resulted in a relatively small (2.6%) reduction in the percentage of illnesses attributed to dairy.

We further assumed outbreak illnesses represented all illnesses and weighted the results for each agent by number of all foodborne illnesses attributed to each agent (*1*). Unweighted outbreak data may be biased toward seafood outbreaks caused by marine biotoxins (e.g., scombroid) that are frequently reported but cause relatively few illnesses. For some agents, foods implicated in outbreaks might not well represent foods responsible for sporadic illnesses. For example, outbreak data underrepresent poultry (8%) and overrepresent dairy (67%) as sources of *Campylobacter* spp. infection; studies of sporadic infections implicate consumption of poultry but not dairy as a major risk factor (*33*). *Campylobacter* spp. are estimated to be the third most common bacterial cause of foodborne illness, but relatively few outbreaks are detected (*1*). For pathogens for which outbreaks are uncommon or do not reflect major modes of transmission, methods that incorporate data from nonoutbreak sources are needed.

We also assumed that, for a given agent, when an outbreak was associated with a complex food, the likelihood that any commodity was the source was proportional to the frequency of illnesses for outbreaks associated with simple foods associated with that commodity. However, when the number of outbreaks associated with simple foods for an etiology is small compared with the number associated with complex foods, the result may be biased toward commodities for which simple foods were vehicles for outbreaks. Other attribution estimates that used outbreak surveillance data have excluded complex foods or have not partitioned them into component commodities (*9*,*34*). Were complex food outbreaks excluded, the result for each commodity would be the same as our minimum estimate. However, inclusion of outbreaks associated with complex foods provides important information. For example, in a review of egg-associated *S. enterica* serotype Enteritidis outbreaks (*35*), eggs were implicated as simple food vehicles in 20% of the outbreaks, but complex foods containing eggs were implicated in an additional 57% of the outbreaks.

Table 3. Estimates of annual deaths resulting from domestically acquired foodborne illnesses attributed to specific food commodities and commodity groups, by pathogen type, United States, 1998–2008*

Commodity or commodity group	No. (%) deaths				
	All agents	Bacterial	Chemical	Parasitic	Viral
Aquatic animals†	94 (6.4)	24 (2.8)	61 (61.6)	2 (0.7)	6 (3.7)
Fish	71 (4.9)	8 (1.0)	60 (59.8)	0 (0.1)	2 (1.4)
Shellfish†	23 (1.6)	16 (1.8)	2 (1.8)	2 (0.6)	4 (2.3)
Crustaceans	3 (0.2)	2 (0.2)	0 (0.5)		0 (0.2)
Mollusks	20 (1.4)	14 (1.6)	1 (1.3)	2 (0.6)	3 (2.1)
Land animals†	629 (43.3)	570 (66.2)	13 (13.3)	0	45 (29.0)
Dairy	140 (9.7)	121 (14.0)	2 (1.5)		18 (11.8)
Eggs	71 (4.9)	57 (6.6)	3 (2.8)		11 (6.8)
Meat-poultry†	418 (28.8)	393 (45.5)	9 (8.9)	0	16 (10.4)
Meat†	140 (9.7)	130 (15.1)	1 (1.0)	0	9 (5.7)
Beef	55 (3.8)	51 (5.9)	0 (0.3)		4 (2.7)
Game	3 (0.2)	2 (0.2)	1 (0.6)	0	0 (0.1)
Pork	82 (5.7)	77 (9.0)	0 (0.1)		5 (2.9)
Poultry	278 (19.1)	262 (30.4)	8 (8.0)		7 (4.7)
Plants†	363 (25.0)	229 (26.5)	25 (25.2)	4 (1.2)	105 (67.4)
Grains-beans	27 (1.9)	16 (1.8)	5 (5.2)		6 (4.1)
Oils-sugars	3 (0.2)		1 (0.9)		2 (1.1)
Produce†	333 (22.9)	213 (24.7)	19 (19.0)	4 (1.2)	97 (62.2)
Fruits-nuts	93 (6.4)	55 (6.4)	12 (11.8)	4 (1.2)	22 (14.2)
Vegetables†	240 (16.5)	158 (18.3)	7 (7.2)	0	75 (48.0)
Fungi	2 (0.1)	0	2 (1.5)		
Leafy	88 (6.0)	27 (3.1)	4 (3.7)	0	57 (36.7)
Root	21 (1.4)	12 (1.4)	0 (0.5)		9 (5.6)
Sprout	27 (1.9)	27 (3.2)			
Vine-stalk	102 (7.0)	92 (10.6)	1 (1.5)	0	9 (5.7)
Undetermined	366 (25.2)	39 (4.5)		327 (98.1)	0
Total	1,451 (100.0)	862 (100.0)	100 (100.0)	333 (100.0)	156 (100.0)

*Most estimates from (*1*); some were made as described in Methods. Numbers of deaths are the most probable estimate, as described in Methods. Estimates are rounded; some row and column sums may differ from their totals. Blank cells indicate no data.
†Indicates commodity group.

A limitation of our study is the absence of outbreaks caused by some agents. None caused by *Toxoplasma* spp. or *Vibrio vulnificus* were reported. The attributable risk for *Toxoplasma* infection is highest for meat (49%) and mollusks (16%) (*36*); most foodborne *V. vulnificus* infections are linked to oysters (*37*). The effect of this absence of data for agents that are uncommon but often cause fatal illnesses is reflected mostly in the number of deaths in our study, 25% of which were not attributed. Attributing an additional 49% of *Toxoplasma* spp.–associated deaths to meats would make meats a more frequent source of foodborne illness–associated deaths than poultry. Attributing all foodborne deaths caused by *V. vulnificus* and 16% of those caused by *Toxoplasma* spp. to mollusks would move this commodity from the thirteenth to the fourth most frequent source of foodborne illness–associated deaths.

Other limitations of our study included the choice not to use the credible interval for the estimated number of illnesses, hospitalization, and deaths (*1*); the lack of published estimates for the number of illnesses caused by chemical etiologies; and the fact that the quality of outbreak data is dependent on the quality and quantity of investigations reported. We maximized the amount of data we compiled by including outbreaks with suspect etiologies or vehicles and developing a method to incorporate data from outbreaks attributed to both simple and complex foods; even so, our study yielded a paucity of data for some agents. Among the agents associated with <10 outbreaks in the dataset, only 1 (non-O157 STEC) is estimated to cause >1% of foodborne illnesses caused by known agents (*1*). Our estimates should be considered an approximation, to be refined by further research and analyses. To improve the quality and accuracy of outbreak attribution, models can be developed that include other types of data (e.g., studies of sporadic cases, isolates from foods and animals, agent subtypes). Measurements that indicate the substantial uncertainty of many of the estimates are particularly critical for agents causing few outbreaks and those for which the major sources for outbreaks are dissimilar to those for sporadic cases. Ultimately, the best data sources and methods for estimating the number of illnesses, hospitalizations, and deaths attributable to each food commodity may vary by etiologic agent, commodity, point of food chain analyzed, and other factors.

For consistency and to obtain sufficient data, we chose to use all years of data for all pathogens, but a shorter, more recent period is desirable when major implicated commodities have changed. For example, outbreaks of *Listeria* spp. infection caused by contamination of ready-to-eat meats markedly decreased after 2002

(*38*). However, using data from only the few listeriosis outbreaks that occurred after 2002 would result in a few commodities having a large effect on results. Developing methods to examine trends should be a high priority. When combined with updated estimates of the number of illnesses, attribution analyses performed at appropriate intervals could help determine the results of prevention efforts. Longer intervals would increase data for agents with few outbreaks, but if the frequency of illness attributed to a commodity changes substantially, results might not reflect the current situation.

In summary, our outbreak-based method attributed most foodborne illnesses to food commodities that constitute a major portion of the US diet. When food commodities are consumed frequently, even those with a low risk for pathogen transmission per serving may result in a high number of illnesses. The attribution of foodborne-associated illnesses and deaths to specific commodities is useful for prioritizing public health activities; however, additional data on the specific food consumed is needed to assess per-serving risk. The risk for foodborne illness is just one part of the risk–benefit equation for foods; other factors, such as the health benefits of consuming a diet high in fruits and vegetables, must also be considered (*39*).

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Dr Painter is an epidemiologist with the Centers for Disease Control and Prevention who worked for the Division of Foodborne, Waterborne, and Environmental Diseases while most of the work for this study was completed. Currently, he is the epidemiology team lead for the Immigrant, Refugee, and Migrant Health Branch of the Division of Global Migration and Quarantine. His research interests include outbreak investigations and the epidemiology of tuberculosis among foreign-born persons.

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Treatment Outcomes for Extensively Drug-Resistant Tuberculosis and HIV Co-infection

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High mortality rates have been reported for patients co-infected with extensively drug-resistant tuberculosis (XDR-TB) and HIV, but treatment outcomes have not been reported. We report treatment outcomes for adult XDR TB patients in KwaZulu-Natal Province, South Africa. Initial data were obtained retrospectively, and outcomes were obtained prospectively during 24 months of treatment. A total of 114 XDR TB patients were treated (median 6 drugs, range 3–9 drugs); 82 (73%) were HIV positive and 50 (61%) were receiving antiretroviral therapy. After receiving treatment for 24 months, 48 (42%) of 114 patients died, 25 (22%) were cured or successfully completed treatment, 19 (17%) withdrew from the study, and 22 (19%) showed treatment failure. A higher number of deaths occurred among HIV-positive patients not receiving antiretroviral therapy and among patients who did not show sputum culture conversion. Culture conversion was a major predictor of survival but was poorly predictive (51%) of successful treatment outcome.

Drug-resistant tuberculosis (TB) is a critical threat to TB control and global public health (*1*–*3*). Nowhere is this threat more pressing than in South Africa, where drug-resistant TB and HIV have converged in a deadly syndemic defined by increased incidences of TB and HIV (*4*), endemic transmission of drug-resistant TB strains (*5*), high mortality rates (*6*), and poor treatment outcomes (*7*). The most drug-resistant form of TB, extensively drug-resistant tuberculosis (XDR TB) (*8*) has been reported in 70 countries and comprises an increasing proportion of drug-resistant TB cases (*1*).

The global epicenter of the XDR TB and HIV syndemic is KwaZulu-Natal Province in South Africa, where nearly 400 XDR TB patients, 70% co-infected with HIV, were admitted to a provincial TB referral hospital for initiation of therapy during 2003–2008 (*9*). To contextualize this incidence, 73% (573/782) of all XDR TB cases reported to the World Health Organization globally during 2002–2009 were from South Africa (*3*,*10*). It is estimated that 50% of patients with a diagnosis of XDR TB in KwaZulu-Natal Province do not survive to treatment referral (*11*). Therefore, hospital-based surveillance represents a major underestimate of cases of co-infection with XDR TB and HIV in the province.

Without adequate second-line TB and HIV treatment, reported mortality rates for persons co-infected with XDR TB and HIV approach 100% (*6*). Among XDR TB patients who survive to initiation of second-line TB therapy, early treatment outcomes reported by our group and others describe low rates of sputum culture conversion, major adverse events, and a substantial number of early deaths (*12*–*14*). To our knowledge there are no published reports of outcomes for patients co-infected with XDR TB and HIV at the end of TB treatment. This report describes treatment outcomes, adverse events, and risk factors for death among patients in South Africa with XDR TB, most of whom were co-infected with HIV.

Methods

Study Participants

Early culture conversion and mortality rate data for the first 12 months after initiation of treatment for

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XDR TB in the first 60 patients in this cohort have been reported (*12*). In brief, patients were adult (≥18 years of age) XDR TB patients consecutively admitted to a public TB referral hospital in KwaZulu-Natal Province in South Africa during December 1, 2006–October 31, 2007 for initiation of treatment for XDR TB. Patients with complications and drug-resistant TB are referred to this facility at the discretion of the treating physician and are admitted depending on patient acuity and bed availability. The practice during the study period was to admit all XDR TB patients for initiation of second-line TB treatment. Eligible participants had culture-proven TB and *Mycobacterium tuberculosis* infection, and drug susceptibility testing results meeting the revised World Health Organization criteria for XDR TB (*10*). In addition, patients agreed to begin appropriate second-line and third-line anti-TB treatment. All anti-TB treatment regimens were determined by treating physicians on the basis of drug susceptibility results and adverse drug reactions. All treatment was provided through the South African public health system and directly observed therapy was provided. However, we did not assess the quality of directly observed therapy support or adherence.

Study Design

Patients who met eligibility criteria were identified retrospectively, and information was collected by chart review and review of an electronic laboratory database. Information on demographics, risk factors and adverse drug reactions, and treatment outcomes were collected retrospectively. Treatment outcomes of enrolled patients were followed through December 31, 2009, to ensure that each patient had ≥24-months of follow-up. Standard drug-resistant TB treatment outcome definitions were used to define outcome (*15*). Treatment outcomes were cure, treatment completion, death, and treatment default (*15*).

All drug-resistant TB treatment outcomes were mutually exclusive and were defined at 24 months except in the case of treatment default and death, which were defined when they occurred. Cure was defined as treatment for 24 months and ≥5 consecutive negative culture results in the final 12 months of treatment. Treatment completion was defined as treatment for 24 months, with clinical improvement, and negative cultures after treatment, but did not meet the definition for cure because of lack of bacteriologic results (<5 cultures performed in the final 12 months of therapy). If 1 culture was positive for TB but there was no clinical deterioration, the patient was still considered cured or that treatment was completed provided that this result was followed by ≥3 monthly negative cultures. Treatment failure was defined as treatment for 24 months but with ≥2 of 5 cultures in the final 12 months positive, or if any 1 of the final 3 cultures was positive for TB, or if clinical failure was indicated by the clinician. Default was defined as treatment interrupted for ≥2 consecutive months for any reason. Death was defined as death of a patient for any reason during treatment.

TB sputum culture conversion was defined as having ≥2 negative consecutive sputum cultures 30 days apart after initiation of treatment. Patients may have subsequently showed reversion to a status of TB sputum culture positive. Adverse events were recorded by clinical staff. Severe adverse events were defined as events causing new hospitalization, stopping a drug in the regimen, urgent/emergent treatment, or death (*16*). In addition, electrolyte abnormalities (potassium level <2.5 mmol/L or magnesium level <1.5 mmol/L) attributed to medication use, were considered to be severe adverse events. The study protocol was approved by the Biomedical Research Ethics Committee of the University of KwaZulu-Natal and the Institutional Review Board of Boston University Medical Center.

Drug Susceptibility Testing

Drug susceptibility testing for first-line and second-line drugs was performed at the provincial TB referral laboratory in Durban, South Africa. Culture positivity was determined by using the BACTEC MGIT 960 fluorometric system (Becton Dickinson Diagnostics, Sparks, MD, USA). Drug susceptibility testing for isoniazid, rifampin, ethambutol, streptomycin, ethionamide, ofloxacin, and kanamycin was performed by using the modified proportional growth method on 7H11 agar according to standard techniques (*17*,*18*). Drug susceptibility testing for capreomycin, *p*-aminosalacylic acid (PAS), terizidone, cycloserine, and pyrazinamide was not available during the study period because of technical and resource availability issues. Sputum samples for culture were routinely obtained on a monthly basis.

Statistical Analysis

All participants in the study were included in an analysis of risk factors for survival and unfavorable treatment outcome. For the survival analysis, we included all patients who died, even if they defaulted before death, as deaths. For the unfavorable treatment outcome analysis, unfavorable treatment outcome included death, treatment failure, and default. Successful treatment outcome included cure and treatment completion. Cox proportional hazards models were used to estimate hazard ratios (HRs) and 95% CIs. Significant variables or variables that caused >10% change in the bivariate HR were included in the multivariate model. We calculated 95% CIs by using a normal approximation of the binomial distribution. The Fischer exact test or χ^2 test was used to compare categorical variables. Medians were compared by using the Wilcoxon

Mann-Whitney U test. Kaplan-Meier survival curves for death and for time to culture conversion were calculated from time of XDR TB treatment initiation with appropriate anti-TB drugs. Participants were censored at time of default or death. The p values for survival analysis were determined by using Cox proportional hazards model adjusted for potential confounding variables, as per above. Analysis was performed by using SAS version 9.2 software (SAS Institute, Cary, NC, USA).

Results

During 2006–2007, a total of 6,127 persons in KwaZulu-Natal Province were given a diagnosis of multidrug-resistant TB (MDR TB) (5,612) or XDR TB (536) (*19*). Of those 6,127 persons, 2,013 (1,771 with MDR-TB and 242 with XDR TB) were admitted to King George V Hospital for initiation of treatment during the 2-year study period (December 2006–October 2007). During this period, 130 consecutive patients with XDR TB were admitted to King George V Hospital for initiation of treatment. Four of these patients refused treatment, 2 patients had insufficient data, 6 patients died before the start of treatment, and 4 patients were <18 years of age. The remaining 114 XDR TB patients were eligible and were analyzed during the study.

The patients included in this study were transferred from 41 distinct municipalities or areas representing 9 (82%) of 11 health districts in KwaZulu-Natal Province. The most common addresses patients reported were in the Tugela Ferry catchment area (31%), metropolitan Durban (14%), and Pietermaritzburg (11%). Most (57%) patients were female patients, young (median age 35 years), and co-infected with HIV (77% with a known test result) (Table 1). Female patients were significantly younger (median age 31 years vs. 39 years; p<0.001) and more likely to be co-infected with HIV (80% vs. 61%; p = 0.01) than male XDR TB patients. Most (81%) patients had been treated previously for TB; fewer (37%) had been previously treated for MDR-TB. Fifty (61%) of 82 patients with known HIV infection were receiving antiretroviral therapy (ART) before hospital admission or initiated ART therapy early during treatment for XDR TB with efavirenz-based regimens. A total of 42 (37%) patients showed TB sputum culture conversion during treatment. Eighteen (43%) of 42 patients who showed culture conversion subsequently showed culture reversion (n = 7), defaulted (n = 7), or died (n = 4).

Patients were treated with a median of 6 drugs (interquartile range [IQR] 5–7 drugs). The most common initial regimens included capreomycin (90%), PAS (90%), pyrazinamide (92%), ethionamide (92%), ethambutol (97%), cycloserine (66%), or terizidone (30%). Moxifloxacin (1%) and leveofloxacin (0%) were not available through the public health care system during the study period.

Adverse events during treatment occurred in 58% of patients; severe adverse events occurred in 25% of patients and were not associated with HIV status, ART, or treatment default. Within the HIV co-infected subgroup, there was an association between adverse events and death, this association was not significant by multivariate analysis. Physicians infrequently recorded a specific drug associated with the adverse event (29%): cycloserine (12%), capreomycin (8%), and PAS (4%) were the most common drugs related to adverse events. There were 8 episodes of psychosis or severe psychiatric illness attributed to cycloserine, which resulted incessation of the use of this drug. There were 4 deaths in the cohort attributed to hypokalemia or hypomagnesemia related to use of capremoycin. These events decreased over the study period as physicians empirically supplemented potassium and magnesium for patients during treatment with capreomycin.

Treatment outcomes were determined 24 months after the initiation of treatment (Table 2). All treatment outcome categories were mutually exclusive. By 24 months, 48 (42%) of 114 patients had died, 25 (22%) of 114 were either cured or completed treatment, 19 (17%) of 114 defaulted, and 22 (19%) of 114 showed treatment failure (Table 2). Kaplan-Meier survival and culture conversion curves from start of XDR TB therapy are shown in Figures 1 and 2. Deaths of patients after they defaulted (n = 1) were counted as deaths in survival analysis. HIV status was not associated with a higher mortality rate or culture conversion, but among HIV-infected XDR TB patients, receiving ART was associated with improved survival but not improved sputum culture conversion. Patients who showed culture conversion early or late during treatment had improved survival by Kaplan-Meier analysis (Figure 2, panel B).

TB culture conversion at 2 months of treatment was associated with survival by bivariate analysis (HR 5.55, 95% CI 1.75–20.0) and multivariate analysis (HR 5.0, 95% CI 1.49–16.67). Sputum culture conversion was not included as a variable in Table 3 because it is an intermediate in the causal pathway. Among all XDR TB patients, none of the included variables were associated with death (Table 3) or unfavorable treatment outcome as a composite outcome of death, treatment failure, and default. Among the subset of HIV co-infected XDR TB patients (Table 4), ART was protective against death by multivariate analysis (HR 0.46, 95% CI 0.22–0.94). When further stratified by CD 4 T-cell count/mm^3, HIV co-infected XDR TB patients with CD4 cell counts >200/mm^3 who received ART had substantially lower risk for death than patients with CD4 cell counts ≤200/mm^3 who were not receiving ART (HR 0.094, 95% CI 0.007–1.22), but this result was not significant.

Table 1. Demographic characteristics of patients with XDR TB, KwaZulu-Natal Province, South Africa*

Characteristic	All patients, n = 114	Female patients, n = 65	Male patients, n = 49	p value
Sex				
M	49 (43.0)	NA	NA	NA
F	65 (57.0)	NA	NA	NA
Age, y				
18–25	16 (14.0)	16 (24.6)	0	<0.0001
26–35	42 (36.8)	27 (41.5)	15 (30.6)	<0.0001
36–50	46 (40.4)	21 (32.3)	25 (51.0)	NA
>50	10 (8.8)	1 (1.5)	9 (18.4)	MA
Median age (IQR)	35 (30–42)	31 (26–37)	39 (35–47)	NA
HIV status				
Positive	82 (71.9)	52 (80.0)	30 (61.2)	0.0153
Negative	25 (21.9)	8 (12.3)	17 (34.7)	NA
Unknown	7 (6.1)	5 (7.7)	2 (4.1)	NA
CD4 cell count/mm^3†				
Known	55 (67.1)	38 (73.1)	17 (56.7)	0.1487
Not determined	27 (32.9)	14 (26.9)	13 (43.3)	0.1426
Median (IQR)	197 (80–300)	222 (71–316)	130 (83–254)	NA
Receiving ART†				
Yes	50 (61.0)	34 (65.4)	16 (53.3)	0.3492
No	32 (39.0)	18 (34.6)	14 (46.7)	NA
Severe adverse event‡				
Yes	29 (25.4)	29 (25.4)	12 (24.5)	1.0000
No	85 (74.6)	48 (73.9)	37 (75.5)	NA
Previous TB treatment				
Yes	92 (80.7)	53 (81.5)	39 (79.6)	0.7216
No	15 (13.2)	9 (13.9)	6 (12.2)	NA
Unknown	7 (6.1)	3 (4.6)	4 (8.2)	NA
Previous MDR TB diagnosis				
Yes	69 (60.5)	41 (63.1)	28 (57.1)	0.5649
No	45 (39.5)	24 (36.9)	21 (42.9)	NA
Health care worker				
Yes	6 (5.3)	4 (6.2)	2 (4.1)	0.6982
No	108 (94.7)	61 (93.9)	47 (95.9)	NA
Type of TB				
Pulmonary	103 (90.4)	58 (89.2)	45 (91.8)	NA
Extrapulmonary	11 (9.7)	7 (10.8)	4 (8.2)	NA
Culture conversion, mo§				
None	72 (63.2)	40 (61.5)	32 (65.3)	0.2523
≤2	16 (14.0)	7 (10.8)	9 (18.4)	NA
>2	26 (22.8)	18 (27.7)	8 (16.3)	NA

*Values are no. (%) unless otherwise indicated. XDR TB, extensively drug-resistant tuberculosis; NA, not applicable; IQR, interquartile range; ART, antiretroviral therapy; MDR TB, multidrug-resistant TB.
†Among HIV-positive patients only.
‡Resulted in changes in clinical status or electrolyte abnormalities or required change of TB treatment regimen.
§After initiation of treatment.

Although ART was protective against death among patients co-infected with XDR TB and HIV, it was not associated with sputum culture conversion. After we adjusted for age, sex, ART use, previous TB treatment, adverse drug reactions, and a baseline CD4 cell count ≤200/mm^3 by using the Cox proportional hazards model, we found that ART use was not associated with culture conversion after 2 months of treatment (HR 0.90, 95% CI 0.23–3.51) or culture conversion during treatment (HR 1.13, 95% CI 0.47–2.7).

When we compared data for HIV co-infected patients hospitalized for initiation of treatment from the first period of the study (December 2006–May 2007) with data of patients hospitalized during the second period (May 2007–November 2007), we found a significant trend toward a higher percentage receiving ART (21/43, 49% vs. 29/39, 74%, respectively; p = 0.02). Multivariate analysis showed that HIV-negative women had higher survival rates than HIV-negative men (HR 0.08, 95% CI 0.01–0.61), Conversely, HIV-positive women had lower survival rates than HIV-positive men (HR 1.82, 95% CI 0.83–4.01), but the difference was not significant.

Data for 109 (96%) patients were analyzed from time of diagnosis. Date of XDR TB diagnosis was unknown for 5 patients. Median time between diagnosis and initiation of therapy was 101 days (IQR 68–150 days). For patients who died, median time between diagnosis and initiation of therapy was 83.5 days (IQR 64–135 days). For patients who survived, median time between diagnosis and initiation of therapy was 118 days (IQR 75.5–163.5 days).

Table 2. Twenty-four month treatment outcomes for 114 patients with extensively drug-resistant tuberculosis, KwaZulu-Natal Province, South Africa*

Treatment outcome	No. (%) patients
Favorable	
Cure	15 (3.2)
Completed	10 (8.8)
Unfavorable	
Defaulted*	19 (16.7)
Failure	22 (19.3)
Died	48 (42.0)

*One patient initially defaulted and subsequently died.

Time to culture conversion appeared to be an insensitive predictor of successful 24-month treatment outcome because for culture conversion at 6 months, sensitivity was only 51% (positive predictive value 85%; negative predictive value 57%) (Table 5). Culture conversion was a better predictor of survival at 24 months because for culture conversion at 6 months, sensitivity was 92% (positive predictive value = 62% and negative predictive value = 97%) (Table 6).

Discussion

The main findings of our study were a high mortality rate (42%) and a low rate of successful treatment outcomes (22%) for XDR TB patients after completion of 24 months of treatment in a setting with a high incidence of HIV. All deaths in this cohort occurred in the first 12 months after start of treatment. Predictors of deaths in this cohort included TB-specific (TB culture conversion) and HIV-specific (ART use) factors. Consistent with findings in other studies of treatment of drug-resistant TB/HIV, HIV was not independently associated with death (*12*,*13*,*20*). Although HIV was not independently associated with death, use of ART among HIV-infected patients was associated with improved survival. Sex appeared to modify the association between death and HIV because female sex was associated with higher survival rates among HIV-negative XDR TB patients but with higher death rates in women co-infected with HIV than in men co-infected with HIV. However, this finding was not significant in all strata. TB culture conversion was a useful predictor of survival and treatment outcome. However, it was not sufficiently sensitive in this cohort to be a surrogate for successful TB treatment outcome, given the number of patients who ultimately showed treatment failure (n = 7), defaulted (n = 7), or died (n = 4) after TB culture conversion.

Recently, 3 large, randomized, control trials of patients with drug-susceptible TB and co-infected with HIV have been conducted that analyzed different starting points for ART (*21*–*23*). Results of these trials showed that ART started early during TB treatment was associated with improved survival and that most decreases in mortality rates were for patients with low CD4 T-cell counts. As ART use increases among patients co-infected with MDR TB and HIV in KwaZulu-Natal Province, survival among these patients will probably improve. Higher rates of TB culture conversion through more effective drug regimens, including second-generation fluoroquinolones, high-dose isoniazid, and clofazimine, may further improve survival among XDR TB patients (*24*). Complete drug susceptibility testing for all drugs used should be performed at baseline and for XDR TB patients who do not show TB culture conversion after treatment to identify baseline and emergent second-line drug resistance. Furthermore, given that many patients showed reversion to sputum cell cultures positive for TB after initial culture conversion, optimal duration of XDR TB treatment remains unclear, Thus, new regimens, including more potent antimycobacterial agents such as linezolid, TMC207, or new nitromidazoles, may further increase sputum culture conversion rates and survival (*25*,*26*).

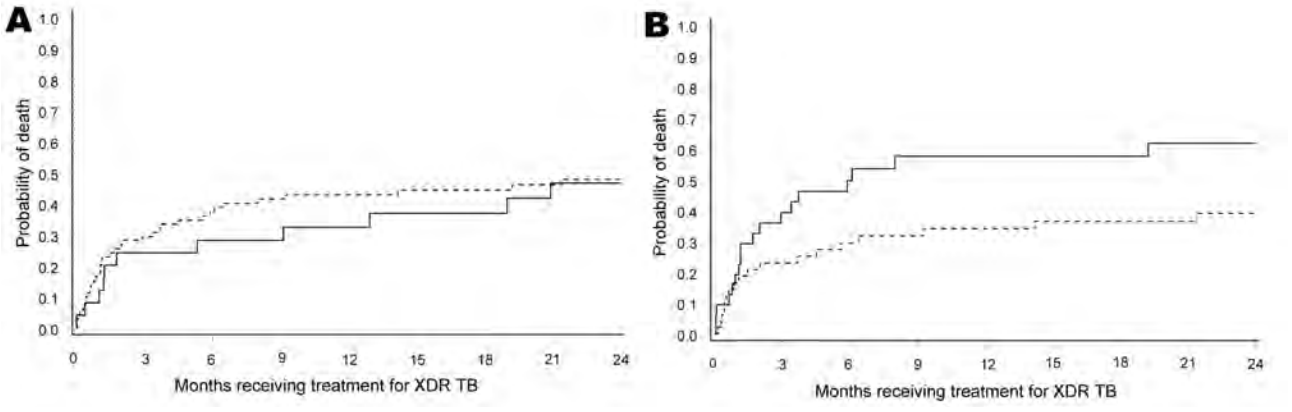

Figure 1. Kaplan-Meier curves for A) 114 HIV-positive (dashed line) and HIV-negative (solid line) patients receiving treatment for extensively drug-resistant tuberculosis (XDR TB) (p = 0.4966); and B) 82 HIV-infected patients with XDR TB receiving (dashed line) and not receiving (solid line) antiretroviral therapy (p = 0.0330), KwaZulu-Natal Province, South Africa. p values were adjusted for sex, TB treatment history, and HIV status.

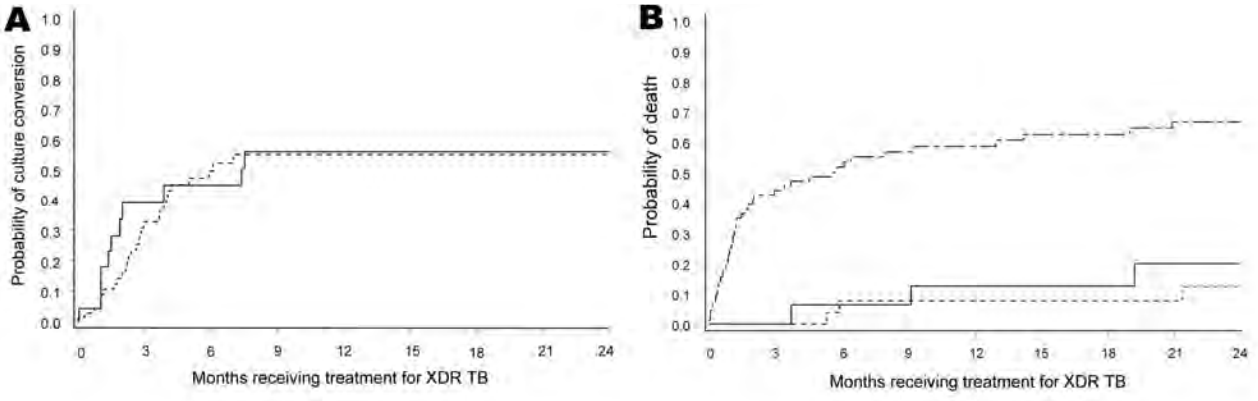

Figure 2. A) Kaplan-Meier curves for sputum culture conversion for HIV-positive (dashed line) and HIV-negative (solid line) patients with extensively drug-resistant tuberculosis (XDR TB) receiving treatment, KwaZulu-Natal Province, South Africa. Sputum culture conversion is defined as 2 consecutive monthly TB cultures with no growth after 6 weeks of incubation after initiation of treatment (p = 0.706). p value was adjusted for age, initial smear result, and HIV status. B) Kaplan-Meier curves for patients receiving treatment for XDR TB stratified by sputum culture conversion status (p<0.0001). Solid line indicates conversion ≤2 months after initiation of treatment, dashed line indicates conversion >2 months after initiation of treatment, and top line with small and large dashes indicates no conversion. p values were adjusted for sex, TB treatment history, and HIV status. There was no significant difference between patients who showed culture conversion ≤2 months and >2 months after initiation of treatment (p = 0.5182).

Although 4 deaths presumed secondary to capreomycin-associated electrolyte abnormalities occurred early in the study period (*12*), clinicians became more vigilant, tested serum electrolytes more often, and used empiric electrolyte supplementation. Overall, adverse effects were not associated with failure to show sputum culture conversion. Treatment adherence was an unmeasured variable that had a major causal role during treatment for XDR TB and HIV infection. Thus, determining operational methods to measure and improve adherence to ART and second-line and third-line anti-TB drugs is critical for improving outcomes.

Co-infection with drug-resistant TB and HIV has emerged as a major syndemic in South Africa and elsewhere (*26*) and has been comprehensively reviewed (*27*), However, to our knowledge, there are only 2 published studies of early results of treatment for co-infection with XDR TB and HIV. One study, published by our group (*12*), reported low rates of culture conversion (20%) and high mortality rates (42%) after a median of 12 months of treatment for the first 60 consecutive XDR TB patients in the current cohort. The second study, published by Dheda et al. (*13*) from Western Cape Province, South Africa,

Table 3. Predictors of 49 deaths at 24 months of treatment for 114 HIV-positive and HIV-negative XDR TB patients, KwaZulu-Natal Province, South Africa*

		Univariate analysis		Multivariate analysis†	
Predictor	No. died/total no. (%)	Hazard ratio (95% CI)	p value	Hazard ratio (95% CI)	p value
Sex					
F	27/65 (41.5)	0.88 (0.50–1.54)	0.6484	0.95 (0.51–1.77)	0.8611
M	22/49 (44.9)	1.0 (referent)	NA	1.0 (referent)	NA
Age, y					
<36	25/58 (43.1)	1.03 (0.59–1.80)	0.9285	NA	NA
≥36	24/56 (42.9)	1.0 (referent)	NA	NA	NA
Previous TB treatment‡					
Yes	38/92 (41.3)	1.47 (0.53–4.13)	0.4614	1.28 (0.45–3.65)	0.6391
No	4/15 (26.7)	1.0 (referent)	NA	1.0 (referent)	NA
Unknown	7/7 (100)	NA	NA	NA	NA
Initial sputum smear result					
Positive	30/67 (44.8)	1.05 (0.59–1.86)	0.8704	NA	NA
Negative	19/47 (40.4)	1.0 (referent)	NA	NA	NA
HIV status‡					
Positive	36/82 (43.9)	1.14 (0.58–2.25)	0.6971	1.30 (0.61–2.78)	0.4966
Negative	11/25 (44.0)	1.0 (referent)	NA	1.0 (referent)	
Unknown	2/7 (28.6)	NA	NA	NA	NA
Adverse event					
Yes	23/52 (44.2)	1.02 (0.58–1.79)	0.9420	NA	NA
No	26/62 (41.9)	1.0 (referent)	NA	NA	NA

*XDR TB, extensively drug-resistant tuberculosis; NA, not applicable.
†Significant variables or variables that caused >10% change in the bivariate hazard ratio were included in the multivariate model.
‡Patients whose HIV status or previous TB treatment history was unknown were excluded from analyses.

Table 4. Predictors of 36 deaths at 24 months of treatment for 82 HIV-positive XDR TB patients, KwaZulu-Natal Province, South Africa*

		Univariate analysis		Multivariate analysis†	
Predictor	No. died/total (%)	Hazard ratio (95% CI)	p value	Hazard ratio (95% CI)	p value
Sex					
F	26/52 (50.0)	1.55 (0.75–3.21)	0.2405	1.82 (0.83–4.01)	0.1349
M	10/30 (33.3)	1.0 (referent)	NA	1.0 (referent)	NA
Age, y					
<36	20/45 (44.4)	1.05 (0.55–2.03)	0.8765	NA	NA
≥36	16/37 (43.2)	1.0 (referent)	NA	NA	NA
Previous TB treatment‡					
Yes	29/66 (43.9)	1.68 (0.51–5.52)	0.3913	1.70 (0.51–5.65)	0.3865
No	3/12 (25.0)	1.0 (referent)	NA	1.0 (referent)	NA
Unknown	4/4 (100.0)	NA	NA	NA	NA
Initial sputum smear result					
Positive	22/48 (45.8)	1.03 (0.53–2.01)	0.9354	NA	NA
Negative	14/34 (41.2)	1.0 (referent)	NA	NA	NA
Initial CD4 cell count/mm^3					
≤200	13/29 (44.8)	1.02 (0.52–2.02)	0.9495	NA	NA
>200	23/53 (43.4)	1.0 (referent)	NA	NA	NA
Receiving ART					
Yes	18/50 (36.0)	0.54 (0.28–1.03)	0.0633	0.46 (0.22–0.94)	0.0333
No	18/32 (56.3)	1.0 (referent)	NA	1.0 (referent)	NA
Adverse event					
Yes	18/35 (51.4)	1.43 (0.74–2.76)	0.2832	1.89 (0.92–3.86)	0.0812
No	18/47 (38.3)	1.0 (referent)	NA	1.0 (referent)	NA

*XDR TB, extensively drug-resistant tuberculosis; NA, not applicable; ART, antiretroviral therapy.
†Significant variables or variables that caused >10% change in the bivariate hazard ratio were included in the multivariate model.
‡Patients whose previous TB treatment history was unknown were excluded from analyses.

reported increased mortality rates (36%) and low culture conversion rates (19%) in 174 XDR TB patients after a median follow-up period of 6.9 months after the start of treatment for co-infection with XDR TB and HIV. This cohort had lower but substantial rates of co-infection with XDR TB and HIV and similarly showed no association between HIV and mortality rates for XDR TB patients receiving treatment but a protective effect for ART. There have been several cohort studies of XDR TB in settings with low incidence of HIV, including South Korea, Europe, Peru, and the United States (*28*–*33*). Successful treatment outcomes at 24 months of treatment ranged from 28% to 60%. Only 2 cohorts from Lithuania (*32*) and the United States (*34*) included patients co-infected with HIV.

Limitations to our study included survival bias associated with an observational study at a TB referral hospital. Median time from diagnosis to initiation of treatment for XDR TB was 101 days, which did not decrease over the time of the study. A total of 50%–70% of patients with a diagnosis of MDR TB in KwaZulu-Natal Province had not initiated treatment (*11*,*35*), and those who survived to study referral are probably unique because they were less immunocompromised and had a higher rate of treatment (*11*). We found no association between HIV status and survival or treatment outcome. This result probably reflects countervailing outcomes of patients who received ART and those who did not receive ART. However, this lack of association might be caused by misclassification (refusal to participate in HIV testing), or survival bias.

Because data were collected retrospectively, there were missing data for ART adherence, repeat CD4 T-cell counts, HIV RNA virus loads, adverse events, and details on ART started subsequent to inpatient hospitalization for XDR TB treatment initiation, which may have led to misclassification bias. This result would presumably bias HIV-associated variables toward the null hypothesis. The study was also limited by lack of full TB drug susceptibility testing for capreomycin, PAS, cycloserine, or terizadone to guide treatment choices. Most concerning

Table 5. Sputum culture conversion at intervals of successful treatment for XDR TB patients, KwaZulu-Natal Province, South Africa*

Time from start of treatment, mo	No. cultures converted/total (%)	No. cultures converted/ total converted (%)	Sensitivity, %	Specificity, %	Positive predictive value, %	Negative predictive value, %
1	4/114 (4)	4/42 (10)	5	98	75	43
2	18/114 (16)	18/42 (43)	19	92	78	42
3	29/114 (25)	29/42 (69)	35	88	79	51
4	33/114 (29)	33/42 (79)	42	88	82	53
6	39/114 (34)	39/42 (93)	51	88	85	57
24	42/114 (37)	NA	NA	NA	NA	NA

*XDR TB, extensively drug-resistant tuberculosis; NA, not applicable.

Table 6. Sputum culture conversion at intervals of survival for XDR TB patients, KwaZulu-Natal Province, South Africa*

Time from start of treatment, mo	No. cultures converted /total (%)	No. cultures converted/ total converted (%)	Sensitivity, %	Specificity, %	Positive predictive value, %	Negative predictive value, %
1	4/114 (4)	4/42 (10)	12	99	75	79
2	18/114 (16)	18/42 (43)	42	92	61	84
3	29/114 (25)	29/42 (69)	65	86	59	89
4	33/114 (29)	33/42 (79)	77	85	61	93
6	39/114 (34)	39/42 (93)	92	83	62	97
24	42/114 (37)	NA	NA	NA	NA	NA

*XDR TB, extensively drug-resistant tuberculosis; NA, not applicable.

was the lack of drug-resistance data for capreomycin. In a study published subsequent to our study period, 17 of 19 *M. tuberculosis* isolates from the site of a well-described TB outbreak in KwaZulu-Natal Province were capreomycin resistant (*36*). Because drug susceptibility testing for capreomycin was not available, we may not have identified all cases of XDR TB. Conversely, complete drug susceptibility testing would not have necessarily led to improved regimens because the availability of second-line and third-line anti-TB drugs was limited during this period. In addition, second-generation fluoroquinolones, which may improve outcomes in XDR TB patients, were not available in the public sector for TB treatment during the study (*37*,*38*).

Although not addressed by our study, improvements in treatment outcomes for patients co-infected with MDR TB and HIV will require changes in HIV- and TB-related factors. For HIV, these include more rapid HIV testing for early initiation of ART, appropriate monitoring of CD4 T-cell counts, HIV virus load testing, appropriate opportunistic infection prophylaxis, and improvement in ART adherence. Although not addressed by our study, we recommend that for TB these improvements include widespread implementation of rapid diagnostics, particularly for smear-negative disease; early drug susceptibility testing for first-line and second line agents; improvement in adherence for second-line TB drugs; development of more effective anti-TB drugs and regimens; and guidance of drug selection by timely and ongoing drug susceptibility testing.

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Foodborne Disease Prevention and Broiler Chickens with Reduced *Campylobacter* Infection

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Studies have suggested that flies play a linking role in the epidemiology of *Campylobacter* spp. in broiler chickens and that fly screens can reduce the prevalence of *Campylobacter* spp. We examined the year-round and long-term effects of fly screens in 10 broiler chicken houses (99 flocks) in Denmark. Prevalence of *Campylobacter* spp.–positive flocks was significantly reduced, from 41.4% during 2003–2005 (before fly screens) to 10.3% in 2006–2009 (with fly screens). In fly screen houses, *Campylobacter* spp. prevalence did not peak during the summer. Nationally, prevalence of *Campylobacter* spp.–positive flocks in Denmark could have been reduced by an estimated 77% during summer had fly screens been part of biosecurity practices. These results imply that fly screens might help reduce prevalence of campylobacteriosis among humans, which is closely linked to *Campylobacter* spp. prevalence among broiler chicken flocks.

Campylobacter spp. is the most common cause of enteritis in humans in the European Union; 190,566 cases were reported in 2008 (*1*). However, it has been estimated that only 2.1% of all cases are reported and that in the European Union the true incidence of campylobacteriosis is ≈9 million cases per year (*2*). From 2008 through 2009, the number of human infections in the European Union increased 4%, although there was no statistically significant trend from 2005 through 2009 (*1*). The incidence of campylobacteriosis seems to differ among European countries (*3*). In addition, campylobacteriosis and its sequelae are calculated to cost 0.35 million disability-adjusted life-years per year, totaling €2.4 billion per year (*2*).

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Campylobacteriosis is largely perceived to be a foodborne disease. Poultry meat is considered the primary source, causing 20%–30% of all cases; and 50%–80% of all cases might be attributed to the chicken reservoir as a whole (*2*). The incidence of campylobacteriosis cases among humans has been shown to correlate with the prevalence of *Campylobacter* spp. among broiler chickens (*4*). The prevalence of *Campylobacter* spp. in broiler chicken batches varies considerably between EU countries; in 2008, prevalence ranged from 2% to 100% (average 71%) (*5*). Therefore, an international priority for ensuring food safety is the elimination of *Campylobacter* spp. from broiler chicken flocks (*6*,*7*). However, even strict compliance with all biosecurity regulations has failed to control infections in broiler chicken houses during peak months in the summer, indicating that transmission routes, and the blocking of these routes, remain to be fully elucidated and understood.

Studies have repeatedly suggested that flies play a linking role in the epidemiology of *Campylobacter* spp. infections by transmitting *Campylobacter* spp. from fecal sources to poultry (*8*–*10*). Moreover, seasonality of infections in humans (*11*) and broiler chicken flocks (*3*,*4*,*12*,*13*) is similar in northern climates; prevalence peaks during the summer, as does abundance of flies (*11*,*14*). In addition, studies have shown that flies can carry *Campylobacter* spp. under natural conditions (*9*,*15*,*16*) and that hundreds of flies per day pass through ventilation inlets into broiler chicken houses (*15*,*17*). The fly that has been found to most often carry *Campylobacter* spp. is the housefly (*Musca domestica*) (*15*). The retention of *Campylobacter* spp. in this species of fly has been found to be relatively short (*18*). Altogether, these findings suggest that flies could explain some aspects of *Campylobacter* spp. epidemiology.

This association between flies and *Campylobacter* spp. is not surprising because flies are natural carriers of many pathogens, including viruses, fungi, bacteria, and parasites (*9*,*16*,*19*–*21*). Studies have shown that different fly species can harbor up to 100 species of pathogenic microorganisms

and that bacteria alone are linked to >65 diseases in humans and animals (*21–23*). Houseflies live in close association with humans and breed in animal manure, human excrement, garbage, animal bedding, and decaying organic matter where bacteria are also abundant (*24*). Houseflies have been suggested to be vectors of bacteria, such as *Shigella* spp., *Vibrio cholerae*, *Escherichia coli*, *Aeromonas caviae*, and *Campylobacter* spp. (*15*,*25–29*).

To test the hypothesis that the influx of flies increases transmission of *Campylobacter* spp. to broiler chickens during the summer, Hald et al. mounted fly screens on 20 broiler chicken houses in Denmark during the summer (June–October) of 2006 (*30*), when the number of *Campylobacter* spp.–positive flocks in Denmark peaks (*4*). The outcome was a statistically significant decrease, from 51.4% to 15.4%, of *Campylobacter* spp.–positive flocks in the fly-screen houses, whereas prevalence for control houses remained unchanged before and after the intervention (51.7% and 51.4%, respectively). During the summer of 2008, the effect of fly screens was also tested on farms in Iceland where prevalence rates of *Campylobacter* spp. among flocks had been high (*31*). That study found a reduction from 48.3% to 25.6% among flocks in 19 houses from one broiler chicken company and from 31.3% to 17.2% in 16 houses from another company. These published results of the fly screen intervention have covered only the summer and only 1 season.

According to the scientific opinion on *Campylobacter* in broiler chicken meat production, published by the European Food Safety Authority Panel on Biological Hazards (*2*), high priority has been given to generating solid long-term data on biosecurity interventions, including the effect of hygiene barriers and fly screens, as a way to reduce prevalence of *Campylobacter* spp. among flocks of broiler chickens (hereafter referred to as flock prevalence) (*2*). Our aim, therefore, was to generate year-round and long-term data on the effect of fly screen interventions. We present 4 years of data (2006–2009) on the long-term effect of fly screens on *Campylobacter* spp. prevalence among broiler chicken flocks.

Materials and Methods

Study Houses

This study was conducted at 10 fly-screened broiler chicken houses situated on 2 one-house farms and 4 two-house farms in Jutland, Denmark. The houses were part of a previous intervention study by Hald et al., conducted in the summer of 2006, in which standard Phiferglass insect screening (Phifer Incorporated, Tuscaloosa, AL, USA) of 18 × 16 mesh/inch^2 had been installed on 20 broiler chicken houses, thereby excluding 95% of all flies from each house (*30*). The remaining 5% of flies were either so tiny that they were able to penetrate the mesh, or they (and larger flies) could enter the house through open gates or doors during stocking of new chicks (*15*). In addition, 10 control houses that were also part of the study by Hald et al. (*30*) were matched and included for comparison in our study. The criteria used to choose houses are described by Hald et al. (*30*). The houses that were chosen were representative in construction and ventilation type of at least 90% of the broiler chicken houses in Denmark (online Technical Appendix, wwwnc.cdc.gov/EID/article/19/3/11-1593-Techapp1.pdf). The houses were equipped with fly screens by June 1, 2006, and data were subsequently obtained through 2009.

Campylobacter spp. Flock Prevalence

Data on *Campylobacter* spp. prevalence among flocks from the 10 houses with fly screens (fly screen houses) during 2006–2009 were compared with data for the same houses during 2003–2005 (before fly screens) and for the 10 control houses (without fly screens) in both periods. In addition, the historical national *Campylobacter* spp. flock prevalence for the 2 periods (2003–2005 and 2006–2009) were included for comparison and are hereafter referred to as national prevalence.

Flock prevalence data were obtained from the national surveillance database (*32*). Since 1998, all broiler chicken flocks in Denmark have been tested for *Campylobacter* spp., and the prevalence of positive flocks has been recorded (*33*). From each flock, 10 pooled cloacal swab samples are obtained at slaughter and analyzed for *Campylobacter* spp. by using a genus-specific PCR (*34*), and results have been collected in the national surveillance database. Data extracted from our study included *Campylobacter* spp. status at slaughter. For flocks that were thinned (part of the flock slaughtered before the end of the rearing period) (*2*), only results from the first slaughter batch were included.

Statistical Analyses

Prevalence was calculated as the percentage of flocks positive by 10 pooled cloacal swab samples at slaughter. The Yates χ^2 test was used to test for differences in *Campylobacter* spp. prevalence, depending on years and treatments. This test was used because of the large sample size of the flocks. Furthermore, odds ratios (ORs) and 95% CIs were calculated. The population attributable fraction (PAF) was calculated according to the method of Webb and Bain (*35*).

Results

Campylobacter spp. Prevalence during Summer

Campylobacter spp. prevalence among flocks from fly screen houses decreased significantly from 41.4% in 2003–2005 (before fly screens) to 10.3% in 2006–2009 (with fly

screens) (p<0.001; OR 6.1; 95% CI 3.1–12.4), whereas the prevalence reduction in the control houses was minor (not significant), from 41.8% in 2003–2005 to 36.0% in 2006–2009 (p = 0.454; OR 1.3; 95% CI 0.7–2.1) (Figure 1). In comparison, national prevalence, obtained from the surveillance data, decreased significantly from 48.6% to 45.6% during 2003–2005 and 2006–2009 (p<0.001; OR 1.1; 95% CI 1.1–1.2). Prevalence rates of *Campylobacter* spp.–positive flocks for the 3 study groups during the summers of 2003–2005 and 2006–2009 are shown in the Table.

Before the fly screen intervention (2003–2005), *Campylobacter* spp. prevalence did not differ between the fly screen houses and the control houses (p = 0.920) or from the national prevalence for the same period (p = 0.188) (Figure 1; Table). During 2003–2005, prevalence for the control houses did not differ from national prevalence (p = 0.221). In contrast, during the period with the intervention (2006–2009), prevalence for fly screen houses was significantly lower than that for the control houses (p<0.001) and lower than national prevalence (p<0.001). During the same period, prevalence was lower for the control houses than nationally (p = 0.036).

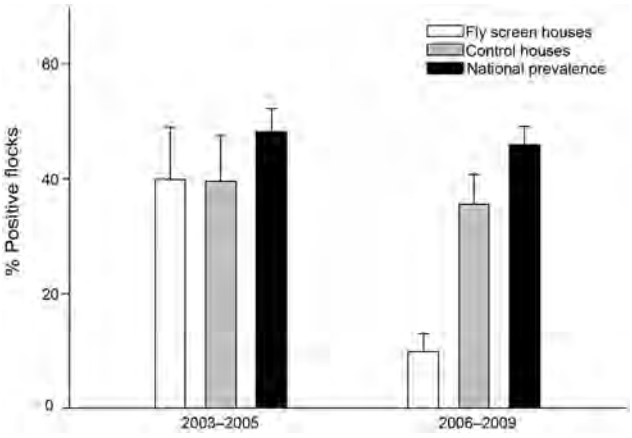

Figure 1. Mean percentage of broiler chicken flocks that were *Campylobacter* spp. positive during summers of 2003–2005 (before fly screens) and 2006–2009 (with fly screens). Prevalence is based on data from June through October each year. Error bars indicate upper limit of SE.

Campylobacter spp. Prevalence Seasonal Trends

Seasonal trends in percentage of *Campylobacter* spp.–positive flocks at the fly screen houses (2003–2005, before fly screens) and the control houses (2003–2005 and 2006–2009) were similar to national prevalence trends (2003–2005 and 2006–2009) (Figure 2). Thus, the number of *Campylobacter* spp.–positive flocks increased during June and July and peaked in August and September. However, the number of *Campylobacter* spp.–positive flocks in fly screen houses during 2006–2009 was lower than that in control houses and than that reported nationally during June–October (Figure 2). During winter, however, flock prevalence of *Campylobacter* spp. was not reduced for the fly screen houses. In fact, flock prevalence in the fly screen houses did not differ significantly between summer (June–October) and winter (November–May) during 2006–2009 (p = 0.129).

PAF

Using the results from the fly screen houses (before and after fly screens had been installed), we calculated the PAF for the national prevalence. We estimated that at the national level, 77% of *Campylobacter* spp. positivity would have been prevented during the summer if fly screens had been part of the biosecurity practice on all broiler chicken farms in Denmark. On a yearly basis, PAF was estimated to be 72%.

Discussion

We found that by using fly screens to prevent flies from entering broiler chicken houses, it was possible to reduce the prevalence of *Campylobacter* spp.–positive flocks from 41.4% to 10.3%. This long-term reduction of prevalence is in accordance with the previous results obtained in the short-term study by Hald et al. (*30*). Prevalence at the control houses and nationally was slightly lower in 2006–2009 than in 2003–2005, a finding that agrees with the general trend in Denmark during this period (*3*). Furthermore, the summer peak in *Campylobacter* spp. flock prevalence observed nationally and in the control houses was absent in the fly screen houses. Summer prevalence at the fly screen houses was equal to the low prevalence levels observed in Denmark during winter. Because only 1 intervention was tested, and because study and control houses were matched thoroughly, the results convincingly attribute the reduction of *Campylobacter* spp. flock prevalence to the use of fly screens. In addition, our results are based on a 4-year dataset, which highlights the robustness of the findings.

Table. *Campylobacter* spp.–positive and –negative broiler chicken flocks in summer (June to October), Denmark

	2003–2005			2006–2009			Odds ratio
Source	No. (%) positive	No. negative	95% CI*	No. (%) positive	No. negative	95% CI	(95% CI)
Fly screen houses	41 (41.4)	58	32.2–51.3	13 (10.3)	113	6.1–16.9	6.1 (3.1–12.4)*
Control houses	41 (41.8)	57	32.6–51.7	48 (36.0)	85	28.4–44.5	1.3 (0.7–2.1)
National prevalence	3,209 (48.6)	3,396	47.4–49.8	3,744 (45.6)	4,471	44.5–46.7	1.1 (1.1–1.2)*

*Significantly different from 1, p<0.001.

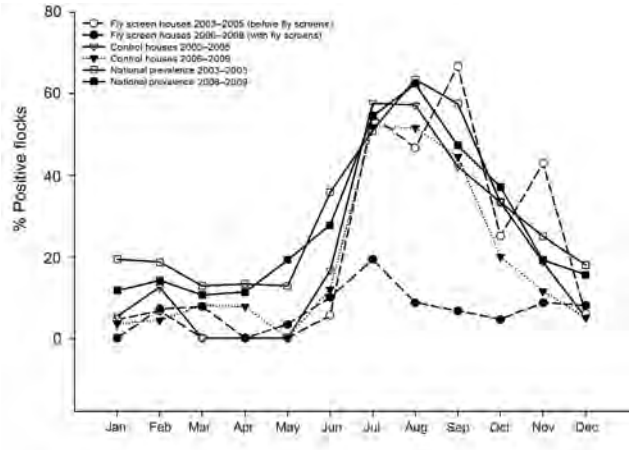

Figure 2. Year-round percentage, by month, of broiler chicken flocks that were *Campylobacter* spp. positive during 2003–2005 (before fly screens) and 2006–2009 (with fly screens).

We are unaware of any studies that have correlated the abundance of flies with the prevalence of *Campylobacter* spp.–positive broiler chicken flocks. Data from field studies suggest, though, that flies play a linking role in the epidemiology of *Campylobacter* spp. infections by transmitting *Campylobacter* spp. to broiler chickens (*9*,*16*,*17*). In agreement, 1 study found that flies outside broiler chicken houses can carry *Campylobacter* spp. and pass through ventilation systems into the broiler chicken houses (*15*). The year-round and long-term data, obtained by blocking access of flies to broiler houses, indicate that flies are responsible for a major part of the *Campylobacter* spp. positivity among broiler chicken flocks during the peak season, June–October (Figure 2). The results also show that fly screens affected *Campylobacter* spp. prevalence only during summer and not winter. This finding agrees with the role of flies as vectors for the transmission of *Campylobacter* spp. because June–September is when the abundance and growth of flies peak, thus increasing the likelihood of transmission (*11*,*14*). Furthermore, the number of flies per animal on pig and cattle farms peaks in July and August (*14*), concurrent with peak *Campylobacter* spp. prevalence for broiler chicken flocks (*3*). The key to understanding these correlations is probably the ambient temperature and humidity. The study by Guerin et al. in Iceland found that temperature played a major role in the colonization of broiler chicken flocks with *Campylobacter* spp. and assumed that *M. domestica* houseflies played a role in the epidemiology and seasonality of *Campylobacter* spp. colonization (*36*).

According to our findings, if prevalence of *Campylobacter* spp. among broiler chicken flocks can be reduced, as we have demonstrated, on a national level, then this would reduce the number of campylobacteriosis cases in humans caused by consumption of broiler chicken meat. Models have predicted that the expected change in prevalence of campylobacteriosis among humans is proportional to a decline in *Campylobacter* spp. prevalence among chicken flocks (*6*,*37*).

According to the scientific opinion published by the European Food Safety Authority Panel on Biological Hazards in 2011 (*2*), placing fly screens in broiler chicken farms that already had a medium level of biosecurity during the rearing period was the intervention strategy calculated to give the highest risk reduction (50% to 90%) in public health. In agreement, we found that an estimated *Campylobacter* spp. positivity of 77% among flocks during summer on the national level would have been prevented through 2006–2009 if fly screens had been part of the biosecurity practice on all broiler chicken farms in Denmark. Combining the fly screen intervention during the rearing period at the farm level with interventions during the slaughter processing should place a substantial improvement in food safety of broiler chicken meat within reach.

Use of fly screens, or other means of fly control, could be an easy and effective way to reduce the number of cases of campylobacteriosis among humans worldwide. However, the degree of success depends on several factors. In general, broiler chicken houses should be under strict biosecurity, otherwise the chickens could become *Campylobacter* spp. positive by other transmission routes. Ventilation systems would also need to be automated to compensate for the slight pressure drop of the airflow through the screen. Any costs of installation and maintenance could limit the adoption of the method. The cost of fly screens has been calculated to be €0.01– €0.02 per kilogram of chicken meat, which would reduce farmers' profits (*38*). On the contrary, fly screens could have other beneficial effects; for instance, fly screens could reduce the prevalence of costly poultry diseases carried by flies. Flies are known to carry other poultry pathogens, such as *Salmonella* spp., *E. coli*, *Pasteurella* spp. and avian influenza virus (*21*,*23*,*39*,*40*). However, such relationships need to be further established and validated by future experiments.

In conclusion, fly screens caused a sustained suppressed prevalence of *Campylobacter* spp. among broiler chicken flocks over 4 years during summer; no seasonal variation was found between summer and winter prevalence among chicken houses with fly screens. Therefore, because the association between *Campylobacter* spp. prevalence among flocks and human health risk has been shown to be linear, fly screens or other equally effective fly control measures might have a substantial reduction effect on the incidence of campylobacteriosis among humans.

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Lack of Norovirus Replication and Histo-Blood Group Antigen Expression in 3-Dimensional Intestinal Epithelial Cells

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Noroviruses (NoVs) are a leading cause of gastroenteritis worldwide. An in vitro model for NoV replication remains elusive, making study of the virus difficult. A previous study, which used a 3-dimensional (3-D) intestinal model derived from INT-407 cells reported NoV replication and extensive cytopathic effects (CPE). Using the same 3-D model, but with highly purified Norwalk virus (NV), we attempted to replicate this study. Our results showed no evidence of NV replication by real-time PCR of viral RNA or by immunocytochemical detection of viral structural and nonstructural proteins. Immunocytochemical analysis of the 3-D cultures also showed no detectable presence of histo-blood group antigens that participate in NV binding and host tropism. To determine the potential cause of CPE observed in the previous study, we exposed 3-D cultures to lipopolysaccharide concentrations consistent with contaminated stool samples and observed morphologic features similar to CPE. We conclude that the 3-D INT-407 model does not support NV replication.

Norovirus (NoV) has been identified as the primary etiologic agent of acute epidemic viral gastroenteritis in industrialized countries (*1*,*2*). Norwalk virus (NV) is the human prototype GI.1 NoV strain; it belongs to the *Caliciviridae* family of positive-sense, single-stranded RNA, nonenveloped viruses (*3*). Human-to-human transmission of NoV occurs primarily through the fecal-oral route, with the small intestine being the initial site of viral replication (*1*,*4*). The lack of an efficient cell culture system in which to study NoV infections has hindered development of antiviral drugs to control or limit NoV outbreaks (*5*).

Currently, animal models are being used to increase our understanding of NoV infections (*6*–*12*); however, no small animal model for NoV mimics the disease manifestations observed in humans (*7*,*11*–*13*). As a result, human outbreaks and volunteer studies have been the primary source for existing knowledge of NV epidemiology and pathogenesis, respectively (*1*,*14*,*15*). A major finding from human NV volunteer studies is that persons with strains containing 2 mutated alleles of the α(1,2) fucosyltransferase (*FUT2*) gene were resistant to NV infection (*16*–*20*). *FUT2* encodes an enzyme that produces histo-blood group antigens (HBGA) on the surface of epithelial cells and in mucosal secretions (*21*,*22*). Persons who lack a functional *FUT2* gene cannot generate ABH antigens in secretions and, thus, are termed nonsecretors (*23*,*24*). HBGA are complex carbohydrates distinguished by different monosaccharides added to a precursor oligosaccharide by fucosyltransferase enzymes (*23*,*24*). In the human gastroduodenal junction where NV has been shown to bind HBGA type 1 (Lewis b [Le^b]), is found exclusively on epithelial surfaces, whereas HBGA type 2 is primarily found at the glandular level (*19*,*24*–*26*). In vitro experiments using NV virus-like particles (VLPs) directly showed NV VLP attachment to HBGA, resulting in VLP internalization into the cell (*19*). In addition, NV VLPs were found to preferentially bind to A and H type 1 and Le^b carbohydrates (*19*,*27*–*29*). From these critical studies, putative NV receptors were identified, and thus it was hypothesized that a successful in vitro cell culture

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system would most likely possess these receptors to support NV replication.

Although most of the understanding of NoV infections in humans has been derived from volunteer studies and authentic sporadic outbreaks, extensive volunteer studies have limitations (variability, cost, and institutional review board considerations). As a result, many attempts have been made to develop a reproducible in vitro model system to culture NoV, but none has yet proved successful (*13*,*30*–*33*). However, one publication has reported establishing a productive NoV infection in a 3-dimensional (3-D) organotypic model of human intestinal epithelium derived from the human embryonic intestinal epithelial cell line INT-407 (*30*). We report here an unsuccessful attempt to replicate these findings using NV.

Materials and Methods

Cell Lines and NV Stocks

INT-407 cells were obtained from the American Type Culture Collection (Manassas, VA, USA) (CCL-6) and grown in GTSF-2 medium (Hyclone, Logan, UT, USA) containing penicillin/streptomycin and amphotericin B. The human colonic adenocarcinoma epithelial cell line (Caco-2; HTB-37) was obtained from the American Type Culture Collection and was cultured in Earle minimum essential medium, supplemented with 10% fetal bovine serum. All cell cultures were grown at 37°C in 5% CO_2.

NV was purified from the stool specimens of infected volunteers by using a CsCl isopycnic ultracentrifugation gradient as described (*5*). Endotoxin levels were determined by using the Pyrotell Gel-Clot method (Associates of Cape Cod Inc., East Falmouth, MA, USA), performed according to the manufacturer's protocol (*31*). NV was inactivated by γ-irradiation at doses of 4.25 kGy at room temperature by using a Gammacell-1000 Irradiator (Best Theratronics Ltd., Ottawa, Ontario, Canada) with a cesium-137 source, at a dose rate of 0.5 kGy/h. The titer of NV RNA in each fraction was determined by immunomagnetic capture reverse transcription (RT) PCR as described (*31*,*34*).

3-D INT-407 Model of Epithelium of Human Small Intestine

The 3-D INT-407 model used in these studies was established by using procedures that were as close as possible to those previously described (*30*). In brief, the INT-407 cells were initially grown as monolayers in GTSF-2 medium (Hyclone) containing penicillin/streptomycin and amphotericin B at 37°C in 5% CO_2 in preparation for seeding into the rotating wall vessel (RWV) bioreactor (*35*). A total of ≈2 × 10^6 cells were subsequently added to the RWV containing 0.25 mg/mL porous Cytodex-3 microcarrier beads (Sigma-Aldrich, St. Louis, MO, USA) at a ratio of ≈10 cells/bead. Cells were then cultured in the RWV at 18–20 rotations/min as described (*30*,*35*). Fresh medium was replenished every 24–72 h, depending on metabolic activity of cultures. Intestinal aggregates were harvested for use in all studies 28–35 days after they were seeded into the RWV.

NV Infection

By using a wide-bore pipette (10-mL wide), mature aggregates in 250 μL of GTSF-2 medium were seeded into a 24-well plate (≈1×10^6 cells/well) and infected with NV at a multiplicity of infection (MOI) of 25 (live or inactivated) for 1 h at 37°C in 5% CO_2. Plates were rocked every 15 min during the 1-h absorption phase and then overlaid with 750 μL of fresh medium. Wells were incubated for 0-, 6-, 24-, 48-, 72-, or 96-h following infection, imaged, and harvested for RNA extraction or immunofluorescence analysis.

NV RNA Extraction

RNA was extracted from samples by using the QIAamp Viral RNA Mini Kit (QIAGEN, Valencia, CA, USA). RNA was treated with DNase (Ambion, Foster City, CA, USA) and converted to cDNA by RT by using the iScript cDNA Synthesis Kit (BioRad, Hercules, CA, USA) according to the manufacturer's instructions. cDNA was stored at −20°C until further analysis.

Quantitative RT-PCR Analysis

Quantitative RT-PCR (qRT-PCR) was carried out in 25 μL of a reaction mixture containing 2.5 μL of cDNA, 12.5 μL of Supermix (BioRad), a 400 nmol/L concentration of each primer designed to bind to the open reading frame 1 and 2 junction (*36*), and 15 pmol of RING1(a)-TaqMan probe and 5 pmol of RING1(b)-TaqMan probe fluorogenic probes for NV genogroup I (GI) detection. PCR amplification was performed with an iQ5 cycler (BioRad) as described (*36*). For each PCR, an NV GI-specific standard curve was generated by a 10-fold serial dilution (10^9–10^3 copies) of purified NV GI cDNA plasmids.

Microscopy Analysis

Phase-contrast images were produced on a Zeiss Axiovert 40 CFL microscope (Carl Zeiss, Thornwood, NY, USA) by using Axiovision 4 software (Carl Zeiss) for image processing. For analysis by confocal microscopy, confluent Caco-2 monolayers, INT-407 monolayers, and 3-D INT-407 aggregates were fixed in 4% paraformaldehyde for 30 min at room temperature. Cells were washed and permeabilized with 0.1% Triton X-100 in Dulbecco phosphate-buffered saline (PBS) and blocked at 37°C for 1 h in 4% bovine serum albumin. Cells were stained with anti-H type 1 antibodies (BG-4 [#SIG-313]; Covance, Princeton, NJ, USA; and UEA-1 lectin [L#8146]; Sigma-Aldrich), anti-H type

2–specific monoclonal antibody (Biogenesis; Poole, UK), anti-Le^a monoclonal antibody (#4861; Immucor Gamma, Atlanta, GA, USA), and anti-Le^b monoclonal antibody (BG-6 [#SIG-3315]; Covance) for 2 h at 37°C at 1:25 dilution. Antiviral protein (VP) 1 and VPg antibodies were used as described (*5*). Unstained control slides and PBS control samples were also used to distinguish background and establish appropriate laser intensity for visualizing experimental slides. Samples were analyzed by using the Zeiss LSM 510 inverted confocal microscope and a 63× objective and Zeiss LSM software.

FUT2 Genotyping Assays

DNA was extracted from 200 μL of INT-407 cell pellets by using the QIAamp DNA Blood Mini Kit (QIAGEN). A region of the *FUT2* gene was amplified, by using the following primers: forward 5′-CCCATCTTCAGAATCACCCTGCCGGTGCTG-3′ and reverse 5′-TCGGCCGGCCCGTGGAAACATCCCC AGGTA-3′, which anneal at positions 280–309 and 535–564, respectively, as described (*18*). Results were analyzed as described; in brief, homozygous amino acid at position 428 or TT at position 385 of the *FUT2* gene defines a secretor-negative genotype (*18*,*31*).

Lipopolysaccharide Treatment

Escherichia coli O111:B4 lipopolysaccharide (LPS) (10^6 EU/mg) was purchased from InvivoGen (LPS-EB; InvivoGen, San Diego, CA, USA). Aggregates were treated with PBS (negative control) or LPS doses ranging from 0.01 to 100.0 μg/mL and incubated for 24 h at 37°C.

Statistical Analyses

Prism software (GraphPad Software, La Jolla, CA, USA) was used to graph all data and to perform statistical analyses. One-way analysis of variance, followed by Bonferroni post hoc analysis, was used to make statistical comparisons for qRT-PCR analysis. For statistical significance, $p<0.05$ was considered significant and $p<0.01$ was considered highly significant.

Results

NV Challenge and CPE-like Effects in 3-D INT-407 Aggregates

Straub et al. (*30*) previously reported the use of RWV-derived 3-D INT-407 models of small intestinal epithelium for successful NV infection, demonstrated in part by cellular vacuolization and detachment after a 24-h challenge with unpurified extracts obtained from human stool specimens that contained genogroup I or genogroup II NoVs (*30*). To test whether exposure to NV led to gross morphologic changes in 3-D INT-407 aggregates, we prepared a highly purified and concentrated (1.63×10^9 RNA genomic copies/μL) stock of NV. (Notably, Straub et al. used virus stocks that were clarified by using a 10,000 molecular weight cutoff filter [*30*]). A γ-irradiated NV stock was used as a negative control for NV infectivity and replication because this treatment inactivates virus but preserves the integrity of the virus and the remaining components of the sample (*31*). Three-dimensional INT-407 aggregates were inoculated with live NV or γ-irradiated NV or mock-inoculated with media alone (uninfected) at an MOI of 25 for 6, 12, and 24 h and monitored for potential CPE by light microscopy (Figure 1). Twelve hours post inoculation (hpi), morphologic changes initially attributed to CPE were observed in the 3-D aggregates. These observed morphologic changes included changes in size, shape, and clumping of cells, and detachment of cells from the beads on which the cells had been cultured. In Figure 1, the arrows indicate dissociated cells. Larger distinct circular beads without attached intestinal cells are more abundant in the samples showing damage. These CPE-like morphologic changes were not observed in the uninfected or inactivated samples, which suggests that the NV stock was able to induce gross morphologic changes in the 3-D intestinal aggregates.

3-D INT-407 Aggregates and NV Replication

To determine whether the CPE-like morphologic changes observed in 3-D INT-407 aggregates were a direct result of NV replication, we performed a kinetic analysis of NV RNA levels by qRT-PCR following NV challenge (Figure 2). The 3-D aggregates were challenged at an MOI of 25 ($\approx4\times10^9$ copies of NV total) with live or γ-inactivated NV or mock-challenged with media alone. At each time point (0, 6, 24, 48, 72, and 96 hpi), cellular pellets (containing possible intracellular and cell-associated virus) and supernatants (containing input and released virus) were harvested for RNA extraction, and viral RNA titers were quantified by qRT-PCR. NV RNA copy numbers (indicative of viral replication) did not significantly ($p>0.05$) increase relative to input copies at any of the time points measured in both cell pellets and culture supernatants (Figure 2). The input virus served as a positive control and for establishing baseline RNA levels for comparison with those of the experimental samples. NV RNA consistently decreased over time in this experiment; however, the differences between these decreases and levels of input virus NV RNA copies over time were not statistically significant for either live or γ-irradiated NV inoculations. These data clearly illustrate that viral RNA levels were not increasing over time as would be expected during a productive viral infection.

To further investigate whether the 3-D aggregates were productively infected, we used confocal immunofluorescence

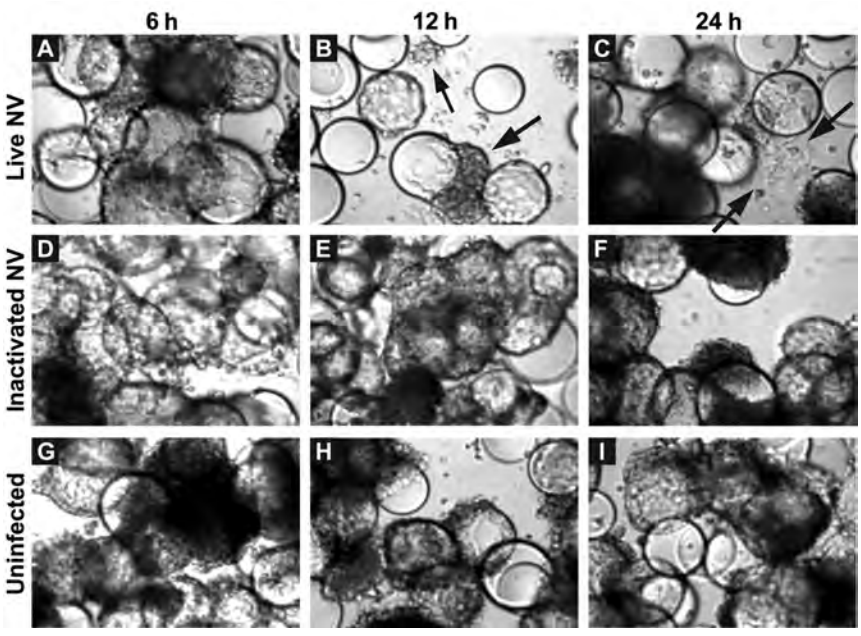

Figure 1. Morphologic changes in Norwalk virus (NV)–infected 3-dimensional INT-407 aggregates. Phase contrast micrographs of 3-dimensional intestinal aggregates cultured in the rotating wall vessel bioreactor and subsequently inoculated with live NV (panels A–C), inactivated NV (γ-inactivated) (panels D–F), or phosphate-buffered saline (mock-uninfected control) (panels G–I) at 6, 12, and 24 h after inoculation. Arrows indicate cells (or cellular debris) that were released from the support beads. The beads appear as large, distinct spheres after the removal of bound cells. Original magnification ×20.

microscopy to analyze 3-D INT-407 aggregates challenged with live NV, γ-irradiated NV, or media alone (mock) and monitored for the presence of NV structural (VP1) and nonstructural (VPg) proteins (Figure 3). During productive NV replication, both structural and nonstructural proteins are detected within cells (*5*). After 24 hpi, VP1 and VPg proteins were detected in the live NV-inoculated aggregates (Figure 3, panels A and B); however, by 48 hpi, evidence for these proteins had markedly diminished and the images resembled the NV-inactivated images (Figure 3, panels C–H). We conclude that the VP1 signal at 24 hpi is most likely a result of input virus and not viral replication, consistent with the qRT-PCR data (Figure 2). The faint VPg signal observed may be nonspecific staining or low-level initial gene expression that does not lead to permissive replication.

3-D INT-407 Aggregates and Surface Carbohydrate Tropism

To better understand why these 3-D intestinal aggregates were not able to productively support NV replication, we characterized them for HBGA expression. NV has been demonstrated to bind to H-type antigens on the surface of mammalian cells, and this binding has been hypothesized to be the mechanism for NV to enter human cells (*5*,*16*,*17*,*19*,*21*). To determine whether 3-D intestinal aggregates contain the *FUT2* gene that converts precursor molecules to H-type antigens (*21*), we performed genotyping analysis on the INT-407 intestinal cells used to produce the organotypic cell culture model. The intestinal cell line was shown to contain the genetic determinants to express a functional *FUT2* (data not shown); therefore, the INT-407 cell line (used to make the 3-D intestinal model) is likely a secretor-positive cell line with the potential to express the H-type receptors necessary for NV binding and, presumably, for infection. However, after performing H-type antigen phenotyping by confocal immunofluorescence microscopy on monolayers and 3-D differentiated intestinal cells, we were unable to detect H type 1 on the surface of the cells by using 2 antibodies specific for different regions of the antigen (Figure 4) (*5*). Further confocal analysis of the INT-407 cells also showed no expression of the other carbohydrate antigens involved in binding of other genogroups of NoV, H type 2, Le^b, although minimal antigen expression of Le^a was observed. Caco-2 cells are shown as a positive

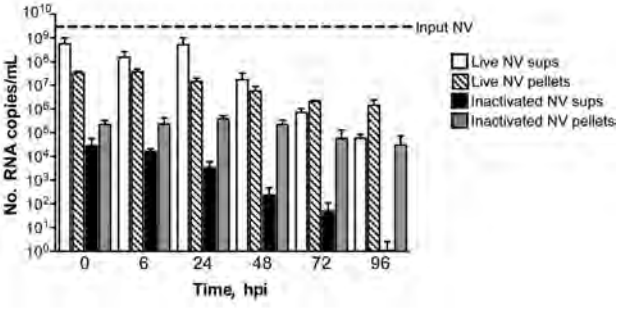

Figure 2. No evidence of productive Norwalk virus (NV) replication in 3-dimensional intestinal aggregates by quantitative reverse transcription PCR analysis. Supernatants (sups) and cell pellets were harvested for RNA at 0, 6, 24, 48, 72, and 96 hours postinoculation (hpi) with live and inactivated NV and analyzed by quantitative reverse transcription PCR. There was no significant increase ($p>0.05$) in NV RNA copy number over time in the supernatants or cell pellets relative to input virus. Error bars indicate SEM.

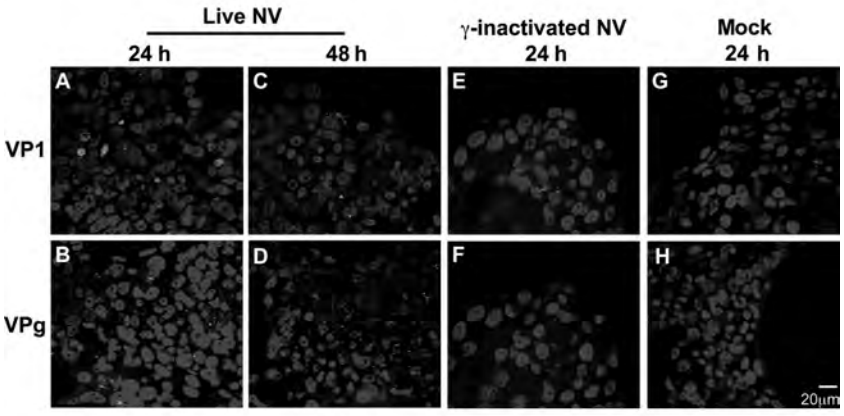

Figure 3. No evidence of productive Norwalk virus (NV) replication in 3-dimensional INT-407 aggregates by confocal microscopy analysis of viral proteins. Three-dimensional INT-407 aggregates 24 h post inoculation (hpi) (panels A, B) and 48 hpi (panels C, D) with live NV, 24 hpi with inactivated NV (panels E, F), or phosphate-buffered saline alone controls (panels G, H). Aggregates were stained for viral capsid protein 1 (VP1) (panels A, C, E, G) or nonstructural protein VPg (panels B, D, F, H). Nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI). Original magnification ×63. A color version of this figure is available online (wwwnc.cdc.gov/EID/article/19/3/12-1029-F3.htm).

control for staining of the surface glycans (*5*). These data demonstrate that both monolayer and 3-D intestinal models lack sufficient expression of the cell surface receptors believed to be required for susceptibility to NV infection.

LPS and CPE-like Morphologic Changes in 3-D INT-407 Aggregates

Our data clearly demonstrate that the 3-D intestinal aggregates do not support a productive NV replication; however, we did observe CPE-like morphologic changes after treatment with highly purified NV (Figure 1). One potential cause of these morphologic changes in the 3-D intestinal aggregates could be the presence of contamination of the NV stocks with a bacterial endotoxin, LPS, or other potentially cytotoxic molecules commonly found in human stool samples (the source of virus used in our studies and those of Straub et al. [*30*]). The highly purified virus preparations used in this study were determined to contain low LPS levels, resulting in 10 ng/mL and 1 ng/mL of LPS for live NV and γ-irradiated NV stocks, respectively, added to the aggregates on NV inoculation. LPS is a known cytotoxic inflammation-inducing product; we therefore hypothesized that the contaminating LPS could be a potential cause of the CPE-like morphologic changes observed after NV inoculation.

To test whether LPS alone induces comparable CPE-like effects at relevant concentrations in the 3-D intestinal aggregates, we treated the aggregates with increasing amounts of LPS (InvivoGen) and monitored for alterations

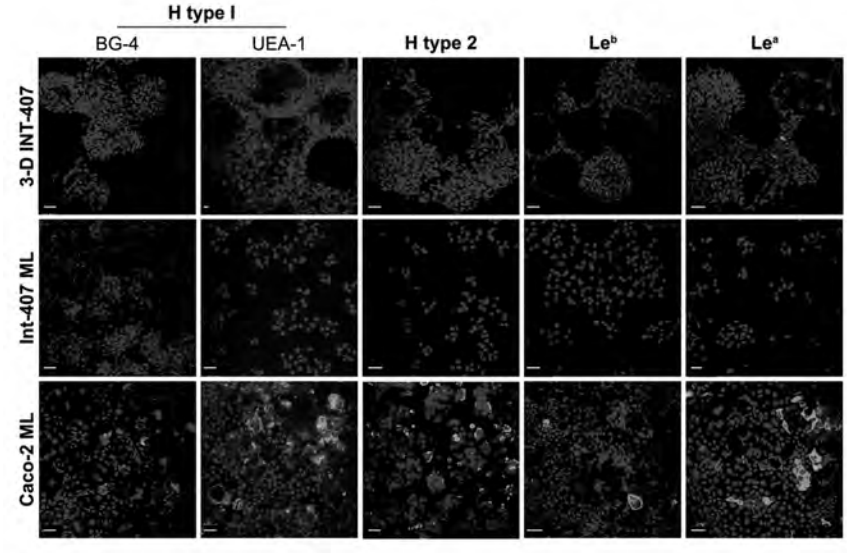

Figure 4. Three-dimensional (3-D) INT-407 intestinal cell aggregates do not express the histo-blood group antigens important for Norwalk virus (NV) attachment. Monolayer (ML) INT-407, 3-D INT-407 aggregates, and Caco-2 ML (positive control) were labeled with 2 different H type 1 (BG-4 and UEA-1) antibodies and with antibodies against H type 2, Le^b, and Le^a and imaged by confocal immunofluorescence microscopy. Nuclei were counterstained with 4′,6-diamidino-2-phenylindole (DAPI). Scale bars = 50 μm. A color version of this figure is available online (wwwnc.cdc.gov/EID/article/19/3/12-1029-F4.htm).

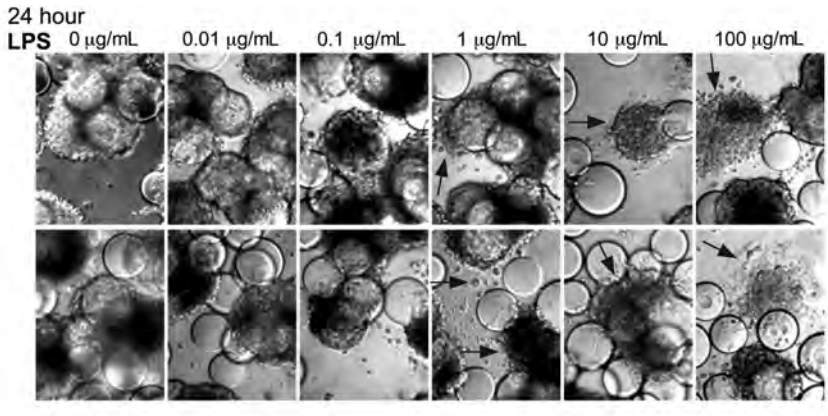

Figure 5. Lipopolysaccharide (LPS) induces morphologic changes consistent with cytopathic effects in Norwalk virus–inoculated 3-dimensional INT-407 aggregates. Two independent sets of light microscopy images show 3-D intestinal aggregates treated with increasing concentrations of LPS for 24 h. Arrows indicate cells (or cellular debris) that were released from the support beads. Original magnification ×20.

in morphology indicative of cytotoxicity (Figure 5). After 24 h, aggregates treated with a range of LPS concentrations (0.01–100.0 μg/mL) exhibited increasing signs of CPE, as shown by cellular dissociation from the support beads and a partial loss of cell viability (online Technical Appendix Figure 1, wwwnc.cdc.gov/EID/article/19/3/12-1029-Techapp1.pdf). In comparison with the morphologic changes shown in the technical appendix figure (observed with NV addition to the aggregates), we found that LPS at levels of ≈1 μg/mL was able to induce the CPE-like morphologic changes equivalent to those induced by inoculation of nonpurified NV. Although these levels are higher than the measured level of LPS in the purified NV samples we used, there might also be additional cytotoxic contaminants that were carried from the infected patients' stool specimens. When these data are taken together, we interpret them as indicating the CPE-like morphologic changes were caused by stool-derived NV samples (online Technical Appendix Figure and [*30*]) contaminated with LPS, and perhaps other cytotoxins, and were not a result of virus replication.

Discussion

Using the same RWV bioreactor cell culture system described by Straub et al. (*30*) to generate well-differentiated 3-D organotypic intestinal epithelial aggregates derived from INT-407 cells, we attempted to validate the published report and further characterize the 3-D intestinal model for NV infection studies. Following a 24-h inoculation of the 3-D INT-407 aggregates with highly purified NV stock, we observed similar morphologic changes to the cells as previously reported (*30*). However, the CPE-like effects observed did not correlate with NV replication, because no measurable increase in viral RNA occurred in a period up to 96 hpi. Rather, CPE-like effects can be mimicked by addition of a known component of human stool samples—LPS. In addition, no increase in viral (structural or nonstructural) proteins could be detected within the aggregates after challenge with NV. Overall, these results support our hypothesis that morphologic changes of the 3-D aggregates after NV inoculation, as observed herein and by Straub et al. (*30*), were most likely a result of LPS within the virus inocula and not due to NV replication.

Our observed lack of HBGA carbohydrates on the surface of the intestinal cells that participate in NV binding to mammalian cells further highlights that this model is likely not a sufficient model system to study NV replication because the cells do not express the putative NV receptor. Previous studies determined that overexpression of FUT2 in natively nonexpressing or low-expressing cell types, or upon differentiation of Caco-2 cells that natively express FUT2, results in a dramatic increase in NV particle binding (*5*,*19*,*32*,*37*,*38*). However, this binding was not sufficient to result in NV infection and replication within these cell types (*5*). Transfecting cells expressing FUT2 with NV RNA (isolated from virus from the stool samples of infected volunteers) did result in NV replication and synthesis of NV proteins within the cells, suggesting that the block in NV replication following virus addition to the cells occurs at a postbinding step and may require an additional cellular factor for viral entry and/or uncoating (*5*,*32*,*38*,*39*). Recently, Lay et al. took a different approach to culture NV: they used primary macrophages and dendritic cells from secretor-positive persons/donors to test their ability to support a NV infection (*31*). Unlike the case with murine NoV, these primary immune cells were unsuccessful in supporting NV infection (*11*,*31*). Future attempts to develop in vitro systems to study NoV replication may include cultures derived from human primary intestinal cells and tissue explants, utilization of Caco-2 cells grown in RWV, use of 3-D intestinal immunocompetent coculture models, or human intestinal organoids derived from pluripotent stem cells (*40*,*41*). These models alone, or in combination, may provide a meaningful way to obtain a system(s) to support productive NoV infection and replication and thus serve as useful tools for studying NoV.

Acknowledgments

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Dr Herbst-Kralovetz is an assistant professor in the Department of Basic Medical Sciences at the University of Arizona, College of Medicine, Phoenix. Her research interests include the use of human mucosal models to analyze host–microbe interactions and the study of novel mucosal immunization strategies for the development of subunit-based vaccines, including NoV VLP.

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Effects of Vaccine Program against **Pandemic Influenza A(H1N1) Virus,** United States, 2009–2010

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In April 2009, the United States began a response to the emergence of a pandemic influenza virus strain: A(H1N1) pdm09. Vaccination began in October 2009. By using US surveillance data (April 12, 2009–April 10, 2010) and vaccine coverage estimates (October 3, 2009–April 18, 2010), we estimated that the A(H1N1)pdm09 virus vaccination program prevented 700,000–1,500,000 clinical cases, 4,000–10,000 hospitalizations, and 200–500 deaths. We found that the national health effects were greatly influenced by the timing of vaccine administration and the effectiveness of the vaccine. We estimated that recommendations for priority vaccination of targeted priority groups were not inferior to other vaccination prioritization strategies. These results emphasize the need for relevant surveillance data to facilitate a rapid evaluation of vaccine recommendations and effects.

On April 26, 2009, the United States declared a public health emergency in response to the 2009 pandemic influenza A(H1N1)pdm09 virus (*1*). The Centers for Disease Control and Prevention (CDC) estimated that in the United States during April 12, 2009–April 10, 2010, there were 61 million clinical cases of influenza and that 274,000 persons were hospitalized and 12,500 died (*2*). For the purpose of this study, we considered clinical cases as influenza-like illness in persons who did or did not seek medical care (*2*).

The US Food and Drug Administration approved multiple formulations of monovalent inactivated, unadjuvanted influenza vaccine, and a monovalent live attenuated vaccine against A(H1N1)pdm09 virus in mid-September 2009 (*3*); a national vaccination program was initiated in October (*4*). In July 2009, estimating that initial vaccine supplies could be insufficient to meet demand, the Advisory Committee on Immunization Practices (ACIP) recommended priority groups for the vaccination program. These priority groups included pregnant women, household contacts and caregivers of children <6 months of age, health care and emergency medical services personnel, all persons 6 months–24 years of age, persons <19 years of age who were receiving long-term aspirin therapy, and persons 25–64 years of age who had health conditions associated with a higher risk for medical complications from influenza. Such complications include asthma; neurodevelopmental conditions; chronic lung disease; heart disease; blood, endocrine, kidney, liver, and metabolic disorders, and a weakened immune system. (*5*,*6*). When a vaccine against the pandemic strain was released for initial use, the supply was only 25%–50% of the amount that had been projected because vaccine production yields were lower than expected (*7*,*8*). By January 2010, when 100 million doses had been delivered and an estimated 57 million doses had been administered (*9*), most states were offering vaccination to anyone ≥6 months of age. By February 2010, 125 million doses, most of which were inactivated, had been made available and ≈69 million persons had been vaccinated (*4*,*9*,*10*). Final estimates indicated that by the end of May 2010, ≈81 million persons had been vaccinated and 90 million doses had been administered (*11*).

We estimated the number of clinical cases, hospitalizations, and deaths prevented in the United States that were directly attributable to the 2009–2010 A(H1N1)pdm09 virus vaccination program. These results can be used by public health officials, policy makers, and the public to evaluate this program and plan for the management of future pandemics.

Methods

Calculation Overview

Using Excel (Microsoft Corp., Redmond, Washington, USA), we developed a tool to estimate the effects of the

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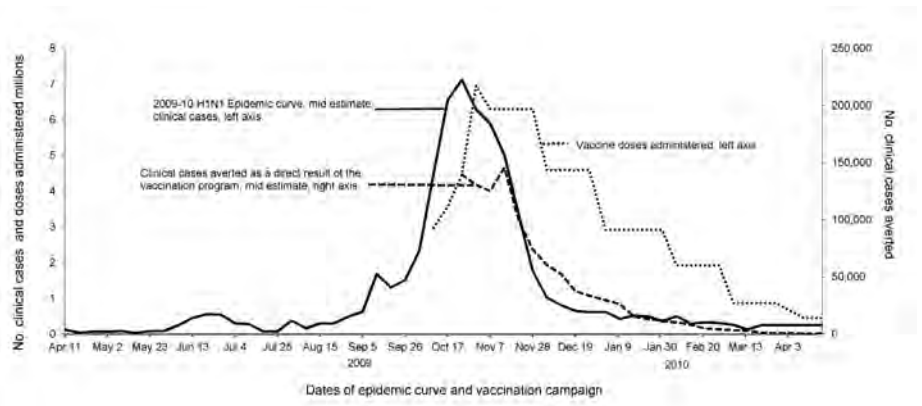

Figure 1. Weekly number of clinical cases of influenza A(H1N1)pdm09 virus infection, the number of vaccine doses administered, and the estimated number of cases averted over time because of the vaccination program. Midranges shown for epidemic curve and clinical cases; ranges provided in Table 3.

vaccination program (online Technical Appendix, wwwnc.cdc.gov/EID/article/19/3/12-0394-Techapp1.xlsx). The estimate was based on the actual epidemic curve in the United States, which included the effects of the vaccination program. We divided the US population into 8 subgroups: 1) persons 6 months–9 years of age; 2) persons 10–24 years (all persons 10–17 years of age and persons 18–24 years, not pregnant); 3) pregnant women, 18–64 years; 4) persons 25–64 years, high risk, not pregnant; 5) persons 25–64 years, health care workers, non–high risk, not pregnant; 6) persons 25–64 years who had contact with a child <6 months of age, non–health care worker, non–high risk, not pregnant; 7) persons 25–64 years who did not have contact with a child <6 months of age, non-health care worker, non-high risk, not pregnant; and 8) persons ≥65 years.

First, we calculated the weekly number of vaccine doses administered within each population subgroup. We then estimated, using the existing epidemic curve, the probability that a person who was vaccinated had not previously been infected with A(H1N1)pdm09 virus and had a clinical or subclinical case and the probability that a person would be infected during the remaining portion of the season. We adjusted our estimates for a 2-week delay in protection against the virus after vaccine administration (*12*). In this initial calculation (phase 1), we based the probability of infection on the actual epidemic curves during the pandemic, April 11, 2009–April 18, 2010 (*13*) (Figure 1), because those were the best sources of data available. This calculation included the effects of the vaccination program, as described below in Equations 1a and 1b.

During phase 2 of the calculation, we adjusted the probabilities of infection over time to include the number of averted events by including the event prevented (i.e., clinical cases, hospitalizations, or deaths) in the epidemic curve (see Equations 2a, 2b below). Our original epidemic curve included the effects of the actual vaccination program; therefore, it was necessary to repeat the second phase (i.e., continue to add the number of clinical events into the epidemic curve) until the number of events in the final epidemic curve (final estimate from Equation 2b), minus the number of events prevented (final estimate from Equation 2a), exactly matched the epidemic curve that existed during the pandemic, week by week, for each population subgroup. This enabled us to estimate the direct effects of the vaccination program.

Table 1. Data used to calculate effects of vaccination program against influenza A(H1N1)pdm09 virus by population subgroup*

Subgroup	Population	% Vaccinated	No. doses recommended for full coverage	% Assumed outcomes: Vaccine effectiveness†	% Assumed outcomes: Clinical cases and hospitalizations	% Assumed outcomes: Deaths
6 mo–9 y	39,429,115	1st dose/45, 2nd dose/23	2 doses, 4 wks apart	1st dose/0, 2nd dose/62	20.1	6.0
10–24 y (10–17 all, 18–24 NP)	59,684,833	27	1	62	22.3	8.0
Pregnant 18–64 y	5,578,782	43	1	62	2.2	4.0
25–64 y, HR, NP	33,949,395	27	1	62	13.5	24.0
25–64 y, HCW, non-HR, NP	17,451,921	36	1	62	5.6	9.0
25–64 y, contact <6 mo, non-HCW, non-HR, NP	8,933,718	23	1	62	2.5	6.0
25–64 y, non-contact <6 mo, non-HCW, non-HR, NP	96,235,755	16	1	62	24.0	30.0
≥65 y	37,989,965	28	1	43	9.8	13.0
Total	299,253,484	27	1–2	NA	NA	NA
References	(*14,28,39*)	(*9,14,28*)	(*6*)	(*24,36,40*)	(*2,19–21*)	(*2,19–21*)

*NP, not pregnant; HR, high risk; HCW, health care worker; contact, household contacts and caregivers of children <6 months of age; NA, not applicable.
†Data are for effectiveness against clinical cases, hospitalizations, and deaths. For population subgroup 6 mo–9 y, we assumed the vaccine reached effectiveness levels 2 wk after full coverage (*12*).

Table 3. Estimated number of cases of influenza prevented by vaccination against influenza A(H1N1)pdm09 virus*

Subgroup	No. clinical cases in absence of vaccination program (range)	No. clinical cases prevented by a vaccination program (range)	No. doses administered to avoid 1 clinical case (range)
6 mo–9 y	12,333,906 (8,766,004–18,088,655)	81,518 (52,081–100,349)	326 (265–511)
10–24 y (10–17 all, 18–24 NP)	13,891,877 (9,879,008–20,374,801)	300,724 (212,953–420,991)	53 (38–75)
Pregnant 18–64 y	1,410,032 (1,004,978–2,062,896)	71,601 (53,084–97,884)	34 (25–45)
25–64 y, HR, NP	8,378,054 (5,957,746–12,286,626)	164,958 (116,575–228,593)	56 (40–79)
25–64 y, HCW, non-HR, NP	3,530,341 (2,510,291–5,178,995)	123,427 (87,287–177,144)	51 (35–72)
25–64, contact with <6 mo, non-HCW, non-HR, NP	1,550,007 (1,101,603–2,276,098)	29,063 (19,904–43,129)	70 (47–102)
25–64 y, noncontact with <6 mo, non-HCW, non-HR, NP	14,734,336 (10,470,235–21,640,930)	163,327 (107,305–248,548)	94 (62–143)
≥65 y	6,038,353 (4,290,972–8,868,687)	94,538 (63,719–142,293)	114 (76–169)
Total	61,866,905 (43,980,837–90,777,687)	1,029,157 (712,908–1,458,930)	86 (61–124)

*All values are estimates. NP, not pregnant; HR, high risk; HCW, health care worker; contact, household contacts and caregivers of children <6 mo of age.

Data

Demographics

The population in each ACIP-defined prioritized target group was estimated by using the National 2009 H1N1 Flu Survey (NHFS) (*14–16*) and CDC's 2008–09 projected influenza vaccination target population sizes (*17*) (Table 1). The total population of pregnant women over the course of the pandemic was based on data from Moro et al. (*18*).

Clinical Cases, Hospitalizations, and Deaths

Three influenza surveillance systems in the United States were used to estimate the incidence and outcomes of A(H1N1)pdm09; the detailed methods are published in Shrestha et al. (*2*). The ranges of our data are based on the ranges of these epidemic curves (Figure 1). We reviewed published estimates and expert opinion (*2, 19–21*) (Table 1) to estimate the proportion (Table 1) and thus the incidence over time (used in Equation 1a) of A(H1N1)pdm09-related clinical cases, hospitalizations, and deaths for each population subgroup after being vaccinated,.

Vaccine-related

Our estimates of vaccination coverage were based on combined monthly data from the NHFS and the Behavioral Risk Factor Surveillance System survey (*9,22*). Children <10 years of age required 2 doses; we assumed that children who received their second dose received it 4 weeks after their first dose (*23*) (Table 1).

Our estimates of vaccine effectiveness are based on studies from Europe and China (*24–27*) and expert opinion based on unpublished internal CDC studies (Table 1). On the basis of these data, we assumed that the vaccine was 62% effective in protecting against clinical cases, hospitalizations, and deaths for all population subgroups except for persons ≥65, for whom we assumed the vaccine to be 43% effective (Table 1). To date, there are no published data from the United States that reflect calculations of vaccine effectiveness of an unadjuvanted A(H1N1)pdm09 virus vaccine on clinical cases, hospitalizations, or deaths. We further assumed that persons vaccinated were not protected from the A(H1N1)pdm09 virus until 2 weeks after the final dose (1 dose for persons ≥10 years, 2 doses for children <10 years) (*12*). We estimated the number of persons vaccinated, by population subgroup, based on data reported to CDC in the NHFS and the Behavioral Risk Factor Surveillance System survey October 3, 2009–April 18, 2010 (*4,9,10,15,22,28*) (Table 2, Appendix, wwwnc.cdc.gov/EID/article/19/3/12-0394-T2.htm).

Estimation of Prior Protection of Vaccinated Persons

Our estimates of the number of persons already immune to the A(H1N1)pdm09 virus are based on data for April 12, 2009–April 10, 2010, and an assumed proportion of subclinical cases; we did not include protection from previous years. For our base estimate, we assumed that 30% of all cases were subclinical; this assumption was held constant throughout the pandemic among all subgroups. Data from numerous countries and influenza challenge studies indicate that 24%–36% of the A(H1N1)pdm09 virus cases were subclinical (*29–36*). We tested the effect of this assumption in our sensitivity analysis. We also assumed that persons who were vaccinated had the same probability of prior infection as the general population.

Equations

We used the following equations to calculate clinical cases prevented. The equations for prevention of hospitalizations and deaths are identical, except that prior clinical or subclinical infections were not included.

Equation 1a

Interim estimated clinical cases prevented by a vaccination program (by population subgroup, at specific points in time, Phase 1) = Doses administered (using estimates from the 2 weeks prior to a specific date) × probability of not having

Table 4. Estimated number of hospitalizations prevented by vaccination against influenza A(H1N1)pdm09 virus*

Subgroup	No. hospitalizations of persons in groups with no vaccination program (range)	No. hospitalizations prevented by a vaccination program (range)	No. doses administered to avoid 1 hospitalization (range)
6 mo–9 y	54,745 (38,826–80,563)	614 (328–1,090)	43,333 (24,421–81,227)
10–24 y, (10–17 all, 18–24 NP)	63,117 (44,761–92,999)	1,838 (1,179–3,032)	8,654 (5,246–13,489)
Pregnant, 18–64 y	6,481 (4,590–9,582)	446 (298–722)	5,396 (3,336–8,072)
25–64 y, HR, NP	38,060 (26,990–56,074)	1,029 (653–1,707)	8,972 (5,409–14,132)
25–64 y, HCW, non-HR, NP	16,082 (11,394–23,734)	721 (469–1,181)	8,679 (5,294–13,324)
25–64, contact with <6 mo, non-HCW, non-HR, NP	7,020 (4,981–10,338)	163 (104–270)	12,478 (7,528–19,516)
25–64 y, noncontact with <6 mo, non-HCW, non-HR, NP	67,249 (47,743–98,922)	902 (558–1,516)	17,005 (10,124–27,524)
≥65 y	27,789 (19,723–40,901)	527 (334–876)	20,444 (12,305–32,278)
Total	280,544 (199,009–413,112)	6,240 (3,923–10,393)	14,193 (8,522–22,575)

*All values are estimates. NP, not pregnant; HR, high risk; HCW, health care worker; contact, household contacts and caregivers of children <6 mo of age.

had a prior clinical or subclinical infection (based on original pandemic data) × probability of having a future clinical infection (based on original pandemic data) × vaccine effectiveness

Equation 1b

Interim epidemic curve = Original epidemic curve + Estimated cases prevented (Equation 1a)

Equation 2a

Estimated cases prevented by a vaccination program (by population subgroup, at specific points in time, Phase 2+) = Doses administered (using the estimates from the 2 weeks prior to a specific date) × probability of not having had a prior clinical or subclinical infection (based on Interim epidemic curve, Equation 1b) × probability of having a future clinical infection (based on Interim epidemic curve, Equation 1b) × vaccine effectiveness

Equation 2b

Epidemic curve without a vaccination program = Interim epidemic curve (Equation 1b) + Estimated cases prevented by a vaccination program (Equation 2a)

Equation 2c

Final check: Estimated number of clinical cases prevented by a vaccination program (final outcome from final repetition of Equation 2a) = Final Epidemic Curve (final adjustment from Equation 2b) – Original epidemic curve (with a vaccination program)

Equation 3

Number needed to treat = number of doses administered/number of medical events (i.e., clinical cases, hospitalizations, or deaths) averted

Sensitivity Analyses

We conducted sensitivity analyses for 8 scenarios (see below); for each scenario the epidemiologic curve used was identical to that for our base case estimates, assuming that a vaccination program did not exist (Figure 1). For all scenarios except scenario 5, the total number of doses administered each week was the same as the number in our base estimate. We assumed that no children 6 months–9 years of age could have received their second dose until the fifth week of the vaccination program. Therefore, for scenarios 1–4, we assumed that only first doses were administered to children in this age group during the first 4 weeks.

Scenario 1: Even Distribution over Time

To assess the effects of accelerated vaccine uptake among specific groups, we calculated the proportion of total doses administered among each population subgroup over the course of the pandemic. We multiplied the result by the number of doses administered each week; e.g., if a subgroup received a total of 20% of the doses, we assumed that they received 20% each week.

Scenario 2: Population Proportions

We assumed that each population subgroup had a proportionately equal demand for the vaccine. For each subgroup, we set the proportion of vaccine equal to the population proportion (e.g., if a population subgroup represented 10% of the populations, we assumed that the subgroup would be administered 10% of the doses each week).

Scenario 3: 2008 Distribution

We used the proportion of doses administered among each subgroup during the 2008 seasonal vaccination

Table 5. Estimated number of deaths prevented by vaccination against influenza A(H1N1)pdm09 virus*

Subgroup	No. deaths without a vaccination program (range)	No. deaths prevented due to a vaccination program (range)	No. doses administered to avoid 1 death (range)
6 mo–9 y	759 (538–1,117)	9 (5–15)	3,087,138 (1,745,154–5,761,939)
10–24 y, (10–17 all, 18–24 NP)	1,028 (729–1,514)	30 (20–50)	525,012 (319,229–814,797)
Pregnant 18–64 y	533 (378–789)	37 (25–60)	64,787 (40,177–96,492)
25–64 y, HR, NP	3,077 (2,182–4,533)	84 (54–139)	109,638 (66,300–171,951)
25–64 y, HCW, non-HR, NP	1,175 (833–1,735)	53 (35–87)	117,312 (71,786–179,325)
25–64, contact with <6 mo, non-HCW, non-HR, NP	766 (544–1,128)	18 (12–30)	112,945 (68,351–175,889)
25–64 y, noncontact with <6 mo, non-HCW, non-HR, NP	3,792 (2,692–5,578)	52 (32–86)	297,838 (177,870–479,999)
≥65 y	1,653 (1,173–2,433)	32 (20–53)	339,494 (204,961–533,711)
Total	12,783 (9,069–18,826)	315 (201–520)	281,305 (170,343–439,832)

* All values are estimates. Vaccinations beginning at week 40 with a distribution of the vaccines as outlined in Table 2, Appendix, wwwnc.cdc.gov/EID/article/19/3/12-0394-T2.htm. NP, not pregnant; HR, high risk; HCW, health care worker; contact, household contacts and caregivers of children <6 mo of age.

campaign. That is, if a population subgroup received 15% of the doses in 2008, we assumed that they used 15% of the doses each week during the 2009 pandemic).

Scenario 4: 2009 ACIP Priority Subgroups

To assess the effects of providing the vaccine only to the aforementioned 2009 ACIP priority subgroups, we used the total percentage of doses administered to each group, based on the total 2009 vaccine uptake estimates, but adjusted the denominator of total doses by excluding the non-ACIP priority subgroups. We applied that percentage to the total number of doses administered each week.

Scenario 5: Accelerated Start Date

We estimated the effects of moving the start date of the vaccination program to begin 8 weeks to 1 week earlier. We did this by moving the date forward in increments of 1 week.

Scenario 6: Vaccine Effectiveness

We examined the outcomes of assuming different vaccine effectiveness. We initially increased vaccine effectiveness to 85% for all health outcomes in population subgroups, except those including persons ≥65 years of age, for which we increased the effectiveness to 55% for all outcomes. Last, we assumed vaccine effectiveness at 40% for all health outcomes in all population subgroups, except those including persons ≥65 years of age, for which we assumed 20% effectiveness for all outcomes.

Scenario 7: Effectiveness of First Dose for Children

We examined the effects of assuming that, among vaccinated children 6 months–9 years of age, the first dose of vaccine was 20%–40% effective 2 weeks after administration and that vaccine effectiveness reached the levels listed in Table 1 by 2 weeks after the second dose was administered. Some evidence in the published literature shows that 1 dose might have provided some protection (*37*).

Scenario 8: Proportion of Subclinical Cases

We varied the range of subclinical cases from 0% to 50%. The base estimate was 30%.

Results

Health Effects of Vaccination Program

We estimate that during October 3, 2009–April 18, 2010, the A(H1N1)pdm09 virus vaccination program directly prevented 712,908–1,458,930 clinical cases of A(H1N1)pdm09 infection, 3,923–10,393 hospitalizations, and 201–520 deaths (Tables 3–5). Based on the number of patients who needed to be treated to prevent 1 additional bad outcome, the vaccination program, as implemented, had the most value for pregnant women and for persons in the ACIP target group who were 25–64 years of age (Tables 3–5).

Effects of Targeting Subgroups

The estimated numbers of clinical cases prevented under different (assumed) prioritization strategies are shown in Table 6. In the 4 sensitivity scenarios related to prioritization strategies, the ranges of estimated total cases prevented overlap substantially. However, the effect on each population subgroup varies considerably. For example, if we focus solely on children ≤9 years, we estimate that during the 2009 pandemic ≈81,518 (range 52,081–100,349) A(H1N1)pdm09 infections were prevented among this population subgroup. However, by entering the same number of doses and same effectiveness, but adjusting the timing of administration by group (Scenario 1), we calculated that the number of cases prevented in this population subgroup would increase to ≈131,000 (range 91,000–164,000). In Scenario 2, in which we assumed children 6 months–9 years of age received 9% of all vaccines administered (i.e., population proportional),

Table 6. Sensitivity analysis showing number of clinical cases prevented by vaccination against influenza A(H1N1)pdm09 virus for different scenarios of vaccine distribution*

Subgroup	Base case estimate (range)†	Scenario 1: even distribution over time (range)‡	Scenario 2: distribution based on population proportion (range)§	Scenario 3: 2008 distribution (range)¶	Scenario 4: ACIP priority subgroups (range)#
6 mo–9 y	81,518 (52,081–100,349)	131,170 (90,932–164,352)	57,511 (39,869–72,060)	65,093 (45,125–81,559)	186,041 (128,970–233,103)
10–24 y (10–17 all, 18–24 NP)	300,724 (212,953–420,991)	279,715 (196,606–392,577)	310,656 (218,355–436,003)	249,981 (175,708–350,847)	396,725 (278,851–556,801)
Pregnant, 18–64 y	71,601 (53,084–97,884)	44,486 (31,726–60,936)	30,506 (21,756–41,787)	14,809 (10,561–20,285)	63,096 (44,998–86,427)
HR, 25–64 y	164,958 (116,575–228,593)	168,521 (119,243–233,197)	183,417 (129,784–253,810)	73,157 (51,765–101,234)	239,017 (169,125–330,749)
HCW, 25–64 y	123,427 (87,287–177,144)	100,229 (69,407–144,610)	82,764 (57,313–119,413)	41,099 (28,460–59,297)	142,157 (98,441–205,104)
Contact with <6 mo	29,063 (19,904–43,129)	28,861 (19,686–42,794)	37,583 (25,634–55,726)	151,525 (103,351–224,675)	40,935 (27,920–60,696)
25–64 y (all others)	163,327 (107,305–248,548)	197,372 (133,316–297,625)	366,354 (247,455–552,439)	278,226 (187,928–419,547)	0
≥65 y	94,538 (63,719–142,293)	99,116 (67,121–148,741)	103,402 (70,023–155,172)	197,547 (133,778–296,454)	0
Total	1,029,157 (712,908–1,458,930)	1,049,470 (728,037–1,484,834)	1,172,194 (810,188–1,686,411)	1,071,437 (736,676–1,553,899)	1,067,971 (748,306–1,472,881)
			Assumed % distribution by week**		
6 mo–9 y, 1st dose††	20	20	9	10	28
6 mo–9 y, 2nd dose	10	10	4	5	14
10–24 y (10–17 all, 18–24 NP)	18	18	20	16	25
Pregnant, 18–64 y	3	3	2	1	4
HR, 25–64 y	10	10	11	5	15
HCW, 25–64 y	7	7	6	3	10
Contact with <6 mo	2	2	3	12	3
25–64 y (all others)	17	17	32	24	0
≥65 y	12	12	13	24	0

*Data reflect calculations made in scenarios 1 –4. ACIP, Advisory Committee on Immunization Practices; NP, not pregnant; HR, high risk; HCW, health care worker; contact, household contacts and caregivers of children <6 mo of age.
†Total number of doses administered to each population subgroup (Table 2, Appendix, wwwnc.cdc.gov/EID/article/19/3/12-0394-T2.htm).
‡For each population subgroup, this scenario assumes that the group received the same proportion of the total number of doses; the proportions were applied to the total number of doses administered each week (Table 2, Appendix).
§ It was assumed that the distribution of vaccines was proportional to the population.
¶Distribution of vaccine was based on estimates of estimated 2008 seasonal vaccine uptake (*17*,*38*).
#Distribution of vaccine was based exclusively on ACIP priority groupings. The proportion of doses administered was based on the proportion of doses administered to persons in each of the subgroups during the A(H1N1)pdm09 virus vaccination program, while excluding the non ACIP subgroups.
**In scenarios 1–4, the epidemiologic curve was based on the estimated A(H1N1)pdm09 vaccination curve, for which no vaccination program was assumed (Figure 1). We also assumed that the total number of vaccines administered each week remained exactly the same as outlined in Table 2, Appendix.
††For scenarios 1–4, we assumed that the 6 mo– 9 y age group required 2 doses and that a 4 wk delay was required between the first and second dose. We also assumed that no children 6 mo–9 years of age could have received their second dose until the fifth week of the vaccination program. Therefore, any doses during the first 4 wk that would have been proportioned as a second dose were added as a first dose.

cases prevented decreased to ≈58,000 (range 40,000–72,000). If no changes had been made to the ACIP recommendations and the rate of vaccine uptake among the different population subgroups had been similar to uptake of the 2008 seasonal influenza vaccine (*17*,*38*) (Scenario 3), we would expect the number of cases prevented among children ≤9 years of age to be ≈65,000 (range 45,000–82,000). This would have been ≈80% of what was estimated during the A(H1N1)pdm09 pandemic. This projected decrease in cases averted indicates that this population subgroup would not have benefitted from such a change in policy. Last, if the A(H1N1)pdm09 virus vaccine had been administered exclusively to those in the ACIP priority groups, we estimate that the number of cases that would have been prevented among children aged ≤9 years would be »186,000 (range 129,000–233,000); under this assumption, 43% of this ACIP target group would be fully vaccinated, compared with an estimated 27% that actually were vaccinated.

Effects of Timing of Vaccination Administration

The effects of earlier vaccine administration on the number of clinical cases prevented are presented in Table 7 and Figure 2. If the entire A(H1N1)pdm09 virus vaccine program had begun 1 week earlier, the number of clinical cases prevented would have increased by »27% more than the base estimate. If it had begun 2 weeks earlier

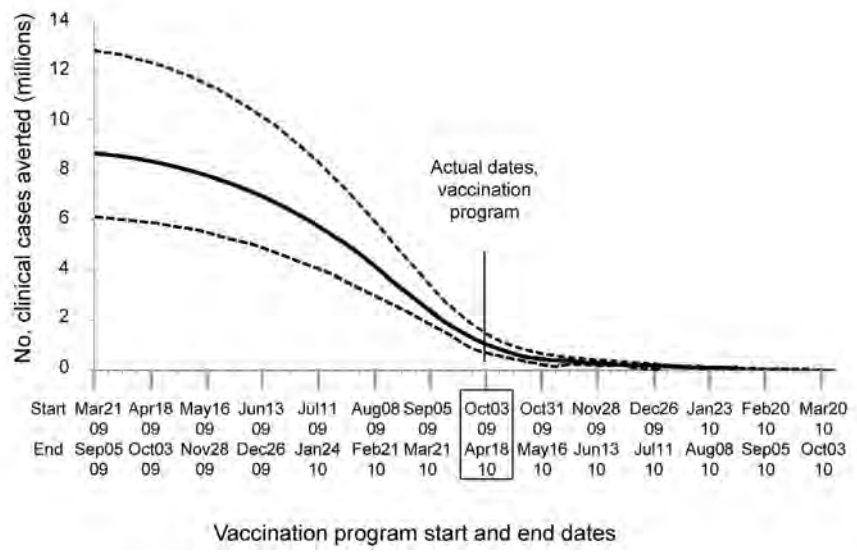

Figure 2. Comparison of the effects of shifting hypothetical start and end dates on the number of clinical cases prevented by the influenza A(H1N1)pdm09 virus vaccination program in the United States. Doses administered by week and program duration were unchanged from actual program (Table 2, Appendix, wwwnc.cdc.gov/EID/article/19/3/12-0394-T2.htm). Solid line represents the best estimate; dotted lines represent ranges. October 3, 2009–April 18, 2010, is actual vaccination program period; all other periods are hypothetical. See Table 7 for additional data.

than the actual date, the number of cases prevented would have been ≈59% greater than the base estimate; moving the program ahead by 8 weeks would have resulted in a ≈306% increase in cases prevented compared with the base estimate.

Outcomes of Vaccine Effectiveness

The vaccine administered during the 2009–2010 A(H1N1)pdm09 vaccine program was 62%% effective, and was calculated to have prevented »1,000,000 (range 712,908–1,458,930) clinical cases. If the vaccine had been more effective (85% effective for all groups, except for persons ≥65 years of age, for whom effectiveness was assumed to be 55%), 983,671–2,004,053 clinical cases would have been prevented (»38% more than in the base estimate). If the vaccine had been less effective (40% effective for all groups, except for persons ≥65 years of age, for whom it was assumed to be 20% effective), 442,971– 907,688 clinical cases would have been prevented (≈38% fewer than in the base estimate) (Table 8).

Effects of the 2-dose Vaccine Program for Children

In our base case estimate, we assumed 0% effectiveness for a single dose of vaccine and 63% effectiveness for a second dose administered 4 weeks later for children ≤9 years, and we estimated that vaccination prevented 52,081–100,349 clinical cases among persons in this age group (Table 9). Assuming that an initial dose was 20% effective, 152,420–268,852 clinical cases would have been prevented, and assuming an initial dose was 40% effective, 256,510–439,714 clinical cases would have been prevented. This striking difference between the base estimate and the other estimates occurred primarily because only ≈51% of the children who received their first dose also received a second dose, and children who received only 1 dose were not considered protected in the base case estimate.

Table 7. Sensitivity analyses showing estimates of clinical cases prevented by acceleration of vaccination against influenza A(H1N1)pdm09 virus*

Dates of vaccination program	Point estimate	Range
Hypothetical dates		
2009 Aug 08–2010 21 Feb	4,176,031	2,974,975–5,970,682
2009 Aug 15–2010 28 Feb	3,742,600	2,674,232–5,322,588
2009 Aug 22–2010 07 Mar	3,299,591	2,366,468–4,668,558
2009 Aug 29–2010 14 Mar	2,855,894	2,054,754–4,020,843
2009 Sep 05–2010 21 Mar	2,422,481	1,747,781–3,398,603
2009 Sep 12–2010 28 Mar	2,010,198	1,450,291–2,817,245
2009 Sep 19–2010 04 Apr	1,633,200	1,171,673–2,292,018
2009 Sep 26–2010 11 Apr	1,303,621	922,931–1,836,514
Actual dates		
2009 Oct 03–2010 18 Apr†	1,029,157	712,908–1,458,930

*The epidemic curve that was used to generate these estimates was the base case estimate, which was based on the assumption that a vaccination program did not exist. Data reflect calculations made for scenario 5 by estimating effects of moving the start date of the program to begin 8 weeks to 1 week earlier.
†See Table 2, Appendix, wwwnc.cdc.gov/EID/article/19/3/12-0394-T2.htm.

Table 8. Results of sensitivity analyses to estimate number of cases, hospitalizations, and deaths prevented by vaccination against influenza A(H1N1)pdm09 virus obtained with various vaccine effectiveness scenarios*

Outcomes prevented	Base estimate of vaccine effectiveness (range)†	Lower vaccine effectiveness (range)‡	Higher vaccine effectiveness (range)§
Clinical cases	1,029,157 (712,908–1,458,930)	639,449 (442,971–907,688)	1,418,678 (983,671–2,004,053)
Hospitalizations	6,240 (3,923–10,393)	3,857 (3,923–6,418)	8,674 (3,923–14,461)
Deaths	315 (201–520)	193 (124–319)	438 (279–723)

*Data reflect calculations made for scenario 6, outcomes of assuming different vaccine effectiveness.
†Assumed 62% effectiveness for all groups except those ≥65 y, for whom 43% effectiveness was assumed.
‡Assumed 40% effectiveness for all groups except the elderly, for whom 20% effectiveness was assumed.
§Assumed 85% effectiveness for all groups except the elderly, for whom 55% effectiveness was assumed.

Effects of Subclinical Cases

In our base estimate we assumed that 30% of all cases were subclinical. When we assumed that 50% of all cases were subclinical, the estimated number of clinical cases prevented was 87% of the base estimate. When we assumed that 0% of all cases were subclinical, the number of clinical cases prevented was 110% of the base estimate (Table 10).

Discussion

We estimated that »1 million clinical cases, 6,000 hospitalizations, and 300 deaths were prevented among persons who received the monovalent A(H1N1)pdm09 virus vaccine. Approximately 60% of clinical cases prevented were among persons 6 months–24 years of age and among those 25–64 years, including pregnant women, who were considered at high risk for influenza-related complications. We found that the effects of the vaccination program were greatly influenced by the timing of vaccine administration and by vaccine effectiveness.

Vaccine prioritization recommendations were made in July 2009 based on limited epidemiologic data, previous experience with immunologic responses to novel vaccine antigens, projections about when and how much vaccine would be initially available, and previous public engagement and expert opinion summaries about public values and preservation of societal functions (*5*,*6*). These factors led to a policy that identified and focused on children, pregnant women, and medical personnel as population subgroups who should receive vaccine as early in the program as possible. Uncertainty in the epidemiologic data makes it difficult to accurately determine exactly how many cases, hospitalizations, or deaths would have been prevented under any given scenario. However, the results of our sensitivity analyses indicate that the effects of the 2009 ACIP recommendations were similar, and for some subgroups even better, than those for other vaccine prioritization strategies.

This study has several limitations. We did not directly account for the effects of any other interventions (e.g., antiviral drugs, school closures, facemasks, improved management of clinical cases); we assumed these to remain constant, with or without a vaccination program. We did not estimate the curve beyond April 10, 2010, which may have resulted in a slight underestimation of the effects. However, influenza-like illness data for the United States indicated that it was unlikely that many cases occurred after April 2010 (www.cdc.gov/h1n1flu/updates/us/051410.htm [cited 2013 Jan 11]). We did not directly account for any vaccine-induced herd immunity. Estimates of A(H1N1)pdm09 virus vaccination coverage were based on survey data and subject to bias from low sample sizes from specific population subgroups and misclassification of vaccination status. Weekly vaccination estimates were interpolated. One of our sensitivity analyses illustrated the importance of the assumed level of vaccine effectiveness (Tables 8, 9). The delays we assumed between vaccination and effective protection could also have affected the estimates.

Table 9. Results of sensitivity analyses to determine the impact of the effectiveness of the first dose of vaccine against influenza A(H1N1)pdm09 virus among children 6 months–9 years of age*

Outcome prevented	Base estimate: 62% vaccine effectiveness 2 wk after dose 2†	Sensitivity estimate (range) 20% Vaccine effectiveness 2 wk after dose 1 and 62% effectiveness 2 wk after dose 2‡	Sensitivity estimate (range) 40% Vaccine effectiveness 2 wk after dose 1 and 62% effectiveness 2 wk after dose 2§
Clinical cases	81,518 (52,081–100,349)	212,363 (152,420–268,852)	347,323 (256,510–439,714)
Hospitalizations	614 (328–1,090)	1,473 (906–2,294)	2,393 (1,520–3,964)
Deaths	9 (5–15)	21 (13–35)	33 (21–55)

*Data reflect calculations made for scenario 7 by estimating changes in assumed effectiveness first dose of vaccine among children 6 months–9 years of age.
†1 dose achieves 0% effectiveness against clinical cases, hospitalizations, and deaths; 2nd dose 4 wk later is 62% effective against hospitalizations and deaths 2 weeks after administration.
‡1 dose achieves 20% effectiveness against clinical cases, hospitalizations, and deaths after 2 wk; 2nd dose 4 wk later achieves 62% against hospitalizations and deaths 2 weeks after administration.
§1 dose achieves 40% effectiveness against clinical cases, hospitalizations, and deaths after 2 wk; 2nd dose 4 wk later achieves 62% against hospitalizations and deaths 2 weeks after administration.

Table 10. Results of sensitivity analyses to determine effects of assumed percentages of subclinical cases of influenza A(H1N1)pdm09 virus infection*

Assumed proportion subclinical cases	No. clinical cases prevented (range)
50%	891,682 (651,567–1,135,546)
30% (base estimate)	1,029,157 (712,908–1,458,930)
0%	1,133,734 (759,341–1,706,714)

*Data reflect calculations made for scenario 8 by changing assumed percentages of subclinical cases of influenza.

This study highlights the benefits of earlier, proactive (as opposed to reactive) vaccination programs. However, current influenza vaccine production technology is limited in how quickly large-scale vaccine production can be achieved, and the public health community cannot accurately predict the arrival of a pandemic. This study also demonstrates that the 2009 prioritization of specific subgroups in vaccine administration was not inferior to other vaccination strategies. In addition, this study highlights the need for better data on the effectiveness of influenza vaccine. Influenza vaccine effectiveness estimates vary considerably according to season, yet clearly they can greatly affect the overall results and conclusions of programs for policy makers.

Conclusions

Future influenza pandemics are likely to differ in several ways, including in severity (patients' signs and symptoms were mild during the 2009 pandemic), basic reproductive rate of the virus, virus subtype, subgroups affected, public acceptance of vaccination, vaccine safety profile, and vaccine effectiveness. The major factor influencing the effects of the 2009 subtype H1N1 vaccination program was that the amount of vaccine available early in the epidemic (when the effects of vaccination would be greatest) was limited. Thus, a major priority is to invest in research that can reduce production time (e.g., developing prepandemic vaccines [*38*] and new types of vaccines and production technologies) and the quantity of vaccine initially available (e.g., through antigen-sparing strategies and adjuvants). Robust immunization programs that can more efficiently provide vaccines to targeted groups, faster production of larger supplies of vaccine, and consistent messaging that engenders public confidence in vaccine programs and demand for vaccination (e.g., messaging from public health officials; the media; and community groups, such as churches, daycare facilities, and schools) are factors that must be addressed in preparing for national outbreaks and pandemics.

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Emergence and Spread of Extensively and Totally Drug-Resistant Tuberculosis, South Africa

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Factors driving the increase in drug-resistant tuberculosis (TB) in the Eastern Cape Province, South Africa, are not understood. A convenience sample of 309 drug-susceptible and 342 multidrug-resistant (MDR) TB isolates, collected July 2008–July 2009, were characterized by spoligotyping, DNA fingerprinting, insertion site mapping, and targeted DNA sequencing. Analysis of molecular-based data showed diverse genetic backgrounds among drug-sensitive and MDR TB sensu stricto isolates in contrast to restricted genetic backgrounds among pre–extensively drug-resistant (pre-XDR) TB and XDR TB isolates. Second-line drug resistance was significantly associated with the atypical Beijing genotype. DNA fingerprinting and sequencing demonstrated that the pre-XDR and XDR atypical Beijing isolates evolved from a common progenitor; 85% and 92%, respectively, were clustered, indicating transmission. Ninety-three percent of atypical XDR Beijing isolates had mutations that confer resistance to 10 anti-TB drugs, and some isolates also were resistant to *para*-aminosalicylic acid. These findings suggest the emergence of totally drug-resistant TB.

The emergence of drug-resistant tuberculosis (TB) is of major concern to TB control in South Africa. A countrywide survey in 2002 revealed that 1.8% of all new TB patients and 6.7% of TB patients who had undergone previous treatment had multidrug-resistant (MDR) TB (resistant to at least isoniazid and rifampin) (*1*). This finding translates to an estimated annual case load of 13,000 MDR TB cases, placing South Africa fourth among countries where MDR TB is highly prevalent (*1*). However, this number may be an underestimation; 2 recent studies (*2*,*3*) suggested that the proportion of MDR TB cases may be substantially higher than the World Health Organization (WHO) estimate (*3*). In addition, only 4,143 of the 9,070 patients (46%) who received a diagnosis of MDR TB in 2009 received treatment, possibly because of resource constraints, creating a situation in which control was bound to fail (*4*). This conclusion is supported by the diagnosis of 594 extensively drug- resistant (XDR) TB cases (MDR plus additional resistance to a fluoroquinolone and any second-line injectable drug) in that year (*4*). The cure rate of patients with drug-resistant TB is <50% for those with MDR TB (*5*), whereas culture conversion was observed in only 19% of XDR TB case-patients during the follow-up period, irrespective of HIV status (*6*).

Most cases of MDR TB and XDR TB in South Africa have been detected in KwaZulu-Natal, Western Cape, and Eastern Cape Provinces (*4*). Statistics from the Eastern Cape showed the largest increase in the number of MDR TB cases, rising from 836 cases in 2006 to 1,858 cases in 2009 (2.2 fold increase) (*4*). The reason for this dramatic increase in MDR TB cases remains to be determined.

Molecular epidemiologic data from the neighboring Western Cape Province have demonstrated that MDR TB is spread by primary transmission (*7*–*9*), which accounts for nearly 80% of reported MDR TB cases (*2*). To date, only 1 molecular epidemiologic study has been reported for the

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Eastern Cape (*10*), and it showed that 50% of rifampin-resistant TB isolates (including MDR TB isolates) belonged to the Beijing genotype and that "atypical" Beijing strains were significantly overrepresented. These strains harbored rare mutations in the *inhA* gene promoter (G-17A) and *rpoB* gene (GAC→GTC nucleotide substitutions in codon 516), which have previously been associated with a high fitness cost (*11*). The authors demonstrated that the spread of these strains was facilitated by HIV co-infection, thereby raising concern for the spread of drug-resistant strains in vulnerable populations (*10*).

A recent epidemiologic study conducted in the Eastern Cape estimated that 75.6% of XDR TB cases with complete data were a result of ongoing transmission (*12*). Treatment outcomes were dismal; 58% of case-patients died within 1 year, and culture conversion was observed in only 8.4% of case-patients after 143 days of treatment (*12*), raising concern that these patients had an untreatable form of TB. This situation is similar to the Tugela Ferry outbreak in KwaZulu-Natal Province (*13*), which highlighted the need for improved basic control measures, including rapid diagnostics and infection control methods (*14*).

This study aimed to describe the *Mycobacterium tuberculosis* strain population structure among MDR TB and XDR TB case-patients in Eastern Cape Province, South Africa, in order to determine whether the epidemic was driven by acquisition or transmission of resistance and to describe the extent of resistance within these strains. These findings will inform TB control efforts to better implement measures to curb emergence or the spread of drug-resistance.

Materials and Methods

Study Population

Sputum specimens were collected from persons at high-risk for suspected TB (previously treated case-patients and close contacts of known patients with drug- resistant cases) in accordance with the National TB Control Program. Specimens that were collected at healthcare facilities in the Eastern Cape Province were submitted to the National Health Laboratory Service (NHLS) in Port Elizabeth for TB drug susceptibility testing (DST). From July 2008 through July 2009, a convenience sample of sputum cultures, shown to be either fully drug-susceptible or resistant to at least isoniazid and rifampin (MDR TB) by the NHLS, was submitted to Stellenbosch University in Cape Town for subsequent genotyping. Only limited demographic and clinical data were available for each patient: a unique identifier (assigned by the NHLS), the date sputum was obtained, the name of the clinic/hospital where the sample originated, and the routine DST pattern. The unique identifier was used to identify the first available isolate from 309 drug-susceptible and 342 MDR TB case-patients included in the study. This study was approved by the ethics committee of Stellenbosch University, Faculty of Health Sciences (N09/11/296).

Drug Susceptibility Testing

Sputum samples were processed by the NHLS for routine TB diagnosis by smear microscopy and culture. Each sputum specimen was decontaminated by using the standard *N*-acetyl-L-cysteine-sodium hydroxide method and cultured in mycobacteria growth indicator tube (MGIT) 960 medium until a positive growth index was observed. DST was done by the indirect proportion method with the BACTEC MGIT 960 system (BD Bioscience, Sparks, MD, USA), according to the manufacturer's instructions. We initially tested resistance against isoniazid and rifampin, followed by testing for resistance against streptomycin and ethambutol if the isolate was resistant to either isoniazid or rifampin. Second-line DST was done in 7H10 medium containing 2 μg/mL of ofloxacin, 4 μg/mL of amikacin, or 5 μg/mL of ethionamide. DST for *para*-aminosalicylic acid was done at Stellenbosch University in MGIT 960 medium containing 4.0 μg/mL, 8 μg/mL, and 16 μg/mL of *para*-aminosalicylic acid (*15*).

Molecular-based Analysis

Crude DNA was prepared by boiling a 200-μL aliquot of a mycobacteria-positive MGIT culture, and this was used as a template for subsequent PCR analysis (*16*). Each isolate was spoligotyped by using the international standardized method (*17*) and grouped into genotypes according to previously described spoligotype signatures (*18*). Beijing genotype strains were subclassified as either "typical" or "atypical," according to the presence or absence of an IS*6110* insertion in the noise transfer function (NTF) region (*19*,*20*). The atypical Beijing genotype strains were further classified by using the international standardized IS*6110* DNA fingerprinting method (*21*). For atypical Beijing strains that were drug-sensitive according to DST, sensitivity to isoniazid and rifampin was confirmed by sequencing the *katG* and *rpoB* genes. In MDR atypical Beijing strains, mutations conferring resistance to isoniazid, rifampin, ethambutol, pyrazinamide, ofloxacin, streptomycin, amikacin, kanamycin, and capreomycin were identified by sequencing the *inhA* promoter and the *katG*, *rpoB*, *embB*, *pncA*, *gyrA*, and *rrs* genes, respectively (*22*,*23*). Isolates were grouped as as follows: MDR TB sensu stricto (MDR TB ss, that is, MDR strains excluding identified pre-XDR, MDR plus additional resistance to either a fluoroquinolone or any second-line injectable anti-TB drug) [*24*] and XDR strains); pre-XDR TB; or XDR TB, according to high confidence mutations. This method of grouping was selected because routine DST was not done for all of the anti-TB drugs on all of the isolates. Furthermore, a poor

correlation was observed between high-confidence mutations and routine second-line DST. Isolates were considered to belong to the same cluster (implying ongoing transmission) if identical mutations were observed in all of the genes sequenced.

Results

A convenience sample of 309 drug-sensitive and 342 MDR TB isolates from patients from Eastern Cape Province was collected during the study period. These isolates were submitted to Stellenbosch University for molecular-based analysis. Analysis of the population structure of these isolates by spoligotyping identified 52 and 29 different spoligotype patterns among drug-sensitive and MDR TB strains, respectively. Among drug-sensitive and MDR isolates, 22/52 and 14/29 spoligotype patterns were previously recorded in the fourth international spoligotyping (SpolDB4) database. These represented 275 (89.0%) of 309 drug-sensitive isolates and 327 (95.6%) of 342 MDR isolates. Notably, 84% of MDR isolates constituted only 3 different spoligotypes (Table 1), namely, Beijing, LAM3, and LAM4. These findings indicate transmission of these strains.

Table 1 shows the classification of spoligotypes, according to the degree of drug resistance, in which drug resistance is expressed as the result of culture-based or molecular-based DST. In this study, we used molecular-based DST to define the extent of drug-resistance in routinely diagnosed MDR TB isolates. Accordingly, 119 (38.5%) of the drug-susceptible isolates and 236 (69.0%) of the MDR TB isolates were of the Beijing genotype. Subclassification of Beijing genotype strains showed that 11(9.2%) of 119 drug-sensitive and 217 (91.9%) of 236 MDR strains belonged to the "atypical" subgroup of the Beijing genotype, as indicated by the absence of an IS*6110* element in the NTF region.

Analysis of mutations conferring resistance to first- and second-line anti-TB drugs enabled grouping of the MDR isolates: 136 MDR ss, 98 pre-XDR, and 108 XDR. Using these groupings, we found that isolates with a higher degree of resistance were more likely to have an atypical Beijing genotype (drug sensitive: 11/309 [3.6%, 95% CI 1.8%–6.3%], MDR ss: 29/136 [21.3%, 95% CI 14.8%–29.2%] vs. pre-XDR: 85/98 [86.7%, 95% CI 78.4%–92.7%] vs. XDR: 103/108 [95.4%, 95% CI 89.5%–98.5%]).

We analyzed DNA sequencing data for the first available isolate from each patient infected with an MDR atypical Beijing strain (n = 217) and performed IS*6110* fingerprinting for a subset of these isolates (n = 110) to establish whether the overabundance of the atypical Beijing genotype among patients with pre-XDR TB and XDR TB strains reflected ongoing transmission. IS*6110* DNA fingerprinting showed that all of these patients were infected with closely related atypical Beijing strains with only minor differences in the banding patterns (online Technical Appendix, Figures 1, 2, wwwnc.cdc.gov/EID/article/19/3/12-0246-Techapp1.pdf), thereby suggesting clonal dissemination.

The online Technical Appendix Table shows that 216 (99.5%) of 217 of the MDR atypical Beijing genotype strains harbored an identical *katG* (AGC315ACC) mutation, whereas 209 (94.9%) of 217 had a distinctive *rrs* (A513C) gene mutation. This finding suggests that these mutations were acquired before dissemination. Subsequently, resistance to rifampin, ethambutol, pyrazinamide, amikacin, and ofloxacin was acquired in various combinations. Of the 29 atypical Beijing MDR ss isolates, 22 (75.9%) were grouped into 4 clusters according to mutations

Table 1. Spoligotype classification of drug-sensitive and MDR TB isolates, Eastern Cape Province, South Africa, 2008–2009*

Spoligotype family†	ST no.	Culture-based DST, no. (%)				Molecular-based DST, no. (%)		
		Sensitive	MDR*ss*	Pre-XDR	XDR	MDR*ss*	Pre-XDR	XDR
Atypical Beijing	1	11 (3.6)	41 (27.0)	98 (92.5)	78 (92.9)	29 (22.5)	85 (87.6)	103 (95.4)
Typical Beijing	1	108 (35.0)	19 (12.5)	0	0	18 (14.0)	1 (1.0)	0
H	36; 47; 50; 62	7 (2.3)	2 (1.3)	1 (0.9)	0	2 (1.6)	1 (1.0)	0
LAM3	33; 130; 211	66 (21.4)	12 (7.9)	2 (1.9)	0	12 (9.3)	0 (0)	0
LAM4	60; 811	6 (1.9)	32 (21.1)	4 (3.8)	2 (1.9)	29 (22.5)	3 (3.1)	2 (1.9)
LAM (other)	4; 20; 42; 398	7 (2.3)	1 (0.7)	0	1 (1.2)	1 (0.8)	0	1 (0.9)
MANU2	1247	0	0	0	2 (2.4)	1 (0.8)	0	1 (0.9)
S	34; 71	8 (2.6)	8 (5.3)	0	1 (1.2)	8 (6.2)	0	1 (0.9)
T	44; 53; 73; 254; 926; 1240	51 (16.5)	18 (11.8)	0	0	13 (10.1)	5 (5.2)	0
U	443; 519; 790	1 (0.3)	2 (1.3)	0	0	2 (1.6)	0	0
X	18; 92; 119; 1751	6 (1.9)	3 (2.0)	0	0	3 (2.3)	0	0
CAS	21; 26; 1092	4 (1.3)	0	0	0	0	0	0
Orphan	Not assigned	34 (11.0)	14 (9.2)	1 (0.9)	0	11 (8.5)	2 (2.1)	0
Total		309	152	106	84	129	97	108
Total MDR				342			334‡	

*MDR TB, multidrug-resistant tuberculosis; ST, shared type (*17*); DST, drug susceptibility testing; MDR*ss*, MDR sensu stricto; Pre-XDR, pre–extensively drug resistant; XDR, extensively drug resistant.
†For Beijing isolates a distinction was made between typical and atypical based on the presence or absence of an IS*6110* insertion in the noise transfer region (*18,19*).
‡Molecular-based DST total differs from culture-based DST total, because some results were not available.

(mutation pattern [MP]) in the *inhA* promoter and the *katG*, *rpoB*, *embB*, *pncA*, *rrs*, and *gyrA* genes (cluster size ranged from 3 to 12 cases; online Technical Appendix Table: MP2, MP17, MP32, MP34), whereas 7 had unique MPs (online Technical Appendix Table 2: MP23, MP25, MP30, MP31, MP41, MP44, MP48). Similarly, the 85 atypical pre-XDR Beijing isolates showed 11 different MPs, of which 81 (95.3%) were grouped into 7 clusters (cluster size ranged from 2 to 62 cases; online Technical Appendix Table: MP3, MP5, MP18, MP26, MP28, MP35, MP38). The genotype of the largest pre-XDR TB cluster was characterized by an *inhA* promoter mutation at position 17 and the *katG* AGC315ACC, *rpoB* GAC516GTC, *embB* ATG306ATA, *rrs* A513C, and *rrs* A1401G nucleotide substitutions as well as an insertion in the *pncA* gene at position 172G. This MP was characteristic of 81 (78.6%) of 103 of the atypical Beijing XDR TB isolates and, for ease of reference, will be called MP5 (online Technical Appendix Table). By contrast, only 3 of the 29 atypical Beijing MDR ss isolates showed the same mutation pattern for these genes, excluding the *rrs*A1401G mutation (MP2). Ten different atypical XDR Beijing MPs emerged from the MP5 progenitor by mutation in the *gyrA* gene. Of these, 6 MPs showed clustering (cluster size ranged from 2 to 46 cases, MP6–11), and 4 had unique mutations conferring ofloxacin resistance (MP12–16). Clustering of both the pre-XDR and XDR genotypes suggests transmission after the acquisition of additional resistance. Of the remaining 22 atypical XDR Beijing isolates, 12 distinct resistance MPs were observed, of which 11 isolates were clustered (MP27) and 11 had unique genotypes (MP19–22, MP24, MP29, MP39–40, MP42–43, MP47).

Spatial analysis of the patients' origins showed that pre-XDR and XDR isolates with an atypical Beijing genotype were found in 5 of 8 district municipalities (Figure; online Technical Appendix Table). The largest atypical pre-XDR Beijing genotype cluster (MP5) was identified in 4 adjacent district municipalities (online Technical Appendix Table), and the largest XDR TB cluster (MP6) was identified in 3 of these districts as well as in an additional district, which suggests the past spread of these genotypes.

The presence of mutations in target genes known to confer resistance with high confidence indicated that 95.1% (98/103) of the atypical XDR Beijing isolates were resistant to at least 10 anti-TB drugs: isoniazid, rifampin, ethambutol, pyrazinamide, streptomycin, amikacin, kanamycin, capreomycin, ethionamide, and ofloxacin. The extent of drug resistance in these isolates was underestimated by routine DST (Table 2). The correlation between molecular-based drug-resistance and routine culture-based DST was 99.6% for isoniazid, 100% for rifampin, 28% for ethambutol, 92% for streptomycin, 93% for amikacin, 27% for capreomycin, 52% for ethionamide, and 86% for ofloxacin (Table 2). Routine DST for pyrazinamide, kanamycin, cycloserine, and *para*-aminosalicylic acid was not performed. DST for *para*-aminosalicylic acid was done at Stellenbosch University on 45 isolates; 9 showed resistance at a level of >4.0 μg/mL.

Discussion

Review of routine DST results highlights the severity of the drug-resistant TB epidemic in South Africa (*4*) and thereby emphasizes the urgent need for curbing the rising incidence of drug resistance in the country. This result can only be achieved by implementing appropriate intervention strategies based on knowledge of the mechanisms fueling this epidemic. Recently, molecular epidemiologic techniques were used in combination with classical epidemiologic data to enhance understanding of the TB epidemic in different settings. Those studies have quantified the relative proportion of acquisition versus transmission and have described the population structure of *M. tuberculosis* over time (*7*,*10*,*22*,*24*,*25*). Using these approaches, we show that patients with MDR TB in the Eastern Cape could be divided into 2 distinct groups: isolates from patients infected with MDR ss showed diverse genetic backgrounds, while isolates from patients infected with pre-XDR TB and XDR TB showed restricted genetic backgrounds.

The finding that the pre-XDR TB and XDR TB strains are genetically distinct when compared to the MDR ss strains is counterintuitive because we would expect all MDR TB strains to have had an equal chance of acquiring

Table 2. Correlation of culture-based and molecular-based drug-susceptibility testing among atypical Beijing isolates, South Africa, 2008–2009*

Drug/gene	CB-DST R, MB-DST R	CB-DST S, MB-DST S	CB-DST R, MB-DST S	CB-DST S, MB-DST R	Total	Correlation, %
INH/*katG*	217	9	1	0	227	99.6
RIF/*rpoB*	219	9	0	0	228	100
STR/*rrs*500	191	6	2	16	215	91.6
EMB/*embB*	56	5	2	152	215	28.4
ETH/*inhA* promoter	76	25	5	86	192	52.6
OFL/*gyrA*	78	93	0	29	200	85.5
AMK/*rrs*1400	167	32	7	9	215	92.6
CAP/*rrs*1400	21	38	1	155	215	27.4

*CB-DST, culture-based drug susceptibility testing; R, resistant; MB-DST, molecular-based DST; S, sensitive; INH, isoniazid; RIF, rifampin; STR, streptomycin; EMB, ethambutol; ETH, ethionamide; OFL, ofloxacin; AMK, amikacin; CAP, capreomycin.

resistance to second-line anti-TB drugs. The absence of second-line resistance among a large number of different MDR TB genotypes suggests that under the current MDR TB treatment regimen, acquisition of additional resistance in MDR ss strains is reduced. Conversely, analysis of the DNA sequencing data showed a significant association between the atypical Beijing genotype and mutations conferring second-line resistance. This demonstrates that this genotype has acquired resistance to the level of pre-XDR TB, which in turn has spread and thereafter has acquired additional resistance to the level of XDR TB, followed again by transmission. An alternative explanation would be that the atypical Beijing genotype acquires resistance by conferring mutations more readily than other genotypes. However, the convergent evolution of 7 different mutations within a single genotype is highly unlikely.

Analysis of the locations of the pre-XDR TB case-patients infected with this clone shows that it had a wide geographic distribution, which suggests that this genotype has been in circulation for an extended period. This conclusion was further supported by the analysis of the evolutionary order in which resistance was acquired (online Technical Appendix Table), which showed that the ancestral clone first acquired resistance to isoniazid and streptomycin. This could be explained by the treatment regimen used in the early 1960s, which was based on the combination of isoniazid and streptomycin (*26*). A similar conclusion was drawn from whole genome sequence data which predicted that mutations conferring resistance to isoniazid and streptomycin were deeply rooted in the atypical Beijing genotype (*27*).

Given the extent of resistance in pre-XDR TB strains and the extremely limited treatment options available, the emergence of ofloxacin resistance was inevitable. This idea was supported by our molecular-based analysis of the XDR TB isolates, which demonstrated that resistance to a fluoroquinolone had been acquired independently on several different occasions (several different *gyrA* mutations were observed), followed by amplification through transmission (clustering of XDR phenotypes was observed). However, the true extent of acquisition may be higher than predicted, given that the XDR TB isolates were cultured from samples from patients who resided in different district municipalities, and contact was unlikely because of the long distances.

We suggest that the absence of routine second-line drug susceptibility testing and the treatment of MDR TB with an inadequate standardized regimen, according to the 2004 guidelines (www.sahealthinfo.org/tb/mdrtbguidelines.pdf) (6 months' intensive phase: kanamycin, ethionamide, pyrazinamide, ofloxacin, and cycloserine or ethambutol; 12–18 months continuation phase: ethionamide, ofloxacin, and cycloserine or ethambutol) (*28*) may have led to the inappropriate treatment of undiagnosed pre-XDR TB cases. This regimen would have prolonged the period of infectiousness

Figure. District municipalities in the Eastern Cape Province, South Africa. Map courtesy of F. W. van Zyl.

leading to transmission to close contacts and increased the risk of amplification of resistance (*28*,*29*). This problem has been recently addressed with the implementation of a revised treatment regimen (*28*) as well as routine second-line DST, which is now done on all isolates shown to be resistant to rifampin. However, these tests are culture-based, which exacerbates diagnostic delay and possible transmission. This situation can be partially resolved with the implementation of a genetic-based second-line drug susceptibility test (*29*). However, the extent of resistance associated with the atypical Beijing genotype makes treatment options extremely difficult as these isolates are resistant to all first-line anti-TB drugs (isoniazid, rifampin, ethambutol, pyrazinamide and streptomycin) and many of the second-line drugs (amikacin, kanamycin, ofloxacin, ethionamide, capreomycin). A limited number of isolates were also resistant to *para*-aminosalicylic acid. This suggests that the atypical Beijing genotype clone is evolving toward total drug resistance (defined as in vitro resistance to all first-line drugs, as well as aminoglycosides, cyclic polypeptides, fluoroquinolones, thioamides, serine analogs, and salicylic acid derivatives [*30*]) with acknowledgment of WHO's concern over the definition (*31*). Our molecular-based results are in accordance with a recent study from the Eastern Cape, which documented extremely poor treatment outcomes for XDR TB case-patients (*12*). The authors found that these patients experienced a high death rate (58.4%) and low culture-conversion rates (8.4%) over a follow-up period of 143 days. They concluded that only 1.7 drugs per patient could be regarded as "effective" on the basis of DST results, previous treatment records, or both. Given that this study was conducted concurrently with ours, it is highly likely that a large proportion of their patients were also infected with XDR TB strains with an atypical Beijing genotype. Thus, the poor treatment outcome may be related to the extent of

drug-resistance; however, we cannot exclude the possibility that the atypical Beijing genotype contributes to illness and death. A further concern is the knowledge that this clone is now spreading to other provinces in South Africa, possibly due to migration. In Western Cape Province, an estimated 55% of XDR TB case-patients harbor isolates with the atypical Beijing genotype (*32*).

We acknowledge that this study has several limitations. First, clinical data were not available for this study, and thus it was not possible to establish the effects of drug resistance on treatment outcome. However, we do not believe that the strains reported by Kvasnovsky et al. (*12*) differ from those analyzed in this study because the studies were conducted concurrently. Second, our analysis of a convenience sample may have led to an overestimation of the proportion of pre-XDR TB and XDR TB cases in Eastern Cape Province. Third, our use of mutational data to categorize patient isolates as MDR ss, pre-XDR, and XDR is not the accepted standard. However, genetic DST has been endorsed by WHO for first-line anti-TB drugs, and mounting evidence indicates that high confidence mutations accurately predict second-line drug resistance (*33*).

The diagnostic dilemma facing TB control managers in Eastern Cape Province is how to rapidly identify case-patients at risk of harboring the atypical Beijing genotype to prioritize DST, ensure patient isolation, and administer appropriate treatment. Previous studies have shown a strong association between *inhA* promoter mutations and pre-XDR TB and XDR TB (*34*). Given that the GenoType MTBDR*plus* test (*35*) has been implemented as the diagnostic standard in most NHLS laboratories in South Africa, we propose that this test could be used as a rapid screening tool to identify patients harboring drug-resistant atypical Beijing strains (*34*). To contain the spread of this virtually untreatable form of TB, control managers must make use of this information.

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Human Betacoronavirus 2c EMC/2012–related Viruses in Bats, Ghana and Europe

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We screened fecal specimens of 4,758 bats from Ghana and 272 bats from 4 European countries for betacoronaviruses. Viruses related to the novel human betacoronavirus EMC/2012 were detected in 46 (24.9%) of 185 *Nycteris* bats and 40 (14.7%) of 272 *Pipistrellus* bats. Their genetic relatedness indicated EMC/2012 originated from bats.

Coronaviruses (CoVs) are enveloped viruses with a positive-sense, single-stranded RNA genome (*1*). CoVs are classified into 4 genera: *Alphacoronavirus*, *Betacoronavirus* (grouped further into clades 2a–2d), *Gammacoronavirus*, and *Deltacoronavirus*. Two human coronaviruses (hCoVs), termed hCoV-OC43 and –229E, have been known since the 1960s and cause chiefly mild respiratory disease (*2*). In 2002–2003, an outbreak of severe acute respiratory syndrome (SARS) leading to ≈850 deaths was caused by a novel group 2b betacoronavirus, SARS-CoV (*3*). A likely animal reservoir for SARS-CoV was identified in rhinolophid bats (*4*,*5*). In the aftermath of the SARS pandemic, 2 hCoVs, termed hCoV-NL63 and -HKU1, and numerous novel bat CoVs were described.

In September 2012, health authorities worldwide were notified of 2 cases of severe respiratory disease caused by a novel hCoV (*6*,*7*). This virus, termed EMC/2012, was related to the 2c betacoronavirus clade, which had only been known to contain *Tylonycteris bat coronavirus* HKU4 and *Pipistrellus bat coronavirus* HKU5 (*8*).

We previously identified highly diversified alphacoronaviruses and betacoronaviruses, but not clade 2c betacoronaviruses, in bats from Ghana (*9*). We also identified sequence fragments from a 2c betacoronavirus from 1 *Pipistrellus* bat in Europe (*10*). In this study, we analyzed an extended sample of 4,758 bats from Ghana and 272 bats from 4 European countries.

The Study

Fecal specimens were collected from 10 bat species in Ghana and 4 *Pipistrellus* species in Europe (Table 1). Bats were caught during 2009–2011 with mist nets, as described (*9*), in 7 locations across Ghana and 5 areas in Germany, the Netherlands, Romania, and Ukraine (Figure 1). The species, age, sex, reproductive status, and morphologic measurements of the bats were recorded. Fecal pellets were collected and suspended in RNAlater Stabilization Reagent (QIAGEN, Hilden, Germany). RNA was purified as described (*11*). CoV was detected by using nested reverse transcription PCR (RT-PCR) targeting the *RNA-dependent RNA polymerase* (*RdRp*) gene (*12*) (see Table 1 for assay oligonucleotides).

A novel CoV was detected in insectivorous *Nycteris cf. gambiensis* specimens (online Technical Appendix wwwnc.cdc.gov/EID/pdfs/12-1503-Techapp.pdf; GenBank accession nos. JX899382–JX899384). A real-time RT-PCR was designed to permit sensitive and quantitative detection of this CoV (Table 1). Only *Nycteris* bats were positive for CoV (46 [24.9%] of 185 specimens) (Table 1). Demographic factors predictive of CoV in captured *Nycteris* bats were assessed. Juvenile bats and lactating females were significantly more likely to be CoV-infected than were adult

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Table 1. Overview of bats tested for 2c betacoronaviruses, Ghana and Europe

Area, bat species	No. bats tested (no. [%] positive)*	Age, juvenile/adult†	Sex, F/M‡	Location§ (no. tested/no. positive)
Ghana				
Coleura afra	108 (0)	2/105	46/59	a, b, e
Hipposideros abae	604 (0)	55/548	207/341	a, b, d, f
H. cf. gigas	28 (0)	7/19	8/11	a, b, d
H. fuliginosus	1 (0)	1/0	Unknown	c
H. jonesi	31 (0)	6/25	1/24	c, d
H. cf. ruber	3,763 (0)	674/3,078	1,109/1,969	a, b, c, d, f, g
Nycteris cf. gambiensis	185 (46 [24.9])	22/161¶	79/82	a# (5/2), b# (65/15), d# (104/29), f (1/0)
Rhinolophus alcyone	4 (0)	2/2	1/1	c
R. landeri	13 (0)	3/10	2/8	b, d, f
Taphozous perforatus	21 (0)	3/18	0/18	e
Total	4,758 (46 [1.0])			
Europe				
Pipistrellus kuhlii	7 (0)	Unknown	3/3	l
P. nathusii	82 (30 [36.6])	15/65	38/43	j (2/0), k# (74/29), l# (6/1)
P. pipistrellus	42 (1 [2.4])	17/25	19/21	i (29/0), k# (7/1), h (6/0)
P. pygmaeus	141 (9 [6.4])	11/127	83/55	j (44/0), k# (91/9), l (6/0)
Total	272 (40 [14.7])			

*The real-time reverse transcription PCR (Ghana) used oligonucleotides 2c-rtF, 5′-GCACTGTTGCTGGTGTCTCTATTCT-3′, 2crtR, 5′-GCCTCTAGTGGCAGCCATACTT-3′ and 2c-rtP, JOE-TGACAAATCGCCAATACCATCAAAAGATGC-BHQ1 and the Pan2c-heminested assay (Europe) used oligonucleotides Pan2cRdRP-R, 5′-GCATWGCNCWGTCACACTTAGG-3′; Pan2cRdRP-Rnest, 5′-CACTTAGGRTARTCCCAWCCCA-3′; and Pan2cRdRp-FWD, 5′-TGCTATWAGTGCTAAGAATAGRGC-3′.
†Excludes bats (all coronavirus-negative) that were missing data for age.
‡ Excludes bats that were missing data for sex.
§a, Bouyem; b, Forikrom; c, Bobiri; d, Kwamang; e, Shai Hills; f, Akpafu Todzi, g, Likpe Todome; h, Province Gelderland; i, Eifel area; j, Holstein area; k,Tulcea county; l, Kiev region; GPS coordinates are shown in Figure 1.
¶For 2 animals, no data on age were available.
#Locations in which coronavirus 2c–positive bats were found.

and nonlactating female bats, respectively (Table 2). Virus concentrations in feces from *Nycteris* bats were high (median 412,951 RNA copies/g range 323–150,000,000 copies/g).

The 398-bp CoV *RdRp* screening fragment was extended to 816 bp, as described (*5*), to enable more reliable taxonomic classification. We previously established *RdRp*-grouping units (RGU) as a taxonomic surrogate to enable prediction of CoV species on the basis of this 816-bp fragment when no full genome sequences could be obtained. According to our classification, the amino acid sequences in the translated 816-bp fragment of the tentative betacoronavirus species (RGU) differed from each other by at least 6.3% (*5*). The new *Nycteris* bat CoV differed from the 2c-prototype viruses HKU4 and HKU5 by 8.8%–9.6% and from EMC/2012 by 7.5% and thus constituted a novel RGU. A partial *RdRp* sequence fragment of a *P. pipistrellus* bat CoV from the Netherlands, termed VM314 (described by us in [*10*]), was completed toward the 816-bp fragment to refine the RGU classification of EMC/2012. EMC/2012 differed from VM314 by only 1.8%.

Because of the genetic similarity between EMC/2012 and VM314, we specifically investigated *Pipistrellus* bats from 4 European countries for 2c betacoronaviruses. We detected betacoronaviruses in 40 (14.7%) of 272 *P.*

Table 2. Possible factors predictive of 2c betacoronavirus detection in *Nycteris cf. gambiensis* bats, Ghana and Europe*

Variable	No. tested	CoV positive, no. (%)	χ^2	p value	Odds ratio (95% CI)
Age					
Juvenile	22	10 (45.4)	5.49	0.02	2.89 (1.16–7.24)
Adult	161	36 (22.4)			
Sex					
F	79	16 (20.3)	0.01	0.91	1.04 (0.50–2.17)
M	82	20 (24.4)			
Lactation status, F					
Lactating	25	11 (44.0)	12.77	0.0004	7.70 (2.29–25.89)
Nonlactating	54	5 (9.3)			
Gravidity, F					
Gravid	13	0	3.95	0.06†	0
Nongravid	66	16 (24.2)			
Reproductive status, M					
Active	56	15 (26.8)	0.55	0.46	1.54 (0.49–4.81)
Nonreproductive	26	5 (19.2)			

*All analyses, except for the gravity parameter (because 1 of the expected values was <5), were done by using uncorrected χ^2 tests (2-tailed) in Epi Info 7 (wwwn.cdc.gov/epiinfo/7). All analyses except age excluded juvenile bats.
†Fisher exact test.

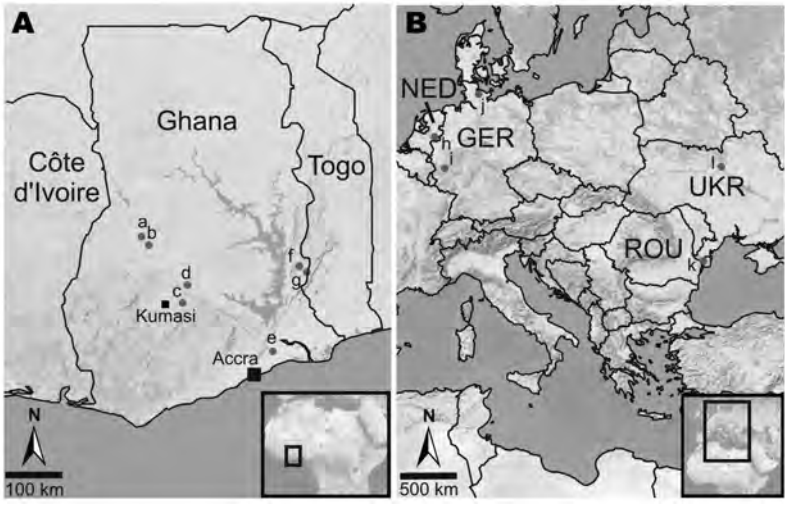

Figure 1. Location of bat sampling sites in Ghana and Europe. The 7 sites in Ghana (A) and the 5 areas in Europe (B) are marked with dots and numbered from west to east. a, Bouyem (N7°43′24.899″ W1°59′16.501″); b, Forikrom (N7°35′23.1″ W1°52′30.299″); c, Bobiri (N6°41′13.56″ W1°20′38.94″); d, Kwamang (N6°58′0.001″ W1°16′ 0.001″); e, Shai Hills (N5°55′44.4″ E0°4′30″); f, Akpafu Todzi (N7°15′43.099″ E0°29′29.501″); g, Likpe Todome (N7°9′50.198″ E0°36′28.501″); h, Province Gelderland, NED (N52°1′46.859″ E6°13′4.908″); i, Eifel area, federal state Rhineland-Palatinate, GER (N50°20′5.316″ E7°14′30.912″); j, Holstein area, federal state Schleswig-Holstein, GER (N54°14′51.271″ E10°4′3.347″); k, Tulcea county, ROU (N45°12′0.00″ E29°0′0.00″); l, Kiev region, UKR (N50°27′0.324″ E30°31′24.24″). NED, the Netherlands; GER, Germany; ROU, Romania; UKR, Ukraine.

pipistrellus, *P. nathusii*, and *P. pygmaeus* bats from the Netherlands, Romania, and Ukraine (Table 1; GenBank accession nos. KC243390-KC243392) that were closely related to VM314. The VM314-associated *Pipistrellus* bat betacoronaviruses differed from EMC/2012 by 1.8%. The difference between EMC/2012 and HKU5 was 5.5%–5.9%. In summary, HKU5, EMC/2012, and the VM314-associated clade form 1 RGU according to our classification system, and the VM314-*Pipistrellus* bat clade contains the closest relatives of EMC/2012. HKU4 and the *Nycteris* CoV define 2 separate tentative species in close equidistant relationship.

We conducted a Bayesian phylogenetic analysis. In this analysis, the *Nycteris* bat CoV clustered as a phylogenetically basal sister clade with HKU4, HKU5, and EMC/2012 and the associated European *Pipistrellus* viruses (Figure 2, Appendix, panel A, wwwnc.cdc.gov/EID/article/19/3/12-1503-F2.htm).

To confirm the *RdRp*-based classification, we amplified the complete glycoprotein-encoding *Spike* gene and sequenced it for the novel *Nycteris* bat virus. The phylogenetically basal position of the novel *Nycteris* bat virus within the 2c clade resembled that in the CoV *RdRp* gene (Figure 2, Appendix, panel B). Partial sequences that could be obtained from the 3′-end of the *Spike* gene of three 2c *Pipistrellus* bat betacoronaviruses confirmed their relatedness to EMC/2012 (Figure 2, Appendix, panel C).

Conclusions

We detected novel clade 2c betacoronaviruses in *Nycteris* bats in Ghana and *Pipistrellus* bats in Europe that are phylogenetically related to the novel hCoV EMC/2012. All previously known 2c bat CoVs originated from vespertilionid bats: VM314 originated from a *P. pipistrellus* bat from the Netherlands and HKU4 and HKU5 originated from *Tylonycteris pachypus* and *P. abramus* bats, respectively, from the People's Republic of China. The *Nycteris* bat virus in Africa extends this bat CoV clade over 2 different host families, Nycteridae and Vespertilionidae (online Technical Appendix). Detection of genetically related betacoronaviruses in bats from Africa and Eurasia parallels detection of SARS-CoV in rhinolophid bats from Eurasia and related betacoronaviruses in hipposiderid bats from Africa (*9*).

The relatedness of EMC/2012 to CoVs hosted by *Pipistrellus* bats at high prevalence across different European countries and the occurrence of HKU5 in bats of this genus from China highlight the possibility that *Pipistrellus* bats might indeed host close relatives of EMC/2012. This suspicion is supported by observations that tentative bat CoV species (RGUs) are commonly detected within 1 host genus (*5*). Within the Arabian Peninsula, the International Union for Conservation of Nature (www.iucn.org) lists 50 bat species, including *P. arabicus*, *P. ariel*, *P. kuhlii*, *P. pipistrellus*, *P. rueppellii*, and *P. savii* bats. Because of the epidemiologic link of EMC/2012 with the Arabian Peninsula (*6*,*7*), bats from this area should be specifically screened.

The genomic data suggest that EMC/2012, like hCoV-229E and SARS-CoV, might be another human CoV for which an animal reservoir of closely related viruses could exist in Old World insectivorous bats (*4*,*9*). Whether cross-order (e.g., chiropteran, carnivore, primate) host switches, such as suspected for SARS-CoV, have occurred for 2c clade bat CoVs remains unknown. However, we showed previously that CoVs are massively amplified in bat maternity colonies in temperate climates (*13*). This amplification also might apply to the *Nycteris* bat CoV because, as shown previously for vespertilionid bats from temperate climates (*14*), detection rates of CoV are significantly higher among juvenile and lactating *Nycteris* bats. In light of the observed high virus concentrations, the use of water from bat caves and bat guano as fertilizer for farming and the hunting of

bats as wild game throughout Africa (*15*) may facilitate host switching events. To our knowledge, no CoV has been isolated directly from bats. Further studies should still include isolation attempts to obtain full virus genomes and to identify virulence factors that may contribute to the high pathogenicity of EMC/2012 (*7*).

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Mycobacterial Lineages Causing Pulmonary and Extrapulmonary Tuberculosis, Ethiopia

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Molecular typing of 964 specimens from patients in Ethiopia with lymph node or pulmonary tuberculosis showed a similar distribution of *Mycobacterium tuberculosis* strains between the 2 disease manifestations and a minimal role for *M. bovis*. We report a novel phylogenetic lineage of *M. tuberculosis* strongly associated with the Horn of Africa.

Ethiopia is among the countries with the highest incidence of tuberculosis (TB) and has a yearly incidence of 261 cases/100,000 population. TB lymphadenitis in cervical lymph nodes (TBLN) accounts for ≈33% of all new cases in this country, which is greater than the global average of ≈15% (*1*). Ethiopia has the largest livestock population in Africa (≈51 million cattle), and recent studies have shown that bovine TB is endemic in this country (estimated prevalence 1%–10%) (*2*).

To explore the public health risk for bovine TB in Ethiopia, we have used molecular typing to characterize mycobacterial isolates from persons with TBLN and pulmonary TB who were visiting hospitals throughout the country. Our aim was to define the role of *Mycobacterium bovis* in human TB and to define the overall structure of the *M. tuberculosis* complex in Ethiopia.

The Study

Patients with suspected TBLN or pulmonary TB who came to hospitals or health centers in study sites and provided voluntary consent were recruited into the study during 2006–2010. Fine needle–aspirate samples and sputum samples were collected from 2,151 patients attending hospitals in Gondar, Woldiya, Ghimbi, Butajira, and Negelle, Ethiopia. In addition, sputum samples were collected from patients at hospitals in Fiche, Jinka, and Filtu and at health centers at 3 suburban sites in Addis Ababa (Holeta, Sululta, and Chancho). Samples were cultured on Löwenstein-Jensen medium supplemented with glycerol or pyruvate and on modified Middlebrook 7H11 medium optimized for culture of *M. bovis*.

We characterized isolates belonging to the *M. tuberculosis* complex by using multiplex PCR for large sequence polymorphisms (*3*,*4*), spoligotyping (*5*), and lineage-specific single-nucleotide polymorphism analysis (*4*,*6*). Isolates of selected spoligotypes were characterized by 24-loci mycobacterial interspersed repetitive unit–variable number tandem repeat (MIRU-VNTR) analysis (*7*). Four *M. tuberculosis* isolates from a group of 36 with unusual spoligotype patterns were further characterized by genome sequencing (Illumina Inc., San Diego, CA, USA). Sequencing reads were mapped to the inferred most recent common ancestor of the *M. tuberculosis* complex (*6*). A final alignment of 13,199 single-nucleotide polymorphism positions was generated and analyzed by using the neighbor-joining method with a Tamura-Nei evolutionary model (www.megasoftware.net/mega_papers.php). Nontuberculous mycobacteria were characterized by sequencing of the 16S rDNA gene.

Characteristics of 964 cultures positive for acid-fast bacilli are summarized in Table 1. Most of these isolates had an intact RD9 region, which identified them as *M. tuberculosis*. Only 4 (0.4%) of 964 isolates had undergone RD9 and RD4 deletions characteristic of *M. bovis* (*3*). The 4 *M. bovis* isolates were obtained from cases of pulmonary TB, 3 of which were from patients living in pastoralist

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Table 1. *Mycobacterium spp.* and strains, Ethiopia, 2006–2010*

	Pulmonary TB							TBLN						
		M. tuberculosis†				*M.*			*M. tuberculosis*†					
Collection site	Total	L4	L3	L7	L1	*bovis*	NTM	Total	L4	L3	L7	L1	*M. bovis*	NTM
Gondar	92	32	58	2	0	0	0	32	21	11	0	0	0	0
Woldiya	23	9	10	4	0	0	0	110	54	43	13	0	0	5
Ghimbi	47	40	6	1	0	0	0	69	58	11	0	0	0	0
Fiche‡	169	128	35	6	0	1	0	NA	NA	NA	NA	NA	NA	NA
Addis Ababa‡	60	50	7	3	0	0	0	NA	NA	NA	NA	NA	NA	NA
Butajira	70	60	9	1	0	0	0	110	91	13	6	0	0	0
Negelle/Filtu/Jinka	161	123	28	0	10	3	3	7	5	1	0	1	0	2
Total isolates	622	442	153	17	10	4	3	328	229	79	19	1	0	7
Total *M. tuberculosis* %	100	71.1	24.6	2.7	1.6	NA	NA	100	69.8	24.1	5.8	0.3	NA	NA

*Values are no. isolates. TB, tuberculosis; TBLN, TB lymphadenitis in cervical lymph nodes; NTM, nontuberculous mycobacteria; NA, not applicable.
†Lineage of *M. tuberculosis* is expressed by an L followed by a digit.
‡TBLN samples were not collected in Fiche and Addis Ababa.

communities in southern Ethiopia. The 10 nontuberculous mycobacterial isolates were identified as *M. intracellulare*, *M. flavescens*, and *M. simiae*; 2 of the isolates were from patients co-infected with *M. tuberculosis*.

Among the 954 isolates belonging to the *M. tuberculosis* complex, 671 (71%) belonged to lineage 4, which was the most common lineage in Ethiopia. However, lineage 3 was most prevalent in the northern sites of Gondar and Woldiya (122/257, 47%). Eleven strains belonging to lineage 1 were isolated in the southern region. Two isolates with a characteristic Beijing family spoligotype (spoligotype international type [SIT] 1) were identified as pseudo-Beijing strains belonging to lineage 3 (*8*). Thirty-six isolates with an unusual spoligotype pattern (missing spacers 4–24) and intact for the TbD1 region could not be assigned to known lineages. Genome sequencing identified these strains as members of a new lineage (lineage 7) localized between ancient lineage 1 and modern lineages 2, 3, and 4 of *M. tuberculosis* phylogeny (Figure 1). This new lineage 7 was prominent among strains collected in the Woldiya region (17/133 strains, 13%) (Table 1).

Lineage distribution was identical between the 2 disease forms at the national level; lineage 4 was isolated from 71% (442/662) and 70% (229/328) of pulmonary TB and TBLN patients, respectively, and lineage 3 was isolated from 25% (153/622) and 24% (79/328), respectively. The *M. tuberculosis* isolates encompassed 176 spoligotypes (online Technical Appendix Table 1, wwwnc.cdc.gov/EID/article/19/3/12-0256-Techapp1.xls), of which 86 patterns were new to the international genotyping database 2 (SITVIT2) (www.pasteur-guadeloupe.fr:8081/SITVITDemo/) (*9*). A total of 11% (101/950) of the isolates represented single spoligotypes, and 62% (591/950) were included in 10 major spoligotype clusters (Table 2).

There was no difference in cluster distribution between pulmonary TB and lymph node TB isolates; 10% and

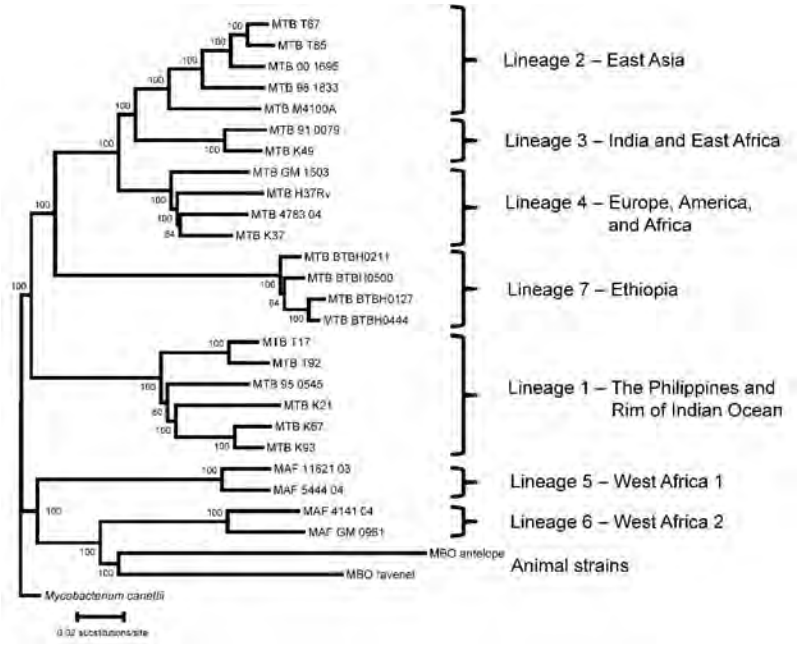

Figure 1. Lineages of the *Mycobacterium tuberculosis* (MTB) complex, Ethiopia, 2006–2010. Genome sequence analysis of 4 strains representative of 36 related isolates identified them as members of a new phylogenetic lineage (lineage 7) of *M. tuberculosis*, which has a phylogenetic location intermediate between ancient lineage 1 and modern Lineages 2, 3, and 4, and a branch point before the deletion of the TbD1 region (*3*). Nomenclatures for lineage names and numbers are as proposed (*4*,*6*). Phylogeny shown is based on 13,199 nt positions that were variable in at least 1 of the 28 *M. tuberculosis* complex strains represented in the tree. Numbers near nodes indicate percentage of bootstrap replicates supporting the topology after 1,000 pseudoreplicates. MAF, *M. africanum*; MBO, *M. bovis*.

Table 2. Major spoligotype clusters of *Mycobacterium tuberculosis*, Ethiopia, 2006–2010*

SIT no.	SITVIT2	Lineage	No. isolates from each site							Disease	
			Go	Wo	Gi	Fi	AA	Bu	NFJ	PTB	TBLN
149	T3-ETH	4	9	17	3	48	15	20	38	125	25
25	CAS1	3	38	32	11	18	3	13	10	77	48
53	T1	4	7	10	17	10	8	32	14	58	40
37	T3	4	2	2	23	8	7	9	10	39	22
3134	New	4	1	1	3	4	2	33	0	26	18
26	CAS1	3	7	3	2	8	4	4	4	25	7
21	CAS1	3	5	2	1	3	0	0	7	16	2
41	LAM7-TUR	4	3	0	3	5	0	0	6	12	5
1729	Undesignated	7	0	13	0	3	0	1	0	5	12
4	LAM3	4	1	3	0	2	0	1	9	11	5
910	Undesignated	7	1	3	1	2	3	4	0	10	4

*SIT, spoligotype international type; SITVIT2, SIT international genotyping database 2; Go, Gondar; Wo, Woldiya; Gi, Ghimbi; Fi, Fiche; AA, Addis Ababa; Bu, Butajira; NFJ, Negelle, Filtu, and Jinka; PTB, pulmonary TB; TBLN, TB lymphadenitis in cervical lymph nodes.

11%, respectively, were single types, and 64% and 60%, respectively, were included in dominant clusters. Two large clusters representative of lineage 4 (SIT 149) and lineage 3 (SIT 25) were further characterized by MIRU-VNTR typing (online Technical Appendix Table 2) and network analysis (Figure 2). In each case, TBLN and pulmonary TB samples were dispersed throughout the network of spoligotype clusters.

All 4 *M. bovis* isolates from humans showed typical bovine spoligotype profiles lacking spacers 3, 9, 16, and 39–43. In addition, they lacked spacers 4–7 and had deletions of RDAf2, which are features that define strains of the African 2 clonal complex of *M. bovis* reported from TB-infected cattle in Ethiopia (*10*).

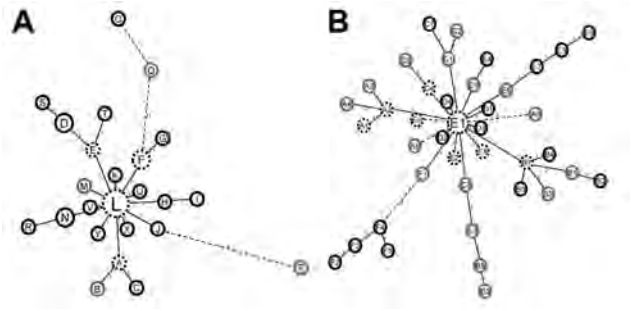

Figure 2. Mycobacterial interspersed repetitive unit–variable number tandem repeat (MIRU-VNTR) networks of major spoligotype clusters, Ethiopia, 2006–2010. Two large sopligotype clusters from lineage 4 (A) (90 isolates of sopligotype 149) and lineage 3 (B) (73 isolates of spoligotype 25) were further characterized by 24-loci MIRU-VNTR typing (online Technical Appendix Table 2, wwwnc.cdc.gov/EID/article/19/3/12-0256-Techapp1.xls). Minimum-spanning trees were calculated for each cluster by using MIRU-VNTR*plus* (www.miru-vntrplus.org). Each circle indicates an individual genotype. Genotypes L and E1 include >15 isolates and remaining genotypes include <15 isolates. Genotypes indicated by a black circle were isolated from patients with pulmonary tuberculosis (TB); those in light gray were isolated from patients with TB lymphadenitis in cervical lymph nodes (TBLN); and those in dashed circles were isolated from patients with pulmonary TB and those with TBLN. Numbers on lines between circles indicate distance between 2 genotypes.

Conclusions

The frequency of *M. bovis* in persons in this study (0.4%) is similar to that found in other studies of human TB in Africa (*11*) and South and Central America (*12*), but much lower than that observed among selected populations in Tanzania (16%) (*13*), Ethiopia (17%) (*14*), and Mexico (28%) (*15*). These findings indicate that the overall contribution of *M. bovis* to human TB is minor but greater in specific areas. In Ethiopia, monitoring of zoonotic transmission is needed in urban areas with high rates of bovine TB associated with intensive farming of imported dairy cattle (R. Firdessa et al., unpub. data) and among pastoralist populations from which human *M. bovis* cases were identified in this study.

Zoonotic transmission of *M. bovis* can be excluded as the predominant cause of the high national incidence of TBLN in Ethiopia. Mapping of disease networks by spoligotyping and MIRU-VNTR analysis showed an integrated distribution of the 2 disease forms, which suggested that cases of TBLN arise from within the pulmonary TB transmission network, rather than from an external zoonotic source.

We identified a novel phylogenetic lineage of *M. tuberculosis* (lineage 7) in multiple sites and at a high frequency in Woldiya in the northeastern highlands of Ethiopia. Screening of the SITVIT2 database (*9*) and the US Centers for Disease Control and Prevention National Tuberculosis Genotyping Surveillance Network Database (L.S. Cowan, pers. comm.) identified 23 (0.03%) of »90,000 isolates as members of lineage 7; all were isolated from patients whose country of origin (when known) was in the Horn of Africa. Lineage 7 is of considerable evolutionary interest because it represents a phylogenetic branch intermediate between the ancient and modern lineages of *M. tuberculosis* (*3*,*4*,*6*).

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Vibrio cholerae Non-O1, Non-O139 Serogroups and Cholera-like Diarrhea, Kolkata, India

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We identified 281 *Vibrio cholerae* non-O1, non-O139 strains from patients with diarrhea in Kolkata, India. Cholera-like diarrhea was the major symptom (66.0%); some patients (20.3%) had severe dehydration. These strains lacked the *ctxA* gene but many had *hlyA*, *rtxA*, and *rtxC* genes. Pulsed-field gel electrophoresis showed no genetic link among strains.

Vibrio cholerae O1 has been responsible for several cholera outbreaks in developing countries. During 1992, a novel serogroup, O139, caused cholera outbreaks in India and other countries in Asia (*1*). These events have shown that serogroups other than O1 have major epidemiologic roles in cholera. *V. cholerae* O1 and O139 serogroups produce cholera toxin (CT), a critical virulence factor and express toxin coregulated pilus (TCP), which are responsible for secretory diarrhea and intestinal colonization, respectively. Serogroups other than O1 and O139 are designated as *V. cholerae* non-O1, non-O139, or nonagglutinating vibrios (NAGs); such serogroups have >200 somatic (O) antigens (*2*) and mostly lack CT- and TCP-coding genes.

Toxigenic and nontoxigenic NAGs have caused several diarrhea outbreaks in India and other countries, including Haiti (*3*–*6*). In non–CT-producing NAGs, other virulence factors such as heat-stable enterotoxin (Stn), hemolysin (HlyA), repeat in toxin (RTX), and type 3 secretion systems (TTSS) have major roles in causing infections (*7*). In this study, we analyzed clinical characteristics of hospitalized patients with diarrhea infected with NAGs and screened strains for antimicrobial drug susceptibility, virulence genes, and genetic relatedness.

The Study

During 2002–2010, a total of 12,719 fecal specimens were collected, which represented every fifth hospitalized diarrhea patient at the Infectious Diseases Hospital in Kolkata and all children at the outpatient unit at B.C. Roy Memorial Hospital for Children in Kolkata. Fecal specimens were screened for *V. cholerae* and other enteric pathogens as described (*8*). NAGs were serotyped by using 206 polyclonal O antisera according to the protocol developed at the National Institute of Infectious Diseases (Tokyo, Japan) (*2*).

Antimicrobial drug susceptibility assays were performed by using the disk diffusion method and commercially available disks (Becton Dickinson, Sparks Glencoe, MD, USA), according to standards of the Clinical and Laboratory Standards Institute (*9*). Because these standards do not include interpretive criteria for *V. cholerae*, breakpoints for *Enterobacteriaceae* were adopted. *Escherichia coli* ATCC 25922 was used as a quality-control strain.

Simplex and multiplex PCRs were performed by using published methods specific for *ctxA, tcpA, rtxA, rtxC, stn*, and *hlyA* genes (classical/El Tor) and the TTSS-coding genes (*7*). Pulsed-field gel electrophoresis was performed according to the PulseNet standardized protocol for *V. cholerae* (www.pulsenetinternational.org/SiteCollectionDocuments/pfge/5.71_2009_PNetStandProtVcholerae.pdf. Gel Compare II software (Applied Maths NV, Sint-Martens-Latem, Belgium) was used for comparison of electrophoresis patterns. This software uses a Dice similarity index and contains an unweighted pair group with arithmetic mean method.

Of the 12,719 diarrhea feces specimens screened, 2,206 (17.3%) contained *V. cholerae*, which included *V. cholerae* O1 in 1,841 (83.4%), and O139 in 84 (3.8%). In the remaining 281 (12.7%) specimens, *V. cholerae* strains did not agglutinate with serogroups O1 or O139. This result was confirmed by species-specific *ompW* PCR, which included strains collected during 2003 (*7*). Among 281 strains, 175 (62.3%) NAGs were the only enteric pathogen found, and 106 (37.7%) of those NAGs were found with other enteric pathogens. The isolation frequency of NAGs ranged from 1.2% to 3.2% (Table 1).

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Table 1. Prevalence rates of *Vibrio cholerae* non-O1, non-O139 strains among patients with diarrhea, Kolkata, India

Year	No. fecal specimens	No. (%) Strains isolated	Infection status	
			Single	Mixed
2002	2,285	49 (2.1)	30	19
2003	1,673	53 (3.2)	29	24
2004	2,430	31 (1.3)	16	15
2005	1,472	38 (2.6)	27	11
2006	930	19 (2.0)	12	7
2007	744	24 (3.2)	23	1
2008	1,124	35 (3.1)	22	13
2009	1,380	17 (1.2)	8	9
2010	681	15 (2.2)	8	7
Total	12,719	281 (2.2)	175	106

Although *V. cholerae* O1 is highly prevalent in Kolkata, more NAGs were detected than in our previous study (*10*). A total of 224 (79.7%) strains were categorized into 80 serogroups; the remaining 57 (20.3%) were untypeable. Among typeable serogroups, 14 (6.2%) strains belonged to the O37 serogroup, and 11 (4.9%) each belonged to serogroups O6 and O34. Serogroups O97 (4.5%), O11 (3.6%), and O59 (3.1%) were also identified in this study. Although serogroups O11, O35, and O37 showed a lower prevalence, their prevalence was higher among patients with diarrhea in Kolkata (*7,10*). The prevalence rate of NAGs (≈2%) in Kolkata is similar to that in the Haizhu District of Guangzhou, China (*11*). However, the number of serogroups identified in China (26 serogroups) was less than in our study (80 serogroups).

Among age groups of patients, NAGs were detected mostly in patients >5 years of age (>72%) than in those <5 years of age (≈28%) (Table 2). NAGs produce a spectrum of gastrointestinal symptoms ranging from asymptomatic infection to severe cholera-like illness or bloody diarrhea. In this study, most (70%) patients had watery diarrhea, which was similar to that for patients with cholera and those exclusively infected by NAGs (Table 2). Other clinical symptoms, such as dehydration status (22.3%) and fever (41.1%), were also high in patients with a single infection. In patients with mixed infections, bloody diarrhea (23.6%) and abdominal pain (41.5%) were the 2 major symptoms (Table 2), perhaps because >1 pathogen was involved.

Most NAGs were resistant to nalidixic acid (57.6%), ampicillin (55.5%), furazolidone (36.6%), and streptomycin (32.4%) and highly susceptible to gentamicin (96%), tetracycline (80%), and chloramphenicol (80.4%). During 1992–1997, antimicrobial drug resistance was high among *V. cholerae* isolated in Kolkata (*12*). In this study, patterns of antimicrobial drug resistance in NAGs were different than those in previous reports (*10,12*). This finding might be caused by discontinuation of ineffective antimicrobial drugs, such as co-trimoxazole and furazolidone, rational use of fluoroquinolones, and introduction of azithromycin for treating diarrhea.

Conclusions

Unlike our previous study (*7*), in this study, factors involved in the virulence of NAGs were not comprehensively elucidated in the study region. Unlike *V. cholerae* O1/O139, the pathogenicity of NAGs has been associated with >1 virulence factor (*7,10*). All NAGs lacked *ctxA* and El Tor *tcpA* genes. However, 6 (2.1%) strains had the *tcpA* gene; in 5 of them it was the only virulence gene detected. Most (94%) strains had the gene encoding El Tor type hemolysin, followed by *rtxA* (91.4%) and *rtxC* (75%) genes; only 5 (1.8%) had the *stn* gene. The RTX family includes a group of protein toxins produced by gram-negative bacteria, including *V. cholerae* with hemolytic, leukotoxic, and actin cross-linking activities, which may play a role in virulence (*13*). In this study, prevalence of NAGs that have genes encoding TTSS as the only virulence factor (41 cases) was higher than in Bangladesh and Argentina (*14,15*).

Strains containing *hlyA*, *rtxA*, and *rtxC* genes were predominant, followed by strains containing *hlyA*-*rtxA*-*rtxC*-TTSS and *hlyA*-*rtxA* genes (Table 3, Appendix, wwwnc.cdc.gov/EID/article/19/3/12-1156-T3.htm). Some gene combinations, such as *hlyA*-*rtxA*-*rtxC*-TTSS, *hlyA*-*rtxA*-*rtxC*, and *hlyA*-*rtxA*, were detected predominantly NAGs as the only virulence factor genes (online Table 3). However, there was no correlation between type of serogroup and prevalence of putative virulence gene(s). Pulsed-field electrophoresis profiles of 70 strains representing the predominant serogroups showed distinct patterns (overall similarity ≈70%) (Figure). There was no profile match among strains belonging to the same serogroup.

Prevalence of NAGs associated with severe traits of infection indicates the role of these pathogens in cholera. The pathogenic mechanism of NAGs is multifarious;

Table 2. Clinical characteristics of *Vibrio cholerae* non-O1, non-O139 strain–infected patients with diarrhea, Kolkata, India

Characteristic	Infection status, no. (%)	
	Single, n = 175	Mixed, n = 106
Type of diarrhea		
Watery	122 (70.0)	63 (59.4)
Loose with bloody mucus	30 (17.1)	25 (23.6)
Other	23 (13.1)	18 (17.0)
Dehydration status		
Severe	39 (22.3)	18 (17.0)
Moderate	136 (77.7)	88 (83.0)
Age, y		
>5	126 (72.0)	75 (70.8)
≤5	49 (28.0)	31 (29.2)
Sex		
M	106 (60.6)	64 (60.4)
F	69 (39.4)	42 (39.6)
Fever		
Yes	72 (41.1)	39 (36.8)
No	103 (58.9)	67 (63.2)
Abdominal pain		
Yes	69 (39.4)	44 (41.5)
No	106 (60.6)	62 (58.5)

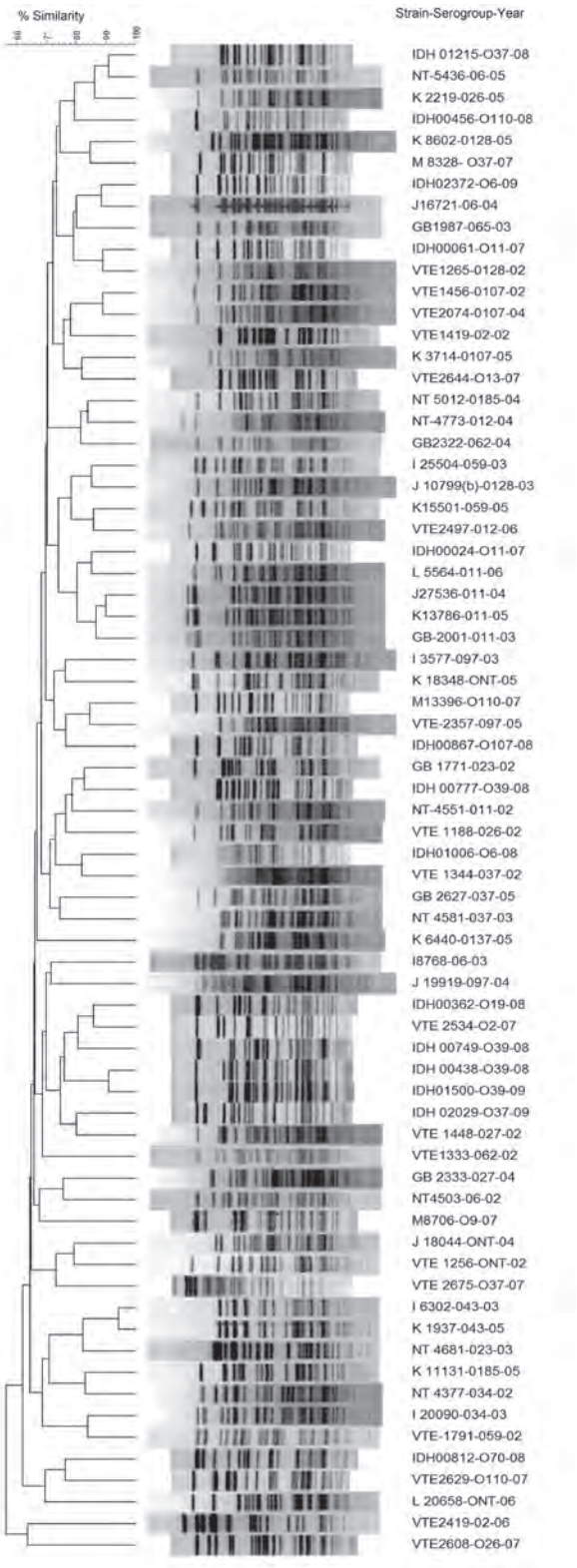

Figure. *Not*I restriction patterns of genomic DNA of representative *Vibrio cholerae* non-O1, non-O139 strains, Kolkata, India. Dendrogram was generated by using the unweighted pair group with arithmetic mean method.

there are several virulence factors in genetically distinct strains. On the basis of our results, current antimicrobial drug therapy in the clinical management of NAG-mediated diarrhea can be continued. Further epidemiologic studies are needed to determine the ecology, virulence factors, and public health role of NAGs.

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Hepatitis E Virus Mixed Infection in Immunocompetent Patient

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We detected 2 hepatitis E virus (HEV) strains in an acutely infected immunocompetent patient. Two populations of genotype 3 virus were observed in the hypervariable regions and, open reading frames 2 and 3, indicating multiple infection with hepatitis E virus. Persons with mixed infections may provide the opportunity for virus recombination.

Most reports of multiple infection with different hepatitis E virus (HEV) variants can be ascribed to immunodeficiency of the host or to high frequencies of infection. For example, 2 different genotype 3 subtypes were detected in samples from an immunocompromised kidney transplant recipient (*1*), and virus from another immunocompromised patient contained an insertion of host-derived sequences or various deletions of this sequence (*2*). Multiple infection or co-circulation of closely related virus variants is not unexpected in such patients with chronic HEV infection (references in [*3*]). Few cases of multiple infection of immunocompetent persons have been reported; 2 different virus genotypes were isolated from a sushi chef in Japan (*4*), and 2 persons in Nepal were each infected by different genotype 1 subtypes (*5*). In the course of our study of the open reading frame (ORF) 1 hypervariable region (HVR) variability in acutely infected persons in Scotland (*6*), we described a person infected with 2 variants of HEV that encoded dramatically distinct HVR sequences. We report here a more complete investigation of this case.

The Study

The patient, a 55-year-old man, sought treatment at a hospital in the southeast of Scotland in April 2012 for nausea, vomiting, anorexia, jaundice, headache, and abdominal pain. At admission, the patient's blood sample was positive for anti-HEV IgM and IgG and raised levels of alanine aminotransferase (ALT) (4,023 IU/L; reference range 5–60 IU/L) and bilirubin (91 μmol/L; reference range 3–17 μmol/L). Over the next 10 days, ALT declined steadily to 404 IU/L and returned to normal levels 1 month later. The patient's abdominal pain had largely resolved by 6 weeks after seeking treatment, but fatigue and lethargy persisted for >8 weeks. There was no evidence that the patient was immunocompromised or was co-infected with other hepatitis viruses. The patient had unexplained jaundice at age 10 years and a history of alcohol abuse as an adult. Symptoms were relatively severe and required 6 days of hospitalization. Informed consent was provided by the patient for laboratory assessment of blood samples.

Virus RNA was extracted from 140 μL of serum and sequenced at limiting dilution in the HVR and ORF2 regions as described (*6*). Analysis of the ORF2/ORF3 overlap region used the primers 5′-CGGGTGGAATGAATAACATGT-3′ (outer sense nucleotides 5098–5118 relative to M73218), 5′-GCRGTYARCGGCGMRGCCCCAGCTG-3′ (outer antisense, 5481–5457), 5′-TYTGCCTATGCTGCCCGCGCCACCG-3′ (inner sense, 5184–5209) and 5′-GGCGCTGGGMYTGGTCRCGCCAAG-3′ (inner antisense, 5426–5403). Negative controls were included in all experiments. GenBank accession numbers for the ORF2 and ORF2/3 sequences described here are JX516004–JX516053 and for the HVR sequences are JX270882–JX270902.

Nucleotide sequence analysis of the HVR from individual virus genomes sampled by limiting dilution of cDNA revealed 2 distinct genotype 3 populations (populations A and B, Figure). Members of these populations all differed by the same 18 nucleotide substitutions, of which 13 were synonymous and 5 nonsynonymous. Two populations (A and B) were also observed among 25 ORF2 sequences (the 2 populations differed by 5 synonymous substitutions) and 26 ORF2/ORF3 sequences (the 2 populations differed by a single substitution, which was nonsynonymous in ORF2 but synonymous in ORF3).

In all 3 regions, 1 of the virus populations represented ≈80%–90% of the sequences: in HVR, 17 sequences were population A and 4 population B; in ORF2, 21 sequences were population A, 2 population B, and 1 mixed; and in ORF2/3, 20 sequences were population A, 4 population B, and 2 mixed. The 3 mixed sequences contained ambiguities at positions where the 2 populations differed, implying that templates from both populations were present in the limiting dilution reaction. The expected number of such mixed-template reactions can be calculated by using the Poisson distribution and the number of PCR-negative replicate reactions, which were 47% (HVR), 50% (ORF2), and 43% (ORF2/3). Thus, 17.5%, 15.4%, and 20.8%, respectively, of reactions would be expected to contain >1 template, of which, given the ratio of populations A and B in each region, 31%, 17.2%, and 28%, respectively, would contain templates from different populations, equivalent

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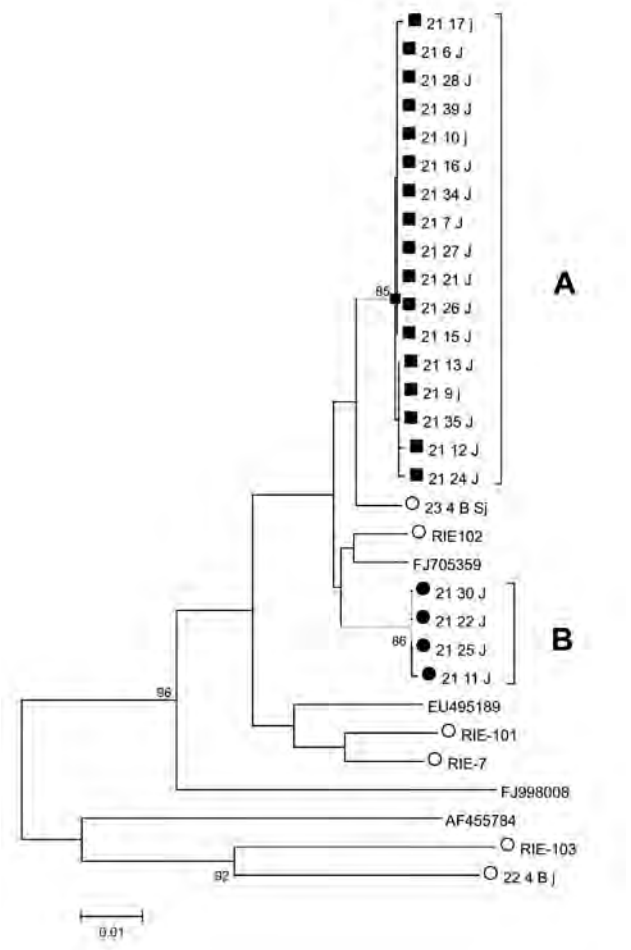

Figure. Phylogenetic tree of hepatitis E virus hypervariable region variants. A neighbor-joining tree of hypervariable region sequences was constructed by using MEGA 4 (*15*). A and B indicate sequences for virus populations A and B. Sequences from the patient studied here (patient 21) belonging to virus population A are indicated by a solid square, and those belonging to virus population B are indicated by a solid circle. Other sequences obtained in the same laboratory are indicated by an open circle. Closely related genotype 3 sequences obtained from GenBank are identified by their accession number. Bootstrap support (1,000 replicates) for branches of >70% are indicated. Scale bar indicates nucleotide substitutions per site.

to 5.4%, 2.6%, and 5.8%, respectively, of positive reactions, compared with the 4.2% (3/71) observed. All other substitutions in these sequence sets were unique to a single sequence. A trivial explanation for the observation of mixed populations in this sample would be contamination during sample collection, processing, or analysis. However, this appears unlikely, given the low frequency of HEV infection in the United Kingdom, the lack of PCR products in negative controls, and the distinctness of the sequences obtained from those previously studied in our laboratory.

Infection of the patient in this study with 2 HEV variants could arise for several different reasons. First, the person could have been exposed to a homogeneous source of HEV, which then diversified during infection. Assuming that infection occurred 1 month before the first symptoms and 6 weeks before sampling, a divergence of HVR sequences at 18 (7.4%) of 243 nucleotide positions equates to a rate of nucleotide substitution from a presumed ancestor of both populations of 0.32/site/year. Not only were these substitutions mostly synonymous, and therefore unlikely to represent selection during infection, but the inferred rate of nucleotide substitution would be several orders of magnitude higher than those previously reported for HEV (0.0014/site/year) (*7*) or for the HCV HVR (0.0043/site/year) (*8*). In addition, the divergence between the 2 virus populations contrasts with the homogeneity observed among 7 other acutely infected patients and between viruses transmitted from 1 person or host to another (*6*).

A second possibility is that this person had a subclinical chronic HEV infection and then became superinfected with a second virus that induced jaundice. No previous blood samples were available for testing, but a prior subclinical chronic infection seems unlikely, given the absence of immunosuppression; the presence of anti-HEV IgM and IgG; the decline and increase, respectively, in these antibody titers in a sample collected 7 days later; and the decline in ALT and bilirubin levels in the weeks following the patient's hospital visit.

Last, the clinical features and our immunologic and virologic findings are consistent with the patient having been infected from a single source containing >1 variant, such as pig-derived figatellu sausage (*9*). Alternatively, he might have been multiply infected from different sources within in a short period and before the development of protective immunity. Follow-up interview revealed no occupational exposure to animals, apart from a domestic dog, and no recent foreign travel; he spent 3 days in a beach resort in southeastern Scotland 12 days before onset of symptoms. The patient ate supermarket-bought prewashed salad vegetables and fresh fruit and drank only bottled or tap water. The patient ate fish, shellfish, chicken, pork, bacon, sausages, ham, Brussels paté, and eggs but not venison or pig liver. He regularly handled uncooked pork and beef at home while preparing food. HEV has been found on the hands and gloves of 17% of persons selling pork products (*10*).

Conclusions

This study suggests that mixed infection with HEV can occur in immunocompetent persons with no obvious high-risk exposure to HEV-infected food or sewage. Only about 300 cases of acute HEV are reported each year in England and Wales (*11*), and the prevalence of HEV PCR-positive blood donors in England is 0.014% (*12*). However, the

seroprevalence of anti-HEV IgG is 1,000× higher (16%) (*13*), and in this context, the identification of sources of autochthonous HEV infection remains an important goal. Our results are also relevant to the suggestion that HEV may undergo recombination (*14*). The case described here suggests that the conditions required for virus recombination may occasionally arise in immunocompetent persons.

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Virulence of Pertactin-Negative *Bordetella pertussis* Isolates from Infants, France

Hélène Bodilis[1] and Nicole Guiso

Bordetella pertussis isolates that do not express pertactin (PRN) are increasing in regions where acellular pertussis vaccines have been used for >7 years. We analyzed data from France and compared clinical symptoms among infants <6 months old infected by PRN-positive or PRN-negative isolates. No major clinical differences were found between the 2 groups.

Bordetella pertussis and *B. parapertussis* are closely related bacterial species, and both cause whooping cough. As early as 1959, whole-cell pertussis vaccine was used intensively in France for primary vaccination of infants at 3–5 months of age and for the first booster at 24 months (*1*). This vaccine program resulted in a dramatic decrease in the incidence of pertussis among young children. Acellular pertussis vaccines (2- and 3–component vaccines) were introduced in 1998 as boosters for vaccinated adolescents and were rapidly adopted for primary vaccination of infants. These vaccines replaced whole-cell pertussis vaccines in 2005, changing herd immunity by specifically targeting the virulence of the bacteria (*2*,*3*).

Since 1996, in France, an active hospital-based surveillance network has performed whooping cough surveillance. The network comprises 42 pediatric hospitals, which participate on a voluntary basis; the National Reference Centre, which is located in the laboratory of the Molecular Prevention and Therapy of Human Diseases Unit at Institut Pasteur; and the French Institute for Public Health surveillance (*3–4*). Participating pediatricians complete a standardized form for every child suspected to have whooping cough. Microbiologists list culture and PCR results and send the clinical isolates to the National Reference Centre for validation of the results. This system of data collection has been unchanged since establishment of the network; data collected is used to analyze trends over time (*3–4*).

We have analyzed the evolution of the bacterial population under vaccine pressure, using pulsed-field gel electrophoresis, genotyping, microarrays, and tests for virulence factor expression (*5–9*). Immunity induced by the whole-cell pertussis vaccine controlled the circulation of vaccine-type isolates but not all types of isolates (*5*,6). The isolates remaining in circulation are as virulent as those circulating during the prevaccine era (*7–9*). Since the introduction of acellular pertussis vaccines, the number of *B. pertussis* and *B. parapertussis* isolates collected that do not express pertactin (PRN), which is used as a vaccine antigen (*7–11*), has steadily increased. The proportion of PRN-negative (PRN–) isolates to the total number of isolates collected each year increased from 2% in 2005 to 14% in 2012 (*8*), indicating that PRN– isolates are transmissible. Studies using animal and cellular models of infection indicate that these PRN– isolates are as virulent as those expressing PRN (PRN+) (*7–9*). However, an analysis and comparison of the clinical symptoms induced by infection with PRN– and PRN+ isolates in infants convey direct information on this strictly human disease. Here, we report a preliminary retrospective comparison of the clinical symptoms of infants <6 months old in France who were infected by PRN– isolates and clinical symptoms of those infected with PRN+ isolates during 2004–2011.

The Study

For the purpose of this study, we used a questionnaire that was more detailed than the one in the standardized form from the hospital-based surveillance program. The questionnaire, including the list of variables described in Table 1, was sent to pediatricians who voluntarily participated. We compared surveys for each patient infected by a PRN– isolate with 2 or 3 randomly selected standardized forms that had been completed by pediatricians and that described patients <6 months of age who were infected by PRN+ isolates during the same period. We sent 68 questionnaires (20 for PRN– isolates, 48 for PRN+ isolates). We received 60 completed questionnaires (40 for infants infected with a PRN+ isolate, 20 for infants with a PRN– isolate).

The available anonymous variables analyzed are shown in Table 1. To compare percentages, we used the χ^2 or Fisher exact test if n<5. To compare means, we used the Mann-Whitney U test. There were no substantial differences in distribution of PRN– and PRN+ isolates among patients in the 2 groups in terms of sex and age

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Table 1. Characteristics and clinical signs and symptoms of patients <6 mo of age infected with *Bordetella pertussis* isolates negative or positive for pertactin, France, 2004–2011*

Variable	Pertactin-negative isolate, n = 20	Pertactin-positive isolate, n = 40	p value
Male sex, %	60	50	0.46†
Age, d (range)	66 (16–147)	61 (23–132)	NA
Year of illness, %			NA
2004	5	2.5	NA
2005	10	7.5	NA
2006	5	5	NA
2007	15	15	NA
2008	5	7.5	NA
2009	25	35	NA
2010	5	22.5	NA
2011	30	5	NA
Previous vaccination	4/19 (21.05)	8/39 (20.51)	0.96†
Vaccinated according to recommendations	n = 19	n = 39	NA
Yes	2 (10.53)	4 (10.26)	1.0‡
No	5 (26.32)	10 (26.54)	NA
Not eligible (<2 mo of age)	12 (63.16	25 (64.10)	NA
Time from onset of signs and symptoms to sample collection, d (range)	14.6 (1–37), n = 13	9.9 (2–35), n = 33	0.04§
Signs and symptoms			
Nocturnal cough	6/7 (86)	19/22 (86)	1‡
Paroxysmal cough	16/16 (100)	35/36 (97)	1‡
Syncope	4/9 (44)	8/25 (32)	0.69‡
Vomiting	3/11 (27)	15/29 (52)	0.29‡
Loss of weight	5/10 (50)	10/25 (40)	0.71‡
Whoop	5/8 (62)	6/14 (43)	0.66‡
Apnea	5/8 (62)	11/24 (46)	0.68‡
Fever	2/11 (18)	3/31 (10)	0.59‡
Bradycardia	4/7 (57)	14/20 (70)	0.6‡
Atypical cough	1/9 (11)	6/17 (35)	0.36‡
Cyanosis/desaturation	9/11 (82)	20/25 (80)	1‡
Deterioration of general condition	4/9 (44)	9/28 (32)	0.77‡
Malignant pertussis	0/9	1/28 (4)	NA
Hyperlymphocytosis	8/9 (89)	17/23 (74)	0.64‡
Hospitalization	16/18 (89)	36/39 (92)	0.65‡
Duration of hospitalization, d (range)	12.6 (1–45), n = 9	16.6 (1–60), n = 28	0.18§
Intensive care	5/10 (50)	13/30 (43)	0.73‡
Duration of intensive care, d (range)	8.2 (2–21), n = 5	5.4 (1–14), n = 12	0.24§

*Values are (no. patients with variable/no. of patients with data) except as indicated. NA, not applicable.
†χ^2 test.
‡Fisher exact test.
§Mann-Whitney U test.

(60% of infants infected with PRN– isolates were boys, as were 50% of those infected with PRN+ isolates; the mean ages of infants in each group were 66 and 61 days, respectively). There was an even distribution of PRN– and PRN+ isolates among the infants across the years studied. Forty-six infants had received no pertussis acellular vaccine, and 11 had received 1 dose. One child >4 months of age received a second dose 4 days before the onset of symptoms. According to information compiled from the survey that used the standardized form, 21.05% of PRN– patients and 20.51% of PRN+ patients had been vaccinated. None of the children had received 3 doses. In each group, ≈10% of infants received vaccinations as scheduled for their age (1 dose of vaccine for each infant). The duration of hospitalization or stay in intensive care was shorter for the group of infants infected with a PRN– isolate, but the difference was not significant (p = 0.18 vs. p = 0.24). The differences found between the 2 groups of infants in terms of the classical symptoms (apnea, vomiting, paroxysmal cough, whoop, bradycardia, and hyperlymphocytosis) were not significant (p = 0.68, p = 0.29, p = 1, p = 0.66, p = 0.6, and p = 0.64, respectively). The only significant difference (p = 0.04) was that the time between the beginning of the cough and hospitalization was longer for infants infected with a PRN– isolate; this finding might reflect less severe disease in this group.

We calculated delay of transmission as the time of onset of coughing by the first member of a household to that by the case-patient. The median delay of transmission was 14.5 and 14.0 days, respectively, in PRN– and PRN+ groups. Among the documented cases, *B. pertussis* was transmitted to the infant by a household member in 84% of the PRN– cases and 91% of the PRN+ cases.

Vaccination was associated with less severe clinical symptoms (Table 2): the proportion of hospitalizations in intensive care units was significantly lower in the

Table 2. Comparison of markers of illness severity for vaccinated versus unvaccinated pertussis patients, France, 2004–2007

Characteristics	No. patients/no. with characteristic (%)		p value*
	Not vaccinated, n = 46	Received 1–2 doses, n = 12	
Intensive care admission	18/34 (53)	0/11 (0)	0.001
Apnea	15/25 (60)	1/7 (14)	0.08
Cyanosis/desaturation	24/28 (86)	5/8 (62)	0.167
Syncopal episodes	12/26 (46)	0/8 (0)	0.03
Bradycardia	15/21 (71)	3/6 (50)	0.367
Deterioration of general condition	12/29 (41)	2/8 (25)	0.68
Malignant pertussis	1/29 (3)	0/8 (0)	1.0

*By Fisher exact test.

vaccinated group (p = 0.001). Clinical symptoms, such as apnea, syncope, cyanosis, and deterioration of general condition, were also less frequent in the vaccinated group (Table 2). This confirms previous findings (*12*) indicating that infants who receive 1 or 2 doses of pertussis vaccine are protected to some extent.

Conclusions

These preliminary data are consistent with those we obtained using murine and cellular models (*8*,*9*). Although the number of infants included in this study is small, we could detect no major difference between the 2 groups; this finding suggests that PRN– isolates are as virulent as PRN+ isolates. This conclusion is also in agreement with data obtained during a clinical trial performed in Italy (*13*). We recommend the continuation of such analyses, and close collaboration of clinicians and microbiologists, to follow the evolution of *B. pertussis* subspecies in terms of virulence. This will help identify strategies to overcome increased adaptive herd immunity induced by acellular pertussis vaccines.

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Unexpected Increase of Alveolar Echinococcosis, Austria, 2011

Renate Schneider, Horst Aspöck, and Herbert Auer

Austria is part of the classical area of central Europe to which classical alveolar echinococcosis (AE) is endemic. Annual incidences in Austria were 2.4 and 2.8 cases/100,000 population during 1991–2000 and 2001–2010, respectively. Hence, the registration of 13 new AE patients in 2011 was unexpected. Increasing fox populations and past AE underreporting might have caused this increase.

Alveolar echinococcosis (AE) is one of the most serious helminthic diseases of humans. It is caused by the larval stages (metacestodes) of the fox tapeworm, *Echinococcus multilocularis*. Final hosts are foxes and, rarely, dogs and cats; intermediate hosts are rodents (voles). Humans are aberrant hosts and acquire the infection by oral ingestion of parasite eggs released in the feces of infected foxes or other carnivores. The metacestodes proliferate in the human liver and induce a hepatic disorder mimicking liver cancer (*1*) that becomes clinically apparent after an incubation period of 5–15 years. The prognosis for untreated AE is poor, and early diagnosis is essential for curative treatment (*2*).

AE has been known to be endemic to Austria, southern Germany, Switzerland, and eastern France since the second half of the nineteenth century. From the Austrian echinococcosis researcher, Adolf Posselt, who documented all reported human AE cases during 1867–1936, we know that the annual incidence of AE in Austria was 1.4 cases at the beginning of the twentieth century and that most patients resided in Austria´s western provinces, Vorarlberg and Tyrol (*3*).

Meanwhile, the parasite and the disease have spread from its classical distribution area in central Europe to at least 11 other European countries (*2*,*4*). Furthermore, several reports from Austria, Germany, and Switzerland document increasing fox populations during the 1990s and 2000s, presumably caused by successful antirabies vaccination (*2*,*5*), which was established in Austria in 1992. In addition, a suspected increase in and/or underreporting of human AE has been discussed within the past few years (*6*,*7*).

The goal of our study was to determine the annual incidence of AE during the past 20 years. We also aimed to discuss possible reasons for the unexpected increase of human AE during 2011.

The Study

Our institute is Austria`s national reference center for echinococcosis (*8*). The study comprised 65 patients in whom AE was diagnosed and registered during 1991–2011; the patients derived from all 9 of Austria's provinces. Inclusion criteria were as follows: 1) AE characteristic imaging findings and 2) *E. multilocularis*–positive species-specific serology and/or 3) AE characteristic histopathologic findings and species-specific molecular analysis. All available details about sex, age, province of origin, results of serologic and molecular biologic investigations, histopathologic findings, and clinical status of AE patients before AE diagnosis were logged into an Excel spreadsheet (Microsoft, Redmond, WA, USA) and analyzed.

Until 2000, we used an in-house crude-antigen ELISA for serologic screening and an in-house immunoblot for species-specific diagnosis (*9*,*10*). In 2000, the in-house immunoblot was replaced by a commercially available immunoblot (LDBIO) (*11*). In 2004, a species-specific PCR was established that detects *E. multilocularis*–specific DNA from fresh or formalin-fixed paraffin-embedded tissue; this method was also applied to retrospectively confirm suspected cases of AE (*12*). To outline the development of the annual incidence of AE in Austria in general and in Vorarlberg and Tyrol Provinces in particular, we compared the annual incidence (patients/year) and the annual incidence rates (patients/100,000 population/year) for 1991–2000 (24 patients) and 2001–2010 (28 patients) with those for 2011 (13 patients) (Table).

During 1991–2000, a total of 24 human AE cases were diagnosed (mean incidence 2.4 new cases/year). The patients derived mainly from the western provinces, Vorarlberg (3 [13%] patients) and Tyrol (12 [50%] patients).

During 2001–2010, we diagnosed 28 AE cases (mean incidence 2.8 new cases/year). The patients derived mainly from Vorarlberg (12 [43%] patients) and Tyrol (5 [18%]).

In contrast to former decades, we registered 13 new AE cases during 2011 (Figure; Table). Four patients resided in Tyrol, and 7 resided in Vorarlberg.

The epidemiologic situation for AE changed most significantly in the most western province (Vorarlberg) during the observation period. The annual incidence rate in Vorarlberg was 0.08 cases per 100,000 population during 1991–2000; it increased to 0.32 cases per 100,000

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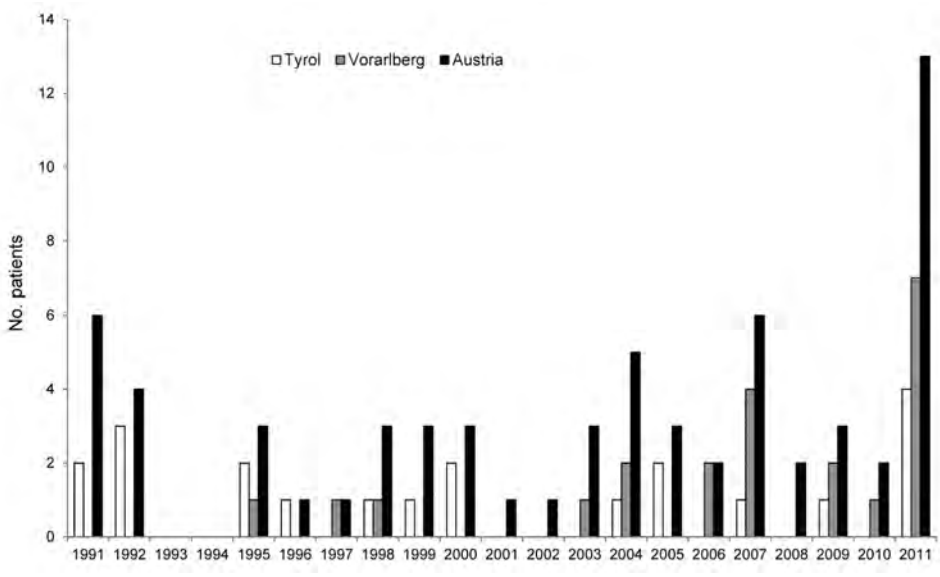

Figure. Incidence of alveolar echinococcosis, Austria and its provinces of Tyrol and Voralberg, 1991–2011.

population during 2001–2010 and peaked in 2011 at 1.9 cases per 100,000 population (Table). Vorarlberg, with 372,001 residents, is one of least populated Austrian provinces. In Tyrol (710,048 residents) the annual incidence rate varied from 0.17 (1991–2000) to 0.07 (2001–2010) to 0.56 per 100,000 population in 2011 (Table). The other 7 provinces showed only sporadic cases during the study period.

Conclusions

Because of the long incubation period of human AE (5–15 years) constant observation of the annual incidence and geographic distribution is critical (*13*). The annual incidence of 2.4 cases (1991–2000) and 2.8 cases (2001–2010) in the past 2 decades represents a moderate increase from the 1.4 cases at the beginning of the twentieth century. Hence, the 13 new cases in 2011 were unexpected, and we assume the following reasons for this increase.

We know from studies in Switzerland and Poland that increasing fox populations changed their population dynamics and live in proximity of villages or even cities (*6*,*14*). The general increase in AE incidence in Austria is mainly due to the increase in Vorarlberg. Duscher et al. (*5*) reported Vorarlberg as the Austrian province with the highest prevalence of *E. multilocularis*–infected red foxes (up to 60%); this high prevalence is a prerequisite for human AE. Although the high incidence in 2011 (7 new cases in Vorarlberg) of such a rare disease can be misinterpreted, data from the past 21 years show a continuous increase of cases in Vorarlberg (Table; Figure). Such an increase in some regions also was observed in neighboring Switzerland (e.g., the Swiss Jura [0.75 cases/100,000 population]) (*13*).

Past underreporting of AE is a problem in Austria (and other European countries) and could partially explain the suddenly increased number of cases (*7*). Although human AE has been reportable in Austria since 2004, only 2 of the 13 cases from 2011 were reported to the Ministry of Health. Our recent data indicate that AE diagnosed only histopathologically is not reported by pathology institutes, a presumption suggested by Jorgerson et al. (*7*).

In addition, our institute provides newly established molecular biologic methods (PCR) enabling not only an exact differentiation between liver cancer or metastases and AE but also species-specific diagnosis based on native

Table. Incidences of alveolar echinococcosis, Austria, 1991–2011

Variable	Observation period		
	1991–2000	2001–2010	2011
Location, no. patients (cases/100,000 population/year)			
Austria, n = 8,217,280 residents*	24 (0.029)	28 (0.034)	13 (0.158)
Tyrol Province, n = 710,048 residents*	12 (0.17)	5 (0.07)	4 (0.56)
Vorarlberg Province, n = 372,000 residents*	3 (0.08)	12 (0.32)	7 (1.9)
Patient characteristics			
Average age (range), y	50.5 (7–78)	56.5 (37–80)	68.6 (45–90)
Sex, no. (%)			
F	12 (50)	10 (36)	6 (46)
M	12 (50)	18 (64)	7 (54)
Asymptomatic, no. (%)	10 (42)	11 (39)	5 (38)

*Census 2011.

material from operations on AE patients and on formalin-fixed paraffin-embedded tissue (*12*). These methods proved to be valuable diagnostic tools.

Because the number of asymptomatic patients remained almost stable during the study period (Table), there is no evidence to suggest that the stage at which AE is diagnosed today is earlier than in past decades. We share this observation with neighboring Switzerland (*6*).

In conclusion, we assume there are several reasons (i.e., increasing fox population, past underreporting, more sensitive laboratory diagnostic tools) for the increasing number of AE cases in Austria within the past decades and especially in 2011. The next years will show whether the high incidence in 2011, which is in accordance with a study from neighboring Switzerland (*6*), is a statistical outlier or reflects a persistent event. We propose that the surveillance system in Austria be improved. Hence, we suggest that serologic screening of exposed groups, such as hunters or farmers, in *E. multilocularis*–endemic regions, such as Vorarlberg, with increasing fox populations could lead to some early diagnosed and therefore successfully treated AE cases (*15*).

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Multidrug-Resistant Tuberculosis, Somalia, 2010–2011

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In a nationwide survey in 2011, multidrug-resistant tuberculosis (MDR TB) was found in 5.2% and 40.8% of patients with new and previously treated TB, respectively. These levels of drug resistance are among the highest ever documented in Africa and the Middle East. This finding presents a serious challenge for TB control in Somalia.

After ≈2 decades of civil war, Somalia is one of the poorest, least developed, and most violent countries in the world (*1*,*2*). The conflict and violence, particularly in the south-central region, have caused massive population movements, exacerbated recently by severe drought, floods, and famine. In 2011, ≈1.5 million internally displaced persons were reported among the country's 9 million inhabitants (*2*). Ratios of maternal mortality and deaths of children <5 years of age are among the highest in the world (*1*,*3*), and communicable diseases are the most common causes of illness and death.

In Somalia, tuberculosis (TB) is a serious public health problem.The estimated incidence in 2011 was 300 cases per 100,000 persons, but fewer than half of the estimated cases are actually detected (*4*). Resistance to anti-TB drugs is considered an emerging problem, but the prevalence of multidrug-resistant (MDR) TB (i.e., resistance to rifampin and isoniazid, the 2 most powerful anti-TB drugs) is unknown. To understand the effect of drug-resistant TB and better plan for treatment needs, Somalia's National Tuberculosis Control Program directly measured drug-resistance prevalence among a representative sample of TB patients in Somalia.

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The Study

During March 2010–October 2011, we conducted a nationwide survey of persons from all 3 zones in the country who had pulmonary sputum smear–positive TB. The survey was designed according to the guidelines of the World Health Organization (WHO) (*5*) by using cluster sampling of 39 diagnostic centers. Patients with sputum smear–negative and extrapulmonary TB were excluded from the study. Cases were classified as newly diagnosed or previously treated according to WHO definitions (*6*). We collected the following variables through a questionnaire administered during sputum collection: patient sex, age, country of birth, and treatment history (new or previously treated). A laboratory in South Africa that is accredited for molecular testing by the National Accreditation System tested sputum samples for resistance to rifampin and isoniazid by using Genotype MTBDR*plus* assay (Hain LifeScience GmbH, Nehren, Germany) (*7*,*8*). The sensitivity of this assay to detect mutations known to confer resistance is higher for rifampin than for isoniazid (98.4% vs. 88.7%, respectively).

The National Ethical Review Board of the Somalia Ministry of Health approved this study. Patients provided informed consent before enrolment. Statistical analyses were performed in Stata (version 12; Stata Corp., College Station, TX, USA). Prevalence estimates were adjusted for fluxes in TB notifications from 2007, the year on which sampling calculations were based, through 2010, the year in which the survey started (Table footnote; online Technical Appendix wwwnc.cdc.gov/EID/article19/3/12-1287-Techapp1.pdf).

A total of 946 patients were consecutively enrolled. Ninety-six enrollees were subsequently excluded because of sample contamination (52 patients), insufficient material to perform the GenoType MTBDR*plus* assay (41 patients), and isolation of *Mycobacteria* spp. other than *M. tuberculosis* (3 patients). The overall total drug susceptibility recovery rate was 89.9%, in line with the country expectations and WHO recommendations (*5*). Of the patients retained in the study (754 persons with new cases and 96 persons with previously treated cases), the male-to-female ratio was 2.4:1.0, and median age was 30 years (range 4–86 years, interquartile range 23–44 years).

MDR TB was detected in 5.2% (95% CI 2.8–7.5) of persons with newly diagnosed TB and 40.8% (95% CI 24.7–57.0) of persons with previously treated TB. Levels of resistance to isoniazid and rifampin and frequencies of any resistance and monoresistance are shown in the Table. The survey detected 87 MDR TB cases.

History of previous anti-TB treatment was the strongest independent factor for MDR TB (odds ratio [OR] 23.0, 95% CI 9.4–56.1, p<0.001), and living in the south-central region or in Puntland was associated with a significantly higher risk for MDR TB than was

Table. Prevalence of resistance to first-line antituberculosis drugs in patients with sputum smear–positive pulmonary tuberculosis, Somalia, 2010–2011*

Drug-resistance pattern	Patients, % (95% CI)		
	New, n = 754	Previously treated, n = 96	All
Susceptible	85.6 (81.5–89.7)	46.2 (29.1–63.2)	81.4 (77.7–85.2)
Any resistance to			
Isoniazid	10.9 (7.8–13.9)	51.4 (35.8–67.0)	15.2 (12.2–18.2)
Rifampin	8.7 (5.2–12.1)	43.2 (25.9–60.6)	12.3 (9.1–15.6)
Total	14.4 (10.3–18.5)	53.8 (36.8–70.9)	18.6 (14.8–22.3)
Monoresistance to			
Isoniazid	5.7 (4.1–7.4)	10.6 (0.0–21.5)	6.2 (4.5–7.9)
Rifampin	3.5 (1.1–6.0)	2.4 (0.0–5.4)	3.4 (1.0–5.8)
Total	9.2 (6.1–12.4)	13.0 (1.4–24.5)	9.6 (6.6–12.6)
Multidrug resistance	5.2 (2.8–7.5)	40.8 (24.7–57.0)	8.9 (6.5–11.4)

*Prevalence estimates were obtained by using logistic regression (Stata's svy: logit command, Stata Corp., College Station, TX, USA) on the binary treatment history variable; each new/retreatment case with a drug-susceptibility test result was weighted by the number of new/retreatment cases notified in its cluster in 2010 (the year in which the survey started), divided by the total number of new/retreated cases with a drug-susceptibility test result in its cluster. The estimation of odds ratios reported elsewhere in the text also included the expansion of categorical sex, age group, and zone variables (*xi: logit*), with clustering and CIs of variance. The findings were robust to multiple imputation of missing data (adding another 78 new and 18 retreatment cases to the sample), use of sampling weights based on 2007 notifications (the year in which cluster samples were calculated), and no use of sampling weights at all; prevalence estimates were equivalent to or slightly higher than those reported here.

living in Somaliland (OR 3.6, 95% CI 1.9–6.9 p<0.001 for living in Puntland and OR 4.3, 95% CI 1.7–11.3, p = 0.003 for living in the south-central region). Associations between MDR TB and sex, age, and country of birth were not significant.

Conclusions

Compared with a study conducted in neighboring Ethiopia in 2005, where MDR TB was found in only 1.6% of new and 11.8% of previously treated TB cases (*9*,*10*), and with surveys conducted in countries of the eastern Mediterranean region, where the average proportion of MDR TB in new and previously treated TB cases was of 3.4% and 29.9%, respectively (*4*), the proportions of MDR TB detected in Somalia are high. At this level of resistance, one would expect that among the 9,760 pulmonary TB cases notified in Somalia in 2011 (*4*), ≈750 were MDR TB and therefore required treatment with second-line drugs; this number does not include other (non-MDR) cases of rifampin-resistant TB that would probably require an MDR TB regimen. This finding presents a real emergency for the National Tuberculosis Control Program considering the duration of second-line treatment (≥2 years) (*11*,*12*), the current availability of such treatment in Somalia for a only few patients, and the country's lack of laboratory capacity to diagnose drug resistance. A systematic review of the cost effectiveness of MDR TB care (*13*) suggests that it would cost US$3,500–5,900 to treat MDR TB on an outpatient basis in Somalia. At <US$400 per disability-adjusted life-year averted, this intervention is cost effective. Efforts are being made to treat MDR TB patients detected in the survey. Because the cost of treating all 750 MDR TB patients is ≈32%–54% of the US$8.2 million (all of it from the Global Fund to Fight AIDS, Tuberculosis and Malaria) that was available to Somalia in 2012 for its entire TB control program (*4*), additional funding will be needed.

In a drug quality survey conducted in Somalia in 2010, 60% of 10 products containing first-line anti-TB drugs that can be easily purchased from pharmacies and informal health care providers met international quality standards (I. Sindani, pers. comm.). The compound most commonly found in insufficient concentration and quality was rifampin. The extensive use of drugs of suboptimal quality, the widespread practice of using wrong medical prescriptions, and incomplete adherence of patients to treatment are the most likely reasons for the high levels of MDR TB in Somalia. These levels appear to be highest in the south-central region, where the security situation is most volatile and disruption of care more frequent. This region also is most affected by recent food shortages (*14*) and has the most internally displaced persons (*15*), factors that are expected to exacerbate disease progression and transmission of *M. tuberculosis*.

This study shows that nationwide surveys to monitor drug-resistant TB are possible even when social conditions are unstable. Two Middle Eastern countries, Afghanistan and Yemen, also were able to conclude nationwide drug resistance surveys in 2011 despite social unrest (*4*). Sample collection, transportation, and monitoring of survey operations in our study have been challenging. The analysis had to account for population movements and changes in the availability of and access to health services.

This study, conducted under difficult circumstances in a country with unstable social conditions, showed that MDR TB is a serious underdetected and widespread public health problem in Somalia. The documented levels of MDR TB are among the highest reported in Africa and the Middle East and suggest that ≈750 patients in the country had MDR TB in 2011. Urgent measures should be introduced to improve access to diagnosis of drug resistance and availability of second-line medication for all patients who need them.

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Swine Influenza in Sri Lanka

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To study influenza viruses in pigs in Sri Lanka, we examined samples from pigs at slaughterhouses. Influenza (H3N2) and A(H1N1)pdm09 viruses were prevalent during 2004–2005 and 2009–2012, respectively. Genetic and epidemiologic analyses of human and swine influenza viruses indicated 2 events of A(H1N1)pdm09 virus spillover from humans to pigs.

Data on swine influenza in southern Asia are limited (*1*–*3*). Sri Lanka is an island in this region with a human population of 21 million and a swine population of ≈83,785 (*4*,*5*). Pigs are not routinely imported into Sri Lanka. Most (61%) swine farms are located in the western costal belt spanning the Puttlam, Gampaha, Colombo, and Kalutara districts. In 2010, for these 4 districts, pig population densities were 7, 15, 12, and 1 animal per km^2, respectively (*4*,*5*). In 2001, for these districts, the human population densities were 246, 1,539, 3,330, and 677 persons per km^2, respectively (*6*).

The Study

During 2004–2005 and 2009–2012, tracheal and nasal swab and serum samples were collected from pigs at government slaughterhouses in Sri Lanka (Table 1). Culture tubes with MDCK cells were inoculated with the swab samples, and 2 blind passages were made. Also, embryonated eggs were inoculated by the allantoic route with swab samples collected during 2004–2005. Virus isolates were subtyped by hemagglutination inhibition (HAI) testing and neuraminidase inhibition testing with reference antiserum as described (*7*,*8*), and results were confirmed by sequencing the hemagglutinin and neuraminidase gene segments.

RNA extraction, cDNA synthesis, PCR, genome sequencing (*9*), and one-step quantitative real-time reverse transcription (RT-PCR) for rapid genotyping of all 8 gene segments (*10*) of A(H1N1)pdm09 isolates were performed as described. Methods used for the phylogenetic analysis are described in online Technical Appendix 1 (wwwnc.cdc.gov/EID/article 19/3/12-0945-Techapp1.pdf). GenBank accession numbers assigned to the sequences determined in this study are KC197816–KC197855 and KC190041–KC190078.

Serum samples were tested by HAI as described (*8*) by using the virus antigens shown in Table 2. The number of human influenza A(H1N1)pdm09 viruses detected by RT-PCR in Sri Lanka during July 2009–March 2012 was obtained from the World Health Organization FluNet and from the Epidemiology Unit, Ministry of Health, Sri Lanka (*11*,*12*). Seven A(H1N1)pdm09 viruses isolated from humans during 2009–2011 were obtained from the National Center for Influenza, Medical Research Institute, Sri Lanka, and were genetically sequenced as described above. Genetic sequence data of the hemagglutinin gene of 5 additional human A(H1N1)pdm09 viruses isolated in Sri Lanka were provided by the World Health Organization Influenza Collaborating Centre, Melbourne, Australia.

One influenza A virus, A/swine/Colombo/48/2004(H3N2), was isolated in MDCK cells from a tracheal swab sample collected in 2004–2005. All genes of this virus were closely related to human influenza (H3N2) virus isolate A/Ragama/190/2003 from Sri Lanka and to other subtype H3N2 influenza viruses isolated worldwide at this time (data not shown). During January 2004–March 2005, a total of 185 (61.6%) of 300 serum samples tested were positive for A/swine/Colombo/48/2004(H3N2); HAI titers ranged from 40 to ≥1,280 (Table 2), indicating that this human-like influenza (H3N2) virus was widespread in the swine population. Serum samples collected from swine during 2009–2012 were also mostly seronegative to this and to more contemporary human influenza (H3N2) viruses.

Table 1. Swine influenza viruses isolated from pigs, Sri Lanka*

Collection years, location	No. pigs sampled/no. viruses isolated (source)
2004–2005	
Welisara†	40/0
Dematagoda‡	260/1 (tracheal swab)
2009–2012, Dematagoda‡	2,710/26 (7 tracheal swabs, 19 nasal swabs)

*From each pig, 1 tracheal swab, 1 nasal swab, and 1 serum sample was collected, except during 2009–2012, when only 1,773 serum samples were collected from 2,710 pigs beginning in February 2010.
†National Livestock Development Board swine farm, Welisara, Sri Lanka (slaughters 2–3 pigs/ wk).
‡Government slaughterhouse, Colombo Municipal Council, Dematagoda, Colombo, Sri Lanka (slaughters 10–20 pigs/d).

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Table 2. Homologous serological reaction profile to subtypes of influenza viruses among pigs, Sri Lanka, 2004–2005 and 2010–2012*

Virus antigen (lineage)	Seroprevalence, no. (%)				
	Jan 2004–Mar 2005, n = 300	Feb–Aug 2010, n = 149	Sep 2010–Mar 2011, n = 284	Apr–Oct 2011, n = 577	Nov 2011–May 2012, n = 763
A/swine/Colombo/48/2004 (H3N2) (human-like)	185 (61.6%)	06 (4.0%)	0	0	0
A/swine/HK/2422/98 (H3N2) (swine) (human)	0	0	0	0	0
A/Sydney/5/97 (H3N2) (human)	0	0	0	0	0
A/swine/HK/1774/99 (H3N2) (European swinelike)	0	0	0	0	0
A/HK/44062/2011 (H3N2) (human)	Not tested	Not tested	0	0	0
A/swine/Colombo/330/2009 (H1N1) (H1N1pdm09)	0	16 (10.7)	95 (33.5)	14 (25.1)	77 (10.1)
A/swine/HK/29/2009 (H1N1) (Eurasian avian)	0	01 (0.6)	0	0	0
A/swine/HK/1110/2006 (H1N1) (North American–triple reassortant)	0	01 (0.6)	0	0	0
A/swine/HK/915/2004 (H1N2) (North American–TR)	0	0	0	0	0
A/swine/HK/4167/99 (H1N1) (classical swine)	0	0	0	0	0
A/swine/Ghent/G112/2007 (Eurasian avian)	0	0	0	0	0

*Hemagglutination inhibition reciprocal antibody titers ≥40 were considered positive. The range of the antibody titers was 40 to ≥1,280. If serum reacted to multiple antigenically related influenza H3 or H1 subtype viruses, we categorized the serum as having a homologous reaction profile to the virus to which titer was ≥4-fold higher than that for other viruses of the same subtype. For example, during 2004–2005, some serum samples were seropositive for influenza A/swine/HK/2422/98 (H3N2) virus; however, because in the same sample, titer to influenza A/swine/Colombo/48/2004 (H3N2) virus was >4-fold higher, reactivity was attributed to the latter.

Of the nasal and tracheal swab samples collected from 2,710 pigs during 2009–2012, a total of 26 (0.5%) viruses were isolated in MDCK cells; all were identified as A(H1N1)pdm09 viruses. All 8 gene segments of these viruses were similar to those of A(H1N1)pdm09 virus; no evidence of reassortment with other swine or human viruses was found. These isolates were collected on 12 sampling occasions from apparently healthy pigs on 7 farms. The 2 peaks of A(H1N1)pdm09 detection in swine followed peaks of human A(H1N1)pdm09 outbreaks that occurred during June 2009–January 2010 and October 2010–February 2011, which represented the first and second waves of the pandemic in Sri Lanka (Figure 1). Overall, virus yield was higher from nasal swab samples than from tracheal swab samples (Table 1).

Phylogenetic analysis showed that the 15 A(H1N1) pdm09 viruses isolated from swine during October 2009–July 2010 clustered together and with other A(H1N1) pdm09 viruses isolated from humans during this period. In contrast, swine A(H1N1)pdm09 viruses isolated in 2011 clustered separately from swine viruses isolated during 2009–2010 and clustered with human A(H1N1) pdm09 viruses isolated in 2010 and 2011 in Sri Lanka and elsewhere (Figure 2). The amino acid signature changes occurring within human A(H1N1)pdm09 viruses in the first and second pandemic waves are reflected in the

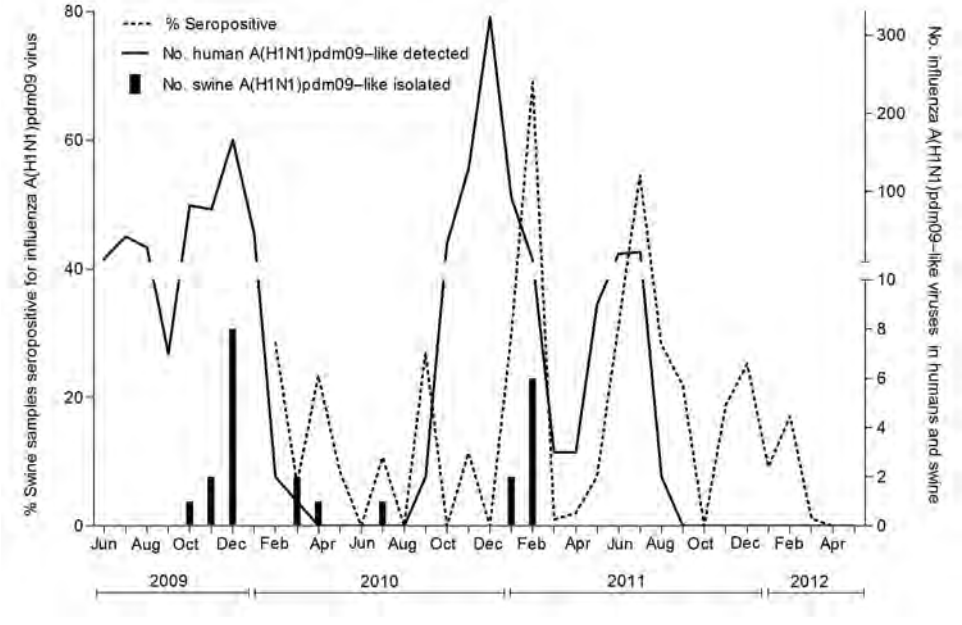

Figure 1. Distribution of percentage of swine serum samples seropositive for influenza A(H1N1) pdm09 viruses, by month, and number of A(H1N1)pdm09 viruses detected in humans and swine. The left y-axis represents the percentage of swine serum samples positive for A(H1N1) pdm09 virus. The right y-axis represents the number of swine A(H1N1)pdm09 isolated in the study and reverse transcription PCR–positive human A(H1N1) pdm09 detected in Sri Lanka.

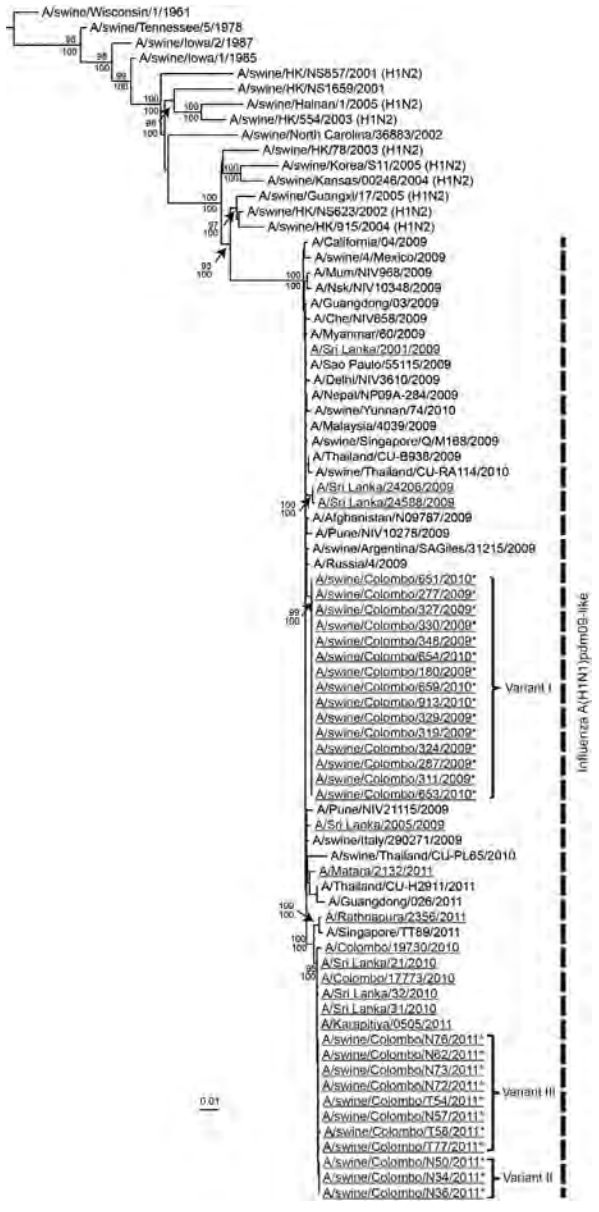

Figure 2. Phylogenetic relationship of the hemagglutinin 1 gene of the human and swine influenza A(H1N1)pdm09–like viruses isolated during 2009–2012 in Sri Lanka. Underlining indicates swine and human viruses characterized in this study; *indicates swine A(H1N1)pdm09 virus isolates. Nucleotide sequences from selected, related avian, equine, swine, and human virus strains available in GenBank are included for comparison. The phylogenetic tree was generated by the maximum-likelihood method and rooted to A/duck/Miyagi/66/77(H1N1) virus (online Technical Appendix 1, wwwnc.cdc.gov/EID/article/19/3/12-0945-Techapp1.pdf). Scale bar represents number of nucleotide substitutions per site. Vertical dashed line indicates influenza A(H1N1)pdm09–like virus lineage. Branch labels record the stability of the branches >500 bootstrap replicates. Numbers above and below branches indicate neighbor-joining bootstrap values and Bayesian posterior probabilities, respectively. Only bootstrap values >70% and Bayesian posterior probabilities >95% are shown. Three genetic variants with >1 aa difference in hemagglutinin 1 are indicated.

corresponding waves of swine infections, and each linage that occurred in swine led to extinction (online Technical Appendix 2, wwwnc.cdc.gov/EID/article/19/3/12-0945-Techapp2.pdf). This finding suggests that the A(H1N1) pdm09 infections among swine that occurred during January–February 2011 were separate spillover events from the second wave of human infections during October 2010–February 2011 rather than from continued epizootic transmission among swine from October 2009. The upper 95% CI of the prevalence of each viral genetic variant, given no positive isolates since the last detection, declined to almost zero during the course of observation, indicating probable extinction of these genetic variants (online Technical Appendix 2).

On some sampling occasions, A(H1N1)pdm09 viruses were isolated from multiple pigs from the same farm, and on 1 sampling occasion, isolates came from multiple pigs (from the same farm) slaughtered 9 days apart. In such instances, with 1 exception, we found viruses from the same farm to be genetically identical, suggesting continued circulation of the virus in swine herds.

Swine serum samples collected in 2004–2005 showed no seroprevalence to A(H1N1)pdm09, A/California/4/2009, and A/swine/Colombo/330/2009 viruses (Table 2). In 2010, seroprevalence to A(H1N1) pdm09 virus was detected (Figure 1). After peaking in February 2011, seroprevalence declined to undetectable levels in April–May 2012, suggesting that the A(H1N1) pdm09 virus was not sustaining transmission among pigs in the absence of continued human infection. The maximum cross-correlation between incidence of human and swine virus isolates was found after an 8-week lag, indicating that the rise in incidence of human virus preceded that in swine by 7–8 weeks (online Technical Appendix 2).

Conclusions

Isolation of human-like influenza A (H3N2) and A(H1N1)pdm09-like viruses from pigs in Sri Lanka probably represents spillover infection from humans, with self-limited transmission and extinction within pig herds. This finding might reflect characteristics of swine husbandry in Sri Lanka, where swine population density in the study area is relatively low (7.7 pigs/km^2), or other factors (*5*,*13*). Genetic characterization of individual gene segments of all influenza (H3N2) and A(H1N1)pdm09 viruses from swine showed no evidence of genetic reassortment. This finding contrasts with those from Hong Kong, Thailand, Argentina, and the United States, where reassortment of A(H1N1)pdm09 with other swine influenza viruses has reportedly occurred (*14*,*15*). This contrast might reflect the low prevalence of other swine influenza virus lineages (e.g., classical swine, Eurasian avian-like and triple-reassortant

swine) endemic to Sri Lanka. With the exception of subtype H3N2 viruses (Table 2), no evidence of other endemic swine influenza viruses circulating in swine in the country before the emergence of the A(H1N1)pdm09 in 2009 was found, and influenza (H3N2) virus in swine became extinct around the time of the spillover of A(H1N1)pdm09 to swine. These observations might explain the lack of emergence of A(H1N1)pdm09 reassortants among swine. It might also indicate that A(H1N1)pdm09, although able to spill over from humans to swine, is not ideally adapted to establish sustained transmission among swine in the absence of further reassortment with other swine influenza virus lineages.

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Tuberculosis Outbreak in a Primary School, Milan, Italy

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Investigation of an outbreak of tuberculosis (TB) in a primary school in Milan, Italy, found 15 schoolchildren had active TB disease and 173 had latent TB infection. TB was also identified in 2 homeless men near the school. Diagnostic delay, particularly in the index case-patient, contributed to the transmission of infection.

Italy has a low incidence of tuberculosis (TB); in 2008, incidence of notified cases was 7.6/100,000 population (*1*). However, higher incidence rates have been reported in some areas. In 2009, in the northern Italy region of Lombardy, incidence of notified cases was 11.7/100,000 population; 58% of cases were in non-Italian nationals (www.dgsan.lombardia.it/malinf/2009/report_sintesi_2009.pdf). Incidence in children 0–14 years of age was 3.38/100,000 (n = 47 cases). In 2009 in Milan, the largest urban area of Lombardy (1.6 million inhabitants), the incidence was 20.44/100,000 population (www.asl.milano.it/user/download.aspx?FILE=OBJ06171.PDF&TIPO=FLE&NOME=report_prevenzione_2011).

In industrialized countries, such as Italy, TB is increasingly associated with specific population subgroups: immigrants from countries with high endemicity (*2*,*5*,*6*), ethnic minorities (*2*), refugees, and the homeless (*2*,*4*). The control of TB in Italy relies on timely diagnosis and adequate treatment of TB cases, screening of persons in at-risk groups and those in close contact with active TB case-patients, and vaccination of at-risk health care workers and children who live in close contact with a reported TB case-patient (*2*,*3*). Factors that influence the effectiveness of TB surveillance and control include lack of prioritization of TB within the health service, difficulties faced by foreign citizens in accessing health care, lack of coordination by a reference center, and diagnostic delay (*7*). One study found that that median diagnostic delay, health care delay, and total delay for TB patients in Italy were 7, 36, and 65 days, respectively (*8*). We investigated an outbreak of TB infection identified in 2010 among children in a primary school in Milan.

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The Study

In November 2010, pulmonary and meningeal TB was identified in a 7-year-old boy in Milan who was in the second year of primary school. The child experienced fever, headache, and asthenia in September 2010 and was treated by a pediatrician; no radiographs were taken. Two months later, the child was hospitalized because the severity of symptoms had increased, and Beijing strain *Mycobacterium tuberculosis* bacteria were isolated from a gastric lavage sample.

As a result of this case, local health authorities conducted a contact investigation. Screening of potential contacts is done by using the Mantoux tuberculin skin test (TST); induration of ≥5 mm is considered positive for this test (*9*). Because a delay of 8–10 weeks after exposure is necessary for a positive skin test, if a contact's exposure to the TB case-patient occurs within that period, TST is repeated 8–10 weeks after the most recent exposure. Persons who have received bacille Calmette-Guerin vaccine and have positive TST results are subjected to the interferon-gamma release assay test. Those with positive results for either test undergo clinical evaluation and chest radiography. If a diagnosis of active TB is ruled out, the person is defined with a case of latent TB infection (LTBI).

In this investigation, the boy's family and friends all had negative TST results. His classmates and the children in the 2 adjacent classrooms were screened; results indicated LTBI for 20% of the students in his classroom and 20% and 14% of students in the other 2 classrooms. His teachers and some other school staff members were also screened (n = 43); no cases of active TB were detected among children or school staff.

In December 2010, pleural TB was confirmed by isolation of *M. tuberculosis* from a secondary school pupil who had attended the same primary school as the first case-patient during the previous year. Genotyping confirmed that the isolate was the same Bejing strain. A search of the regional strain database found that this genotype had also been identified in a case of pulmonary TB reported in November 2009 in a homeless person who lived in the

Table. Distribution of prevalence rates of TB and LTBI among current and former students at primary school, Milan, Italy, 2011*

Class	No. students	No. (%) undergoing TST	No. (%) TST positive†			Total no. (%) active TB cases‡
			First test	Second and third tests	Total positive	
First year	162	161 (99.4)	3 (1.9)	0	3 (1.9)	1 (0.6)
Second year	174	172 (98.9)	47 (27.3)	3 (1.7)	50 (29.1)	5 (2.9)
Third year	170	170 (100)	11 (6.5)	0	11 (6.5)	0
Fourth year	156	156 (100)	30 (19.2)	3 (1.9)	33 (21.2)	2 (1.3)
Fifth year	151	149 (98.7)	57 (38.3)	2 (1.3)	59 (39.6)	4 (2.7)
Former fifth year§	156	156 (100)	29 (18.6)	ND	29 (18.6)	3 (1.9)
Transferred	20	13 (65)	3 (23.1)	ND	3 (23.1)	0
Total	989	977 (98.8)	180 (18.4)	8 (0.8)	188 (19.2)	15 (1.5)

*Because a delay of 8–10 weeks after exposure is necessary for a positive skin test, follow-up screening was performed for students whose initial test occurred <10 weeks after exposure. TB, tuberculosis; LTBI, latent TB infection; TST, tuberculin skin test; ND, not done because initial screening was >10 weeks after exposure.
†At the first screening, 3 students refused to submit to TST; 11 refused at the second, and 11 at the third.
‡All other infections were LTBI.
§Those who were fifth-year students during the previous school year.

city park in front of the school. This man had been lost to therapeutic follow-up but had infected his daughter.

After the second school case was identified, health authorities extended TST testing to all children who had attended the school during 2010, including those who had moved to other schools (Table), and to all school staff members. Thirteen homeless persons who frequented the area around the school were also screened; 1 was found to have TB, and the *M. tuberculosis* isolated was of the same Bejing strain as that isolated from the other homeless man and the first 2 infected schoolchildren.

A total of 15 cases of TB were identified among current and former students at the school, including 2 pulmonary forms, 1 extrapulmonary form confirmed bacteriologically, and 12 early pulmonary forms without *M. tuberculosis* isolation. The latter type is defined as clinical evidence of the disease and any of the following: contact with an adult with TB, positive TST results, suggestive appearances for TB on chest x-ray, and favorable response to antituberculous therapy (*10*). A total of 173 pupils were found to have LTBI. No active TB was found among the school's staff members.

In February 2011, another case of pulmonary TB was reported. The patient, a 17-year-old boy who had been coughing for ≈1 month, was in the fifth year of primary school with younger children because of disabilities (spastic tetraplegia). He had been in the primary school for 3 years. He had the same *M. tuberculosis* genotype (isolate from gastric lavage) as found in the previous cases. The collected data suggest that this boy was the index case-patient for this outbreak: 90.9% of his classmates were infected, and a relevant ratio of TB infection was found among pupils of the other classrooms on the same floor (Figure 1). The spread of infection was probably favored by the fact that, because of his disability, this student was part of an integration program that included taking part in activities in other classes and areas of the school. In addition, positive TST results among former fifth-year primary pupils (in the first year of secondary school at the time of testing) show that the disease was already active during the previous school year.

Genotyping has proved to be an essential tool in TB contact investigations (*11*), especially in the presence of clusters. Genotyping of *M. tuberculosis* isolates during this investigation enabled the establishment of a connection between the cases of TB among the schoolchildren. However, no contact between the 17-year-old boy and the first homeless man has been identified (Figure 2).

Conclusions

The results of this investigation indicate that a diagnostic delay for the index case-patient played a primary role in the transmission of infection inside the school. The main cause of this delay was the low degree of diagnostic suspicion toward the disease; however, TB can also be difficult to diagnose in children because children are less able to produce sputum. Physicians should be aware of the signs and symptoms of early TB infection and should consider this diagnosis accordingly. Furthermore, routine screening for TB could be considered for persons with disabilities or special needs who take part in recreational

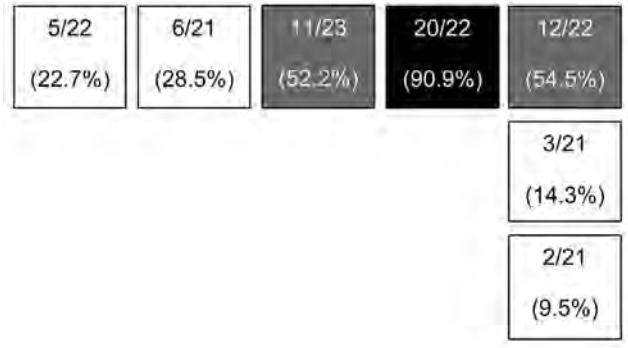

Figure 1. Floor plan of classrooms at primary school in Milan, Italy, 2010. Values are no. tuberculosis infections/total no. pupils in classroom (% pupils infected). Shading indicates classrooms with highest rates of infection: the classroom of the 17-year-old student determined to be the index case-patient (black shading) and the 2 adjacent classrooms (gray shading).

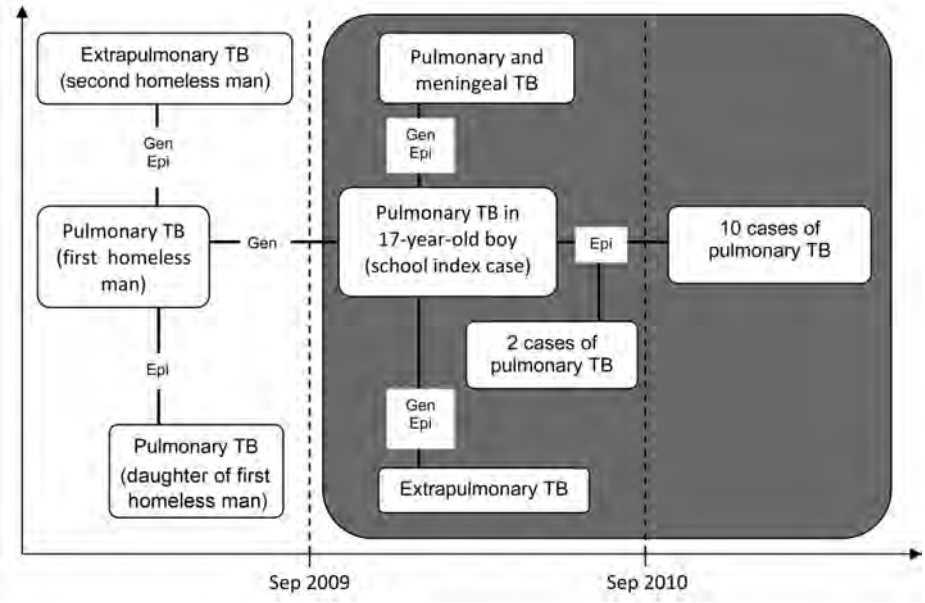

Figure 2. Hypothetical timeline of tuberculosis (TB) outbreak at primary school in Milan, Italy, 2009–2011. Vertical dashed lines indicate school year delineation. The connection among cases established through epidemiologic investigation (epi) or genotyping of Mycobacterium tuberculosis strains (gen) is indicated.

and educational activities in which they come into close contact with more susceptible groups, such as children.

In addition, cases of TB among the homeless, particularly those in close proximity to susceptible groups such as schoolchildren, highlight the problem of therapeutic monitoring among persons who may be lost to follow-up. Careful evaluation of compliance at time of discharge from health care is critical, and social protection programs are needed to improve rates of follow-up care. In particular, in urban areas where risk factors for transmission of TB are highly concentrated, a TB reference center may improve collaboration between local health authority, physicians, and social services.

Dr Faccini works for the Local Health Authority in Milan. His research interests are management of measures of surveillance and prophylaxis of infectious diseases.

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Lymphogranuloma Venereum in Men Screened for Pharyngeal and Rectal Infection, Germany

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To determine prevalence of lymphogranuloma venereum among men who have sex with men in Germany, we conducted a multicenter study during 2009–2010 and found high rates of rectal and pharyngeal infection in men positive for the causative agent, *Chlamydia trachomatis*. Many infections were asymptomatic. An adjusted *C. trachomatis* screening policy is justified in Germany.

Lymphogranuloma venereum (LGV) is a sexually transmitted disease caused by infection with *Chlamydia trachomatis* bacteria, genotypes L1–L3. An outbreak of proctitis cases caused by *C. trachomatis* genotype L2 in men who have sex with men (MSM) became apparent in the Netherlands in 2003; subsequently, awareness of this disease increased throughout Europe (*1*).

In the United Kingdom and the United States, guidelines recommend rectal *C. trachomatis* screening for MSM (*2*). In Germany, no screening recommendations for asymptomatic MSM exist, and nationally, no *C. trachomatis* prevalence data are available. We investigated the prevalence of pharyngeal and rectal *C. trachomatis* infection and LGV among MSM in Germany.

The Study

We conducted a prospective, multicenter study during December 1, 2009–December 31, 2010, by recruiting a convenience sample of MSM at sentinel sites for sexually transmitted infections throughout Germany. Inclusion criteria were being MSM, having ≥1 male sexual partner within the previous 6 months, and agreeing to provide a rectal and/or pharyngeal swab specimen. To measure factors associated with HIV status, enrollment at sites providing HIV care was enhanced.

Rectal and pharyngeal specimens were collected according to standardized protocols; urine testing or collection of urethral swabs was optional. All specimens were sent to a privately owned laboratory (Laboratoriumsmedizin Koeln, Cologne, Germany), and tested for *C. trachomatis* by using the APTIMA Combo 2 Assay (GenProbe Inc., San Diego, CA, USA), based on RNA amplification. Specimens positive for *C. trachomatis* were sent to the Institute of Medical Microbiology and Hospital Hygiene of Heinrich-Heine University in Duesseldorf, Germany, for L genotyping, based on a DNA test (*3*). Persons who had a sample positive for LGV genotype L were defined as LGV-positive; those positive for other genotypes were defined as LGV-negative.

Data on sexual history, behavior, and symptoms were collected from participants through a self-administered questionnaire. Information on HIV status was self-reported or obtained from primary care providers. Results were assessed with 95% CIs, and significance level was set at 0.05. The study protocol was approved by the ethical review committee of Charité University Hospital, Berlin. Data were anonymized, participation was voluntary, and no financial incentives were provided.

Of 1,883 MSM recruited at 22 sites in 16 cities, 1,848 agreed to a pharyngeal swab and 1,754 to a rectal swab. An additional 522 samples from either urine or urethral swab were obtained. Of those recruited, 166 (8.8%) tested positive for *C. trachomatis* by rRNA-based assay (Figure). A total of 632 (33.6%) participants were HIV-positive. *C. trachomatis* prevalence was 10.8% among HIV-positive and 7.8% among HIV-negative or untested participants (odds ratio [OR] 1.42, 95% CI 1.03–1.96).

For logistical reasons, only 154 *C. trachomatis*–positive specimens underwent genotyping. Nineteen samples were LGV-positive: 17 genotype L2 (16 rectal, 1 pharyngeal), 1 genotype L3 (pharyngeal), and 1 genotype L2/L3 (rectal). For genotyped specimens, LGV prevalence was 16.5% in rectal specimens and 15.4% in pharyngeal specimens. Overall, LGV prevalence was 1.7% (11/632) among HIV-positive and 0.6% (8/1,251) among HIV-negative or untested MSM (OR 2.75, 95% CI 1.10–6.88).

Eight (53.3%) of 15 LGV-positive MSM did not report recent rectal symptoms (Table 1). HIV-negative MSM more often met 1 of their last 3 sexual partners in a bar, pub, or club than did HIV-negative MSM ($p = 0.03$ by *t* test). However, we found no substantial differences in sexual practices between HIV-positive and HIV-negative MSM positive for

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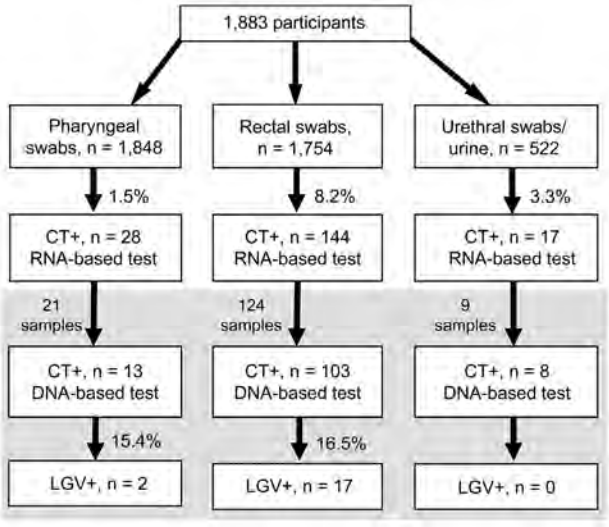

Figure. Flowchart of testing of 1,883 men who have sex with men for *Chlamydia trachomatis* (CT) and lymphogranuloma venereum (LGV) by RNA- and DNA-based assays, Germany, December 1, 2009–December 31, 2010. Gray shading indicates samples positive for CT that were sent for L genotyping. Most participants provided >1 type of sample.

LGV and no differences between LGV-positive and LGV-negative MSM (data not shown).

Overall, 70.2% of *C. trachomatis*–positive MSM were asymptomatic (Table 2). In multivariable logistic regression analysis, only history of *C. trachomatis* infection was associated with LGV infection. In a model not considering history of *C. trachomatis* infection, the number of male sex partners in the previous 6 months was associated with outcome (OR 1.03, 95% CI 1.01–1.06).

Conclusions

Our study showed rectal and pharyngeal LGV prevalences of 16.5% and 15.4%, respectively, among *C. trachomatis*–positive MSM in Germany. Previous reports have found that 75% of all LGV cases in MSM were among HIV-positive men (*1*,*4*); a meta-analysis found HIV prevalence of 67%–100% among LGV-positive men (*5*). In our study, 58% of LGV-positive MSM were HIV positive.

In a screening study conducted in London, an 8% (247/3,017) prevalence of rectal chlamydia was detected; among these infections, 14% were L genotype (*6*). The coinfection rate of HIV in men with rectal *C. trachomatis* in that study was 38% (94/247), comparable to the 44% in our study. HIV-positive status may be associated with having more sexual partners, more frequent unprotected receptive anal intercourse, and higher susceptibility to LGV infection (*4*). Because of a relatively small number of observations, however, our study lacks the power to detect these differences.

Although the finding was not significant, HIV-negative MSM who had higher numbers of sexual partners in the 6 months before the study were more likely to be LGV-positive. These men were also more likely to having met 1 of their previous 3 partners in a bar, pub, or club, settings in which explicit HIV serostatus communication is less likely to occur (*7*). This finding indicates that the spread of LGV is not confined to sex networks of HIV-positive MSM (*8*).

The prevalence of rectal and pharyngeal *C. trachomatis* infection we found in MSM in Germany is comparable to previously reported rates (*9*–*11*). However, because our study used a convenience sample of health care–seeking men, MSM who have poor health care–seeking behavior might be underrepresented, which could mean *C. trachomatis* prevalence is higher than we found. A total of 70% of *C. trachomatis*–positive persons in our study were asymptomatic, similar to the 69% reported from a study in the United Kingdom (*6*).

Our observed proportion of LGV subtypes among *C. trachomatis*–positive persons is in line with published data (*6*). However, the high proportion of asymptomatic cases and LGV-positive cases among HIV-negative MSM we found is in contrast to other recent findings (*6*,*12*), although considerable percentages of asymptomatic LGV infections have been reported elsewhere (*13*).

Although it is not licensed for extragenital use, sensitivity and specificity of the RNA-based assay we used is high (*10*,*14*). Nucleic acid amplification tests may also be used for detection of *C. trachomatis* in pharyngeal and rectal specimens (*15*). Our initial testing used the APTIMA Combo 2 Assay, for which the transport media was adopted. Because of the lysing effect of the transport medium, concentration of pathogens before DNA preparation was disqualified and *C. trachomatis* detection was restricted to samples with higher DNA concentrations, leading to a different number of positive samples. To avoid potential bias, only specimens that tested positive in both assays were included in the analyses.

Another limitation of our study is that not all 166 samples were sent for further subtyping. The genotype for 12 specimens remains unknown.

We found no major predictors for LGV infections in *C. trachomatis*–positive MSM. This finding points to 2 options for control: 1) all MSM diagnosed with *C. trachomatis* should receive treatment adequate to cure LGV (that is, 3 weeks of doxycycline rather than 1), or 2) all MSM-derived specimens positive for *C. trachomatis* should further be genotyped to exclude infection with LGV genotypes. Physicians should be aware of possible L-genotype infection in symptomatic or HIV-positive patients and should initiate further diagnostic tests. In the absence of commercially available LGV sequencing tests, clinicians should

use in-house PCR tests to detect LGV strains. In addition, the observed rate of rectal *C. trachomatis* and LGV infections in MSM justifies the implementation of a *C. trachomatis* screening policy for MSM in Germany.

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Table 1. Characteristics of MSM patients with LGV, by HIV status, Germany, 2009–2010*

Characteristics	HIV negative, n = 8	HIV positive, n = 11	p value
Median age, y (range)	33 (27–55)	41 (31–46)	0.48
Origin			
Germany	7	9	1.0†
Abroad	1 (United Kingdom)	2 (Turkey, Colombia)	
Location of LGV			
Rectal	7	10	1.0†
Pharyngeal	1	1	
Genotype			
L2	7	10	
L2/L3	1	0	
L3	0	1	
Symptoms	n = 8	n = 10	
None	5	3	0.34†
Symptomatic	3	7	
Anorectal symptoms‡	1	12	
Night sweats	1	0	
Median no. male sex partners in past 6 mo (range)			
All partners	11 (3–180)	2.5 (1–1,000)	0.25
Unprotected anal sex partners	2 (0–77)	1 (0–80)	1.0
Meeting place for ≥1 of last 3 partners, %	n = 8	n = 8	
The Internet	63	63	1.0†
Bar/pub/club	75	25	0.13†
Sauna	0	13	1.0†
Sex party	25	13	1.0†
At friends' homes	25	0	0.47†
Other	0	13	1.0†
CT test ever, yes/no	2/3	7/1	0.22†
If yes, when			
Past 3 mo	1	5	
Past 12 mo	1	2	
History of CT	2	5	0.32†
If yes, when			
Past month§	1	3	
Past 12 mo	0	1	
>12 mo	0	1	
If history of CT in past month, location of current LGV			
Rectal§	1	3	
If history of CT in past month, site of last examination			
Urine	0	2	
Rectum	1	1	
Pharynx	1	0	
Blood	0	2	
Do not remember	0	2	
Type of last examination	n = 8	n = 8	
Urine	1	6	
Urethral swab	1	0	
Rectal swab	5	4	
Pharyngeal swab	5	3	
Blood	5	6	
Do not remember	0	3	
HIV test ever, yes/no	7/1	NA	
If yes, when			
Past 3 mo	2	NA	
Past 6 mo	1	NA	
Past 12 mo	1	NA	
ART, yes/no	NA	7/1	

*Values are no. patients except as indicated. MSM, men who have sex with men; LGV, lymphogranuloma venereum; CT, *Chlamydia trachomatis*; NA, not applicable; ART, antiretroviral therapy.
†By Fisher exact test.
‡Anal pain, anal burning/itching, anal inflammation, (bloody) discharge, defecation problems (multiple answers possible).
§Of these patients, 1 HIV negative and 2 HIV positive patients were from 1 proctologist referral practice.

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The authors are aware that the conclusion regarding implementation of *C. trachomatis* screening policies will have financial impacts for companies producing respective diagnostic tests. However, neither GenProbe nor any of its employees had any influence on the analysis and interpretation of the data or on the conclusions. None of the authors has any shares or other investments in companies producing diagnostic tests for *C. trachomatis* or LGV.

Table 2. Characteristics of MSM patients with CT infection, by LGV status, Germany, 2009–2010*

Characteristic	LGV positive, n = 19	LGV negative, n = 95	p value	OR (95%CI)
Median age, y (range)	37 (27–55)	30 (19–67)	0.004	
Origin	n = 16	n = 80		
Germany, % (no.)	87.5 (14)	80.0 (64)	0.73†	1.75 (0.34–17.33)
Reported symptoms	n = 15	n = 69		
None, % (no.)	53.3 (8)‡	73.9 (51)	0.13	2.48 (0.79–7.81)
Median no. male sex partners in past 6 mo (range)				
All partners	9 (1–1,000)	5 (1–150)	0.11	
Unprotected anal sex partners	1.5 (0–80)	1 (0–150)	0.43	
Meeting place for ≥1 of last 3 partners, %	n = 16	n = 82		
The Internet	62.5	65.9	0.80	0.86 (0.29–2.62)
Bar/pub/club	50.0	26.8	0.08†	2.73 (0.91–8.15)
Sauna	6.3	13.4	0.68†	0.43 (0.01–3.43)
Sex party	18.8	3.7	0.05†	6.08 (0.71–49.10)
Pornography cinema	0	7.3	0.59†	0.60 (0.00–4.47)
Cruising	0	4.9	1.0†	0.95 (0.00–8.02)
At friends' homes	12.5	11.0	1.0†	1.16 (0.11–6.52)
Other	6.3	7.3	1.0†	0.84 (0.02–7.81)
Location of sexual contact with last 3 partners, %§	15 patients/33 answers	57 patients/144 answers		
Germany	100.0	95.1	0.33	2.18 (0.29–∞)
Abroad	0	4.9		
HIV status				
% Positive	57.9	41.1	0.18	1.97 (0.73–5.36)
If positive				
Median time since diagnosis, y (range)	4.5 (2.75–13.67)	2.1 (0–20.25)	0.22	
HIV therapy, %	n = 8	n = 25		
Combination therapy	87.5	52.0	0.11†	6.46 (0.63–312.96)
No therapy	12.5	48.0		
Viral load, copies/mL, %	n = 8	n = 22		
Undetectable or <1,000	100.0	54.5	0.03†	0.12 (0.00–0.94)
≥1,000	0	45.5		
History of CT testing and past infections	19	95		
CT test ever, yes/no	9/4	27/26	0.35†	2.17 (0.52–10.73)
If yes, when				
Past 3 mo	6	11		
Past 6 mo	1	3		
Past 12 mo	2	5		
History of CT infection	n = 16	n = 82		
Yes, % (no.)	43.8 (7)	12.2 (10)	0.01†	5.60 (1.40–21.24)
If yes, when				
Past month	4¶	2	0.57†	3.0 (0.26–35.33)
>1 mo	2	3		
If history of CT infection in past month, type of current CT infection, no.				
Anorectal	4	1		
Urethral	0	1		

*No. indicates no. persons. MSM, men who have sex with men; CT, *Chlamydia trachomatis*; LGV, lymphogranuloma venereum; OR, odds ratio.
†Exact logistic regression
‡3 of these patients were from 1 proctological referral practice
§Multiple answers possible.
¶Of 2 pharyngeal LGV cases, the HIV-positive patient was asymptomatic and the HIV-negative patient reported night-sweats.

H.W., F.W., and B.H. work in medical laboratories that also offer *C. trachomatis* and/or LGV testing on a commercial basis and therefore may financially benefit from broadening *C. trachomatis* screening recommendations to MSM; none of them, however, has any influence on the decision-making process of health authorities in Germany.

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Prioritizing Tuberculosis Clusters by Genotype for Public Health Action, Washington, USA

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Groups of tuberculosis cases with indistinguishable Mycobacterium tuberculosis genotypes (clusters) might represent recent transmission. We compared geospatial concentration of genotype clusters with independent priority rankings determined by local public health officials; findings were highly correlated. Routine use of geospatial statistics could help health departments identify recent disease transmission.

Mycobacterium tuberculosis genotyping has been applied to tuberculosis (TB) control activities for >2 decades, and epidemiologic or genotyping data can confirm or disprove outbreaks (*1*–*4*). Investigation of genotype clusters can identify unrecognized transmission and lead to interventions that interrupt further transmission (*5*,*6*). However, cluster investigations are complex, requiring patient interviews and field observations. Focusing resources on clusters that most likely represent recent TB transmission could reduce the number of unnecessary investigations.

Geospatial statistics can identify higher-than-expected concentrations of TB cases with indistinguishable genotypes (*7*). We describe a comparison of a quantitative geospatial statistic analysis with qualitative expert opinion for prioritizing TB cluster investigations in Washington, USA, a state with moderate TB incidence (3.5 cases/100,000 persons) (*8*). The comparison was performed for initial and follow-up 3-year periods, 2005–2007 (period 1) and 2008–2010 (period 2).

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The Study

TB genotype clusters were defined as groups of ≥3 TB case-patients whose isolates had matching spoligotyping and 12-locus mycobacterial interspersed repetitive unit–variable number tandem repeat (MIRU-VNTR) (*9*) genotyping results that were reported in the same county within Washington. A log-likelihood ratio (LLR) was calculated for each genotype cluster identified during each of the two 3-year periods (Figure). The larger the LLR, the greater the possibility the cluster represented geographically concentrated TB cases, a proxy for recent TB transmission. The cutoff point for the LLR was set to 5.0, based on the value used by the national TB Genotyping Information Management System (*10*).

Qualitative analysis came from a 5-member expert panel of TB public health officials in Washington. In 2008, the panel participated in a discussion of all county-level TB clusters, ranking each as high or low priority for additional investigation. Priority was determined on the basis of a review of patient characteristics, epidemiologic links from field investigations, and maps of genotype distributions. The panel also had information from enhanced contact investigations from local public health investigation teams that included the ability to order IS*6110* restriction fragment-length polymorphism (IS*6110* RFLP) and 24-locus MIRU-VNTR testing for clusters of concern, but results from these tests were not universally available. The ranking exercise with the same 5-member panel was repeated after period 1 for clusters from period 1. The expert panel was blinded to the LLR.

Geographic area	Cluster genotype		Total
	Yes	No	
Inside	*a*	*b*	*a*+*b*
Outside	*c*	*d*	*c*+*d*
Total	*a*+*c*	*b*+*d*	N

$$\mathrm{LLR} = a \times \log\left[\frac{\frac{a}{(a+b)}}{\frac{(a+c)}{N}}\right] + c \times \log\left[\frac{\frac{c}{(c+d)}}{\frac{a+c}{(N)}}\right]$$

Figure. Formula used to calculate geospatial statistic (a modified log-likelihood ratio [LLR]) on the basis of geographic distribution of *Mycobacterium tuberculosis* genotype clusters, Washington, USA. Variables are classified as follows: *a* = number of tuberculosis (TB) cases with the genotype of interest in the selected county; *b* = number of cases with the genotype of interest in the United States; *c* = number of cases without the genotype of interest in the selected county; *d* = number of cases without the genotype of interest in the United States; *N* = total number of TB cases.

LLRs were compared with the expert opinion ranking to assess concordance. With expert opinion as the standard, negative and positive predictive values (NPV and PPV, respectively) were calculated for period 1 using a cutoff point of LLR ≥5.0. Alternative cutoff points were evaluated to maximize NPV and PPV. Sensitivity and specificity of the ≥5.0 LLR cutoff point and exact binomial 95% CIs were calculated for period 1 clusters. An alternative cutoff point to maximize sensitivity and specificity was also determined.

A total of 806 TB cases were reported in Washington during period 1. Of 659 culture-positive cases, 642 (97.4%) had genotyped isolates; of these, 318 cases formed 21 clusters. Five of these clusters had a high LLR; the expert panel ranked all 5 of these clusters high priority and identified them as clusters of concern. Of the 16 clusters with LLR <5.0, the expert panel ranked 12 (75.0%) as low priority (Table).

A total of 723 TB cases were reported in Washington during period 2. Of 592 culture-positive cases, 576 (97.3%) had genotyped isolates. The expert panel reexamined the 21 clusters identified during period 1 and focused on new activity within those areas during period 2. Two clusters with a high LLR during period 1 continued to have a high LLR during period 2; the expert panel continued to rank these high priority. The 3 other clusters that had a high LLR during period 1 had a low LLR for period 2; one of those was still considered a high priority by the panel. Of the remaining 16 clusters, which continued to have a LLR <5, 14 (87.5%) were still ranked low priority by the panel.

Two clusters in the same county, PCR00309 and PCR00803, had low LLRs but were considered high priority by the expert panel. For cluster PCR00309, the panel cited high levels of homelessness among case-patients as reason to rank it high priority. For cluster PCR00803, the panel cited a highly mobile population from a TB-endemic country that regularly traveled into and out of the United States as reason to rank it high priority. However, the travel history among case-patients in this cluster made it difficult for investigators to determine whether transmission was occurring within Washington or abroad.

The NPV and PPV for a LLR cutoff point of 5.0 were 75% and 100%, respectively. Lowering the cutoff point to a LLR ≥2.0 increased the NPV to 92.3%, but the PPV remained at 100%. For period 1 clusters only, a cutoff point of LLR ≥5.0 generated a sensitivity of 55.6% (95% CI 21.2%–86.3%) and specificity of 100% (95% CI 73.5%–100.0%) for identifying clusters for further investigation. Decreasing the cutoff point to ≥2.0 increased the sensitivity to 88.9% (95% CI 51.8%–99.7%) but did not change the specificity (100%; 95% CI 73.5%–100.0%).

Conclusions

The geospatial statistic in this study was highly correlated with experts' perceived need for public health action. This finding indicates that automated alerts generated on the basis of geospatial concentration of TB cases might help the state TB program identify clusters that would

Table. Comparison of geospatial analysis results and expert panel priority status rankings for county-level genotype clusters of TB cases, Washington, USA, 2005–2010*

			Period 1†			Period 2†			Key
County	Spoliogtype	12-locus MIRU-VNTR	No. cases	LLR	Expert priority	No. cases	LLR	Expert priority	epidemiologic features‡
A	000000000003771	223321153643	32	**31.8**	High	19	**18.3**	High	H, SA
	000000000003771	223325173533	17	0.1	High	8	0.4	Low	FB
	677777477413771	254326223432	14	0.2	Low	14	0.1	Low	FB
	703377400001771	227425113434	4	1.2	Low	6	2.9	Low	FB, H
	000000000003771	223325153533	3	0.5	Low	1	<0.01	Low	FB
	000000000003771	223325163533	3	0.2	Low	7	1.7	Low	FB, SA
	677777477413771	254326223422	6	1.3	Low	4	0.4	Low	FB
	777000377760771	225125113322	3	0.6	Low	3	1.0	Low	FB
	000000000003771	222325173543	9	2.4	High	3	0.1	High	H, SA
	777777777760771	225125113322	3	0.7	Low	2	0.4	Low	FB
	000000000003771	222325173533	7	2.6	High	8	2.3	High	
	000000000003771	223325173433	3	1.5	Low	1	0.2	Low	FB
	000000000003771	223325133533	3	1.4	Low	0	<0.01	Low	FB
	000000000000000	223325123534	4	**9.2**	High	0	<0.01	Low	H, SA
	777776777620601	224325153323	3	4.4	High	0	0.03	Low	H, SA
B	000000000003771	223425173563	13	**19.5**	High	8	**13**	High	H, SA
C	677777477413771	254326223432	6	1.3	Low	3	<0.01	Low	FB, SA
	777776777760771	125325153225	7	**9.8**	High	2	1.8	High	H, SA
D	677777477413771	254326223432	5	0.8	Low	4	0.4	Low	FB
	777776757760771	223325143324	6	**8.7**	High	1	1.2	Low	
E	677777477413771	254326223432	3	1.0	Low	2	0.9	Low	FB

*LLRs >5.0 (**boldface**) indicate greater possibility that the cluster represents geographically concentrated TB cases, a proxy for recent TB transmission). TB, tuberculosis; MIRU-VNTR, mycobacterial interspersed repetitive unit–variable number tandem repeat; LLR, log-likelihood ratio, a measure of geospatial concentration; H, homelessness; FB, foreign born; SA, substance abuse.
†Period 1, January 1, 2005–December 31, 2007; period 2, January 1, 2008–December 31, 2010.
‡Blank cells indicate none present.

benefit from additional investigation. Automated alerts can be generated by using routinely collected surveillance data and are currently part of the national TB Genotyping Information Management System (*10*).

Patient and contact characteristics, transmission venues, and temporality all contribute toward prioritization determination. For example, during period 1, a total of 6 (66.6%) clusters ranked high priority by the expert panel were characterized by homelessness or substance abuse among case-patients, and 8 (88.9%) were characterized by US-born case-patients (Table).

Conversely, 11 (91.7%) clusters ranked low priority were characterized by case-patients who were foreign-born, a known risk factor for latent TB infection (*7*). None of the period 1 clusters with LLR ≥5 and only 1 of 9 clusters ranked as high priority by the expert panel were characterized by foreign-born case-patients. These results indicate the need for further study to identify the limitations of the LLR score in detecting localized and recent TB transmission among foreign-born case-patients.

The availability of IS*6110* RFLP or 24-locus MIRU-VNTR testing results to the expert panel is the current standard for fieldwork and could have introduced an information bias for the panel in this study. Although this effect is unknown, lack of universal IS*6110* RFLP and 24-locus MIRU-VNTR test results is a limitation of this study.

We found that geospatial statistics based on TB genotyping and surveillance data could help identify and prioritize likely recent disease transmission events in Washington. In addition, LLR values should be incorporated into ongoing evaluation by the expert panel; in fact, LLR is now included in routine genotype and cluster reviews. Geospatial statistics are an attractive approach to prioritization, but additional field-based research is needed to assess whether factors such as epidemiologic characteristics could be used to further develop a prioritization algorithm. Integrating these factors and determining ideal cutoff points in different settings will increase predictive value.

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Anisakiasis and Gastroallergic Reactions Associated with *Anisakis pegreffii* Infection, Italy

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Human cases of gastric anisakiasis caused by the zoonotic parasite *Anisakis pegreffi* are increasing in Italy. The disease is caused by ingestion of larval nematodes in lightly cooked or raw seafood. Because symptoms are vague and serodiagnosis is difficult, the disease is often misdiagnosed and cases are understimated.

Human anisakiasis is a seafoodborne parasitic zoonosis caused by larval nematodes of the genus *Anisakis*. Humans are accidental hosts of the nematodes; they become infected by consuming raw or undercooked seafoods that harbor the nematode larvae in their flesh and muscle (*1*). The larvae do not further develop in humans; however, they can penetrate the gastrointestinal tract and form eosinophilic granulomas, often with pathologic consequences. There is a growing awareness that these parasites generate potentially life-threatening allergic reactions (*2*) when the live parasite attempts to penetrate the gastric mucosa. These reactions, termed gastroallergic anisakiasis, are characterized by urticaria, occurring generally on the arms and abdomen, and by angioedema or anaphylaxis (*3*).

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The development of molecular tools for the diagnosis of human anisakiasis and greater awareness of this parasitic disease have led to an increase in the recorded number of cases of the disease during the past 20 years in many parts of the world (*4*). Two species of the genus *Anisakis* have been found to cause infections in humans: *A. simplex* sensu stricto and *A. pegreffii* (*1*), as has been confirmed by molecular markers (*4–7*). Although *Anisakis* spp. larvae are found in fish and squid worldwide, the prevalence of human infection is highest in countries where eating raw fish is widespread. However, the molecular identification of human cases is still scarce, especially in some European countries where allergic symptoms and hypersensitivity associated with the parasite have been reported (*2*).

In this study, we report several new cases of gastric anisakiasis in Italy. Identifying the etiologic agent is challenging because *Anisakis* spp. larvae lack morphologic features that could be used to identify them at the species level. When larvae infest humans, they can become spoiled or fragmented, making it impossible to identify them at the genus level. We performed sequencing of nuclear and mitochondrial genes to identify the parasites and to gather data on the possible association between pathologic findings of human anisakiasis and different *Anisakis* spp. or haplotypes. In addition, serum samples from the patients were tested for IgE reactivity against specific antigens or allergens (IgE-As) of *A. pegreffii*.

The Study

The 8 patients studied during June 2011–June 2012 were from the following regions of Italy: Abruzzo (4), Latium (1), Campania (2), and Tuscany (1). All of these patients experienced acute gastric pain and nausea for a period ranging from 2–3 hours to 2 days after they had eaten raw fresh marine fish (marinated anchovies). Three patients had allergic reactions that had different manifestations, but no anaphylactic shock occurred (Table). The parasites were observed penetrating the gastric wall in 2 patients.

Nematodes and nematode fragments were endoscopically removed from the stomach and were stored in 99% ethanol or 10% formalin solutions. Total DNA was extracted from 2 mg of tissue from a single nematode or from a fragment removed from each patient (Table). We sequenced the mitochondrial gene (cytochrome c oxidase subunit II [*cox2*]) and nuclear genes (internal transcribed spacer [ITS] region, including ITS1, 5.8S, and ITS2 of rDNA). Amplification of the mitochondrial DNA (mtDNA) *cox2* gene (629 bp) and the ITS region (908 bp) of rDNA was performed on the 8 specimens as described (*8*,*9*). In addition, larval *Anisakis* specimens that were collected from anchovies (*Engraulis encrasicolus*) from the Tyrrhenian Sea, used

Table. Clinical features of patients who had gastroallergic anisakiasis associated with *Anisakis pegreffii* infection, Italy*

Code	Home area of patient, province (region)	Location of nematode	Time since raw seafood ingested	Clinical features	Whole larval nematode or fragment, suspension, condition of specimen	DNA extraction method	Total IgE, IgE-As†
HuC1	Benevento (Campania)	Submucosal layer of gastric wall	2 d	Epigastric pain, edema of oral mucosa	Whole, 99% ethanol, very well conserved	CTAB	1,479, >100
HuC2	Pescara (Abruzzo)	Gastric mucosa	24 h	Epigastric pain, urticaria, generalized edema	Whole, 99% ethanol, well conserved	CTAB	2,180, >100
HuC3	Pescara (Abruzzo)	Lumen of stomach	24 h	Epigastric pain	Fragment, 99% ethanol, well conserved	CTAB	4,727, >100.0
HuC4	Pescara (Abruzzo)	Lumen of stomach	1 d	Epigastric pain, digestive symptoms	Fragment, 99% ethanol, well conserved	CTAB	511, 21.2
HuC5	Pescara (Abruzzo)	Lumen of stomach	1 d	Epigastric pain	Fragment 99% ethanol, well conserved	CTAB	Serum not available
HuC6	Rome (Latium)	Lumen of stomach	1 d	Epigastralgia, urticaria	Fragment 99% ethanol, well conserved	CTAB	Serum not available
HuC7	Pisa (Tuscany)	Lumen of stomach	2 d	Epigastric pain, vomiting	Fragment spoiled, 10% formalin, poorly conserved	Maxwell 16 Promega‡	2,062, 89.3
HuC8	Naples (Campania)	Lumen of stomach	2–3 h	Epigastralgia	Fragment spoiled, 10% formalin, poorly conserved	Maxwell 16 Promega	Serum not available

*Patients are indicated by their codes, HuC1–HuC8; all patients reported having eaten marinated anchovies before onset of illness. CTAB, cetyltrimethylammonium bromide (*8*).
† Determined by ImmunoCAP ISAC diagnostic test (Phadia, Uppsala, Sweden).
‡Maxwell 16 Instrument System (Promega, Madison, WI, USA)

for antigen preparation, were identified by using sequence analysis of the same genes.

Serum samples from patients HuC1, HuC2, HuC3, HuC4, and HuC7 were tested for IgE antibodies to *Anisakis* (IgE-As) by using ImmunoCAP (Phadia, Uppsala, Sweden). The IgE threshold level was defined by an antibody level of >0.35 kilounits of antibody per liter, as stated by the test manufacturer. Serum specimens were also analyzed by Western blot (WB) testing to detect specific IgE-As against antigens or allergens of *A. pegreffii*. For WB analysis, excretory or secretory antigens were obtained from larvae of *A. pegreffii* in stage 3 of 4 larval stages; the larvae were obtained from anchovies and cultured in vitro.

Sequences of the ITS region of the rDNA (908 bp) obtained were aligned with those of *Anisakis* spp. stored in GenBank (www.ncbi.nlm.nih.gov/genbank/) by using ClustalX (www.clustal.org) as described (*10*). Overall, the highest nucleotide homology was observed with nucleotide homologs of *A. pegreffii*. Sequences from larval specimens showed 100% identity (GenBank accession nos. EU624343 and EU718479) with sequences available for *A. pegreffii*. Genetic variation was not observed in the 8 analyzed specimens. One sequence of the ITS region of rDNA, obtained during the current study, was deposited in GenBank under accession no. JQ900763. Similarly, the mtDNA *cox2* sequences obtained from the 8 nematodes or nematode fragments removed from the patients showed 99% homology to those deposited for *A. pegreffii*.

A minor genetic differentiation (p-distance = 0.001) was found between *A. pegreffii* nematodes from the specimens isolated from humans and *A. pegreffii* nematodes from the Mediterranean Sea (*8*,*11*). In *A. pegreffii* (*7*), mtDNA cox2 sequences from 5 specimens (from patients HuC1, HuC2, HuC3, HuC7, and HuC8) corresponded to the most frequent haplotype, designated as H1. Sequences for patients HuC4, HuC5, and HC6 corresponded to 3 common haplotypes detected in *A. pegreffii* throughout its distribution range. Phylogenetic analysis performed by maximum-parsimony (*12*) showed that the larval specimens of *Anisakis* spp. nematodes from these 8 patients clustered in a well-supported clade, which includes specimens of *A. pegreffii* nematodes previously sequenced for the same gene (*7*,*8*,*11*) (Figure 1).

Serum specimens were available for 5 patients. In those specimens, a high level of IgE-As was found by using ImmunoCAP testing (Table). WB analysis revealed that 2 specimens (from patients HuC1 and HuC2) had IgE specific for the allergen Ani s1 at 24 kDa (Figure 2); the remaining serum specimens tested did not show reactivity.

Conclusions

The first known case of anisakiasis among humans in Italy was described in 1996 (*13*). Since then, several cases of gastrointestinal anisakiasis have been reported there (*7*). The evident increase in the number of cases during recent years suggests that anisakiasis is an emergent zoonosis in Italy. In the present study, based on results

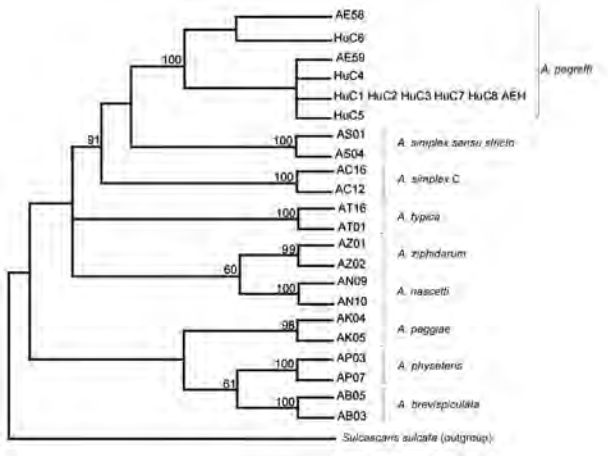

Figure 1. Maximum-parsimony bootstrap consensus tree inferred by PAUP* (12) for specimens of *Anisakis pegreffii* nematodes from patients with gastric anisakiasis (HuC1–HuC8) in Italy. Phylogenetic tree was obtained by mitochondrial DNA cox2 sequences analysis (629 bp) of 1,000 pseudoreplicates related to *A. pegreffii* previously sequenced and deposited in GenBank. AEH indicates *A. pegreffii* associated with a previously reported case of intestinal anisakiasis (7). Bootstrap values are reported at the nodes. Sequences at the mitochondrial DNA *cox2* shown here have been deposited in GenBank under accession nos: JQ900759, JQ900760, JQ900761, and JQ900762.

of multiple gene sequence analyses, we identified several new cases of gastric anisakiasis caused by *A. pegreffii* nematodes. Among them, the mtDNA *cox2* sequence revealed a polymorphic gene, as previously shown in this group of parasites (*8*,*11*,*14*). Previously, the most common haplotype associated with gastric and intestinal anisakiasis in humans in Italy was designated as H1 (*7*). According to network analysis of the intraspecific genetic variation of the mtDNA *cox2* gene performed on *A. pegreffii* nematode populations from different geographic areas, H1 is likely the ancestral haplotype. This molecular marker could facilitate investigation of the possible association between mtDNA *cox2* haplotypes and pathologic features of anisakiasis.

Previous reports of allergic reaction related to *Anisakis* infections have not been associated with larval detection and identification of the parasite (*14*). Two serum specimens recognized Ani s1, a major secretory/escretory antigen/allergen of *Anisakis* spp., have been identified as the causal agent for 85% of allergic reactions (*2*,*3*). Gastroallergic anisakiasis associated with *A. pegreffii* nematodes was likely facilitated by a hypersensitivity reaction in those patients; the mechanism involved is probably an allergic reaction induced in the submucosal layer of the gastric wall around the penetration site of the helminth. The high level of IgE-As observed in the remaining serum specimens was likely related to cross-reactive antibodies against *Anisakis* antigens considered to be panallergens (*3*).

The public should be cautioned against eating raw marinated anchovies, the main source of human anisakiasis in Italy, and other raw or lightly cooked seafoods. Clinicians should be made aware of the potential risk for severe allergic reactions in patients with gastric anisakiasis, and encouraged to make and report molecular identification of the helminths to increase knowledge about the association between different *Anisakis* spp. and their pathogenic effects on humans.

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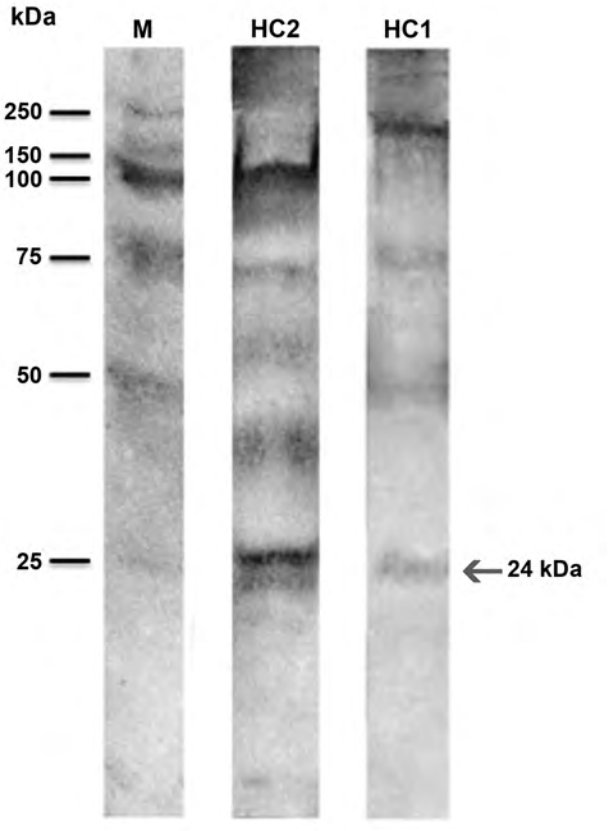

Figure 2. Western blot reaction of the serum samples from patients HuC1 and HuC2 from Italy showing allergic reaction against *Anisakis pegreffii* antigens and allergens. M indicates molecular marker; arrow indicates the reaction at 24 kDa (Ani s1). IgE determination was performed with alkaline phosphatase conjugates obtained from goat anti-human IgE. Antigen-antibody binding was visualized by the alkaline phosphatase 5-bromo-4-chloro-3-indolyl phosphate p-nitroblue tetrazolium chloride system until bands appeared. Human serum specimens negative for *A. pegreffii* were used as controls. HC1 and HC2 represent patient identification numbers HuC1 and HuC2.

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HUS Surveillance Notes—Sarah's Story

Kevin G.J. Pollock

Hemolytic uremic syndrome (HUS) is a rare disorder characterized by microangiopathic hemolytic anemia, microthrombi, and multiorgan injury. Although infection with Shiga toxin–producing *Escherichia coli* is the most frequently identified cause, *Streptococcus pneumoniae* is increasingly recognized as a cause of HUS, and increasing illness and death have been documented for this rare precipitant (*1*).

Cases of pediatric HUS are ascertained prospectively from an active, ongoing national surveillance program (*2*). Clinicians approach parents to seek consent for study enrollment when they deem the child is clinically stable. Parents of children affected by HUS are given a patient information sheet, a consent form, and a questionnaire if consent is given. As part of the surveillance, parents are asked to answer several open-ended questions at year 0, and 1 and 5 years after enrollment. Questions pertain to the status of the child's health and whether visits to doctors and ongoing hospital treatments are still needed. The questionnaire also asks parents to assess their child's illness since the initial visit to the hospital and the effects of this illness on the family.

In 2006, a 1-year-old girl was admitted to a pediatric hospital in Glasgow, Scotland, because of microbiologically confirmed pneumococcal infection and superimposed HUS. Such cases are typically characterized by empyema, meningitis, and bacteremia in children, and also by major renal and neurologic injury requiring extensive dialysis (*3*). Unfortunately, this was true for Sarah.

The following account is the child's story as told by her mother.

Year 1

The pneumococcal infection that caused Sarah's HUS left her severely brain damaged. She has virtually no voluntary movement, cannot walk, talk, speak, or sit unaided, and she cannot feed orally. Sarah attends physiotherapy and speech and language therapy at a hospital for rehabilitation after her brain injury. It is hard to know how much of Sarah's disability was caused by HUS and how much by the pneumococcal meningitis we also believe she had. Obviously, having a severely disabled child has affected the family. I have given up work to be Sarah's full-time caregiver. We also lead a different sort of family life from what we had hoped to have, although our second child is due to be born at the end of the month. We are so proud of Sarah for making it through to celebrate her second birthday last week.

Year 5

Sarah is thriving: she was 6 years old on her birthday and is big sister to Peter, now 4 years old. I am still full-time at home but have started an Open University degree and have several voluntary jobs with organizations in our local community. This enables me to maintain my skills but also be available for Sarah. As you know, with complex needs, her health can change dramatically. However, we are lucky that she keeps generally in good health.

Sarah attends a full-time specialist school and is making small improvements in the consistency of her responses and communication, although she is still nonverbal. We have had her sight and hearing tested and, although she has 100% normal hearing, Sarah is registered as blind (seeing only light and dark). She also goes to a residential respite center twice a month, so between that and school she has an active independent social life away from us, which we believe is essential for her. Sarah adores her brother Peter and has infinite patience with his play and noise and physical contact.

Peter also adores his sister and is protective of her and matter of fact about explaining to friends that Sarah cannot talk because her brain is broken (how we have explained things to him). Five years later, many of the emotions are the same, but many have changed, as have the difficulties.

The effect of pneumococcal HUS on the parents of children with this disease has never been documented. These brief notes convey the remarkable fortitude shown by Sarah's family and the difficulties experienced by parents coping with the needs of a child affected by the disease. Early administration of antimicrobial drugs and dialysis considerably improve prognosis in this severe and rare infectious complication, but the long-term effects on young children and their families remain.

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The names of the children have been changed. I thank the parents of Sarah for their consent in allowing their experiences to be shared.

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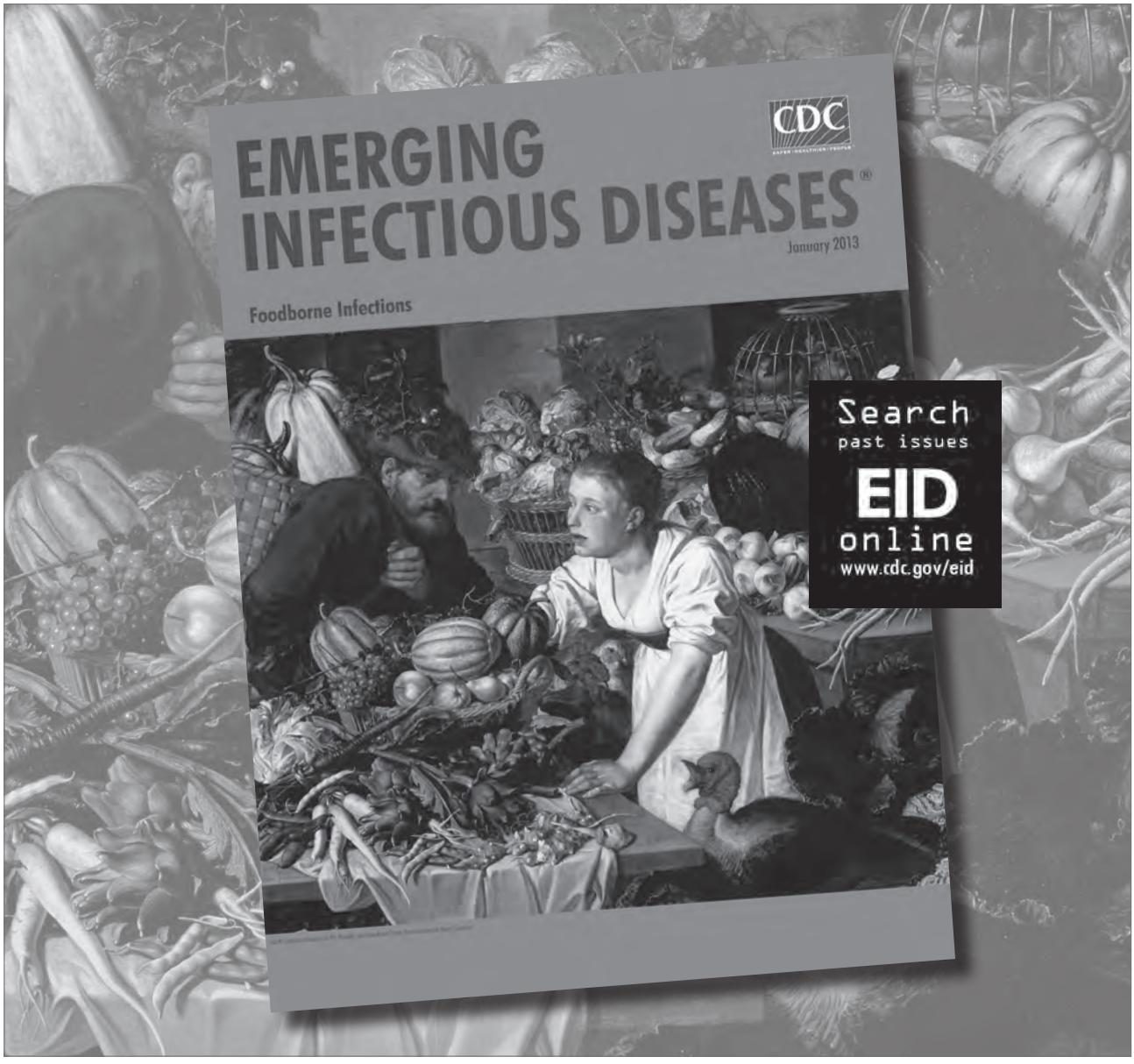

Bordetella hinzii in Rodents, Southeast Asia

To the Editor: Bacteria of the genus *Bordetella* are gram-negative, rod-shaped organisms that cause respiratory tract diseases in humans and animals. In 1995, *Bordetella hinzii* was isolated from poultry and 2 patients in the United States and France (*1*). This pathogen colonizes the respiratory tract of poultry and is closely related to *B. avium*, which is a commensal species in poultry. However, information on the etiologic role, hosts, and transmission routes of *B. hinzii* is incomplete because infections in human who did not have any close contact with poultry have been reported, mainly in immunocompromised patients (*1–5*). We obtained a single isolate of *B. hinzii* from blood agar culture during screening for bacterial zoonotic diseases in blood samples of rodents in Southeast Asia during the Ceropath project (www.ceropath.org).

During 2008–2010, we collected rodents along the Mekong River areas of 3 countries in Southeast Asia (Cambodia, Laos, and Thailand). Rodents were trapped in urban areas and in rural areas, which consisted of forests, upland and dry agricultural areas (orchards, cassava fields), unirrigated and irrigated agricultural areas (rice fields), and domestic areas (isolated farms and villages). Each animal was identified at the species level by using morphologic or molecular methods. Two hundred six blood samples were cultured on Columbia agar containing 5% sheep blood and incubated at 37°C for 3–7 days. A single atypical isolate was observed after 2 days of culture. This isolate was identified by using matrix-assisted laser desorption ionization time-of-flight mass spectrometry as described by Seng et al. (*6*). However, this isolate was identified only at the genus level as a *Bordetella* sp. (score 1.7).

To identify the *Bordetella* species, DNA from the isolate was extracted by using the QIAamp DNA Kit (QIAGEN, Hilden, Germany). Partial PCR amplification and sequencing of 16S rRNA gene was performed as described (*7*). Sequence analysis showed that the isolate was closely related to *B. hinzii* LMG 13501 (99.0% homology), which was isolated from the blood of a patient who died of septicemia in 2000 (*2*). The 16S rRNA sequence of our isolate (*B. hinzii* L135) has been deposited in GenBank under accession no. JX188059. A phylogenetic analysis of the new sequence and sequences of other bacteria in the genus *Bordetella* is shown in the Figure.

B. hinzii is a causative agent of respiratory tract illnesses in birds and has been described as an emerging and opportunistic pathogen in immunocompromised patients; and in patients with AIDS, cystic fibrosis, and fatal septicemia (*1–5*). However, the source of transmission is not clear. Although *B. hinzii* is commensal in birds, several cases were reported in persons who did not have any close contact with birds (*2–5*), suggesting alternative sources of contamination. Thus, transmission routes and reservoirs of *B. hinzii* infection are ambiguous. *B. hinzii* infection has also been reported in rabbits and laboratory mice in Hungary and Japan (*8–10*). Rodents were suspected to be potential reservoirs but, to the best of our knowledge, this emerging pathogen has not been reported in wild rodents.

We detected in *B. hinzii* in a *Rattus tanezumi* rat that was trapped in upland agricultural area in Laos. *R. tanezumi* rats are the most common rodent in southeastern Asia and can be found in various habitats, including forests, agricultural areas, and houses. In Southeast Asia, human populations in several countries (Cambodia, Laos, and Thailand) live in close contact with rodents (including *R. tanezumi*) or share the environment with them. These rodents are known to be a reservoir and possible source of bacterial zoonoses such as leptospirosis, plague, scrub typhus, and murine typhus.

Our findings suggest that *B. hinzii* isolated from wild rodents may serve as reservoir for this bacterial species that could be transmitted to human or pets. *B. hinzii* should be added to

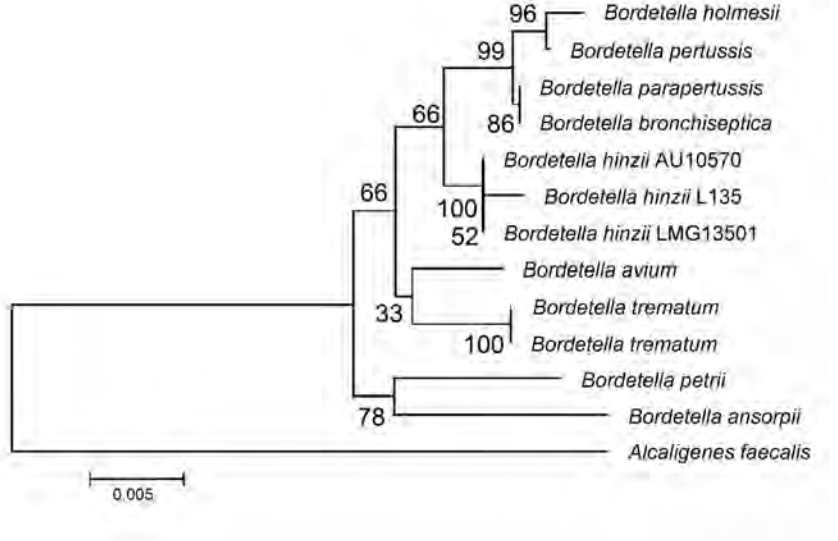

Figure. Maximum-parsimony phylogenetic tree of 16S rRNA gene of *Bordetella hinzii* isolate from this study (L135) and validated *Bordetella* species. Numbers along branches indicate bootstrap values. Scale bar indicates nucleotide substitutions per site.

the list of emerging bacterial zoonotic agents in wild rodents that could be pathogenic for humans. Further studies are warranted to evaluate the prevalence of this bacterium in rodents in other countries and to demonstrate that rodents may be a source of transmission of this bacterium to humans, especially immunocompromised patients.

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Melioidosis and Hairy Cell Leukemia in 2 Travelers Returning from Thailand

To the Editor: Patients with underlying medical conditions travel more than ever (*1*), and such travelers may be exposed to uncommon infections (*2*). We report 2 cases of melioidosis and hairy cell leukemia in travelers returning from Thailand.

Case-patient 1 was a 48-year-old man hospitalized in Paris with fever, asthenia, chills, and pancytopenia after returning from a 1-week visit to Thailand where he had been in flooded regions (Koh Samui and Koh Samet). Clinical examination showed a temperature of 40°C and mucocutaneous pallor. Laboratory tests showed a hemoglobin level of 7.9 g/dL, a platelet count of 33×10^9/L, a leukocyte count of 1.3×10^9 cells/L, a polymorphonuclear cell count of 0.77×10^9 cells/L, a monocyte count of 0, and a C-reactive protein level of 158 mg/L. Results of tests for HIV, dengue, and malaria were negative.

Presumptive antimicrobial drug treatment with piperacillin/tazobactam (12 g/1.5 g/d) was initiated at admission. A blood smear showed 10% hairy cells, and a bone marrow biopsy confirmed a diagnosis of hairy cell leukemia and interstitial infiltration of CD20-positive, monoclonal antibody DBA.44–positive, and tartrate-resistant acid phosphatase–positive cells.

Because of persistent unexplained fever, full-body computed tomography (CT) was performed and showed multiple liver, spleen, and lung abscesses (Figure, panels A and B). Culture of a CT scan–guided liver abscess puncture specimen was positive for *Bukholderia pseudomallei* after 12 days of antimicrobial drug treatment. Treatment was changed to ceftazidime (120 mg/kg/d) trimethoprim/sulfamethoxazole

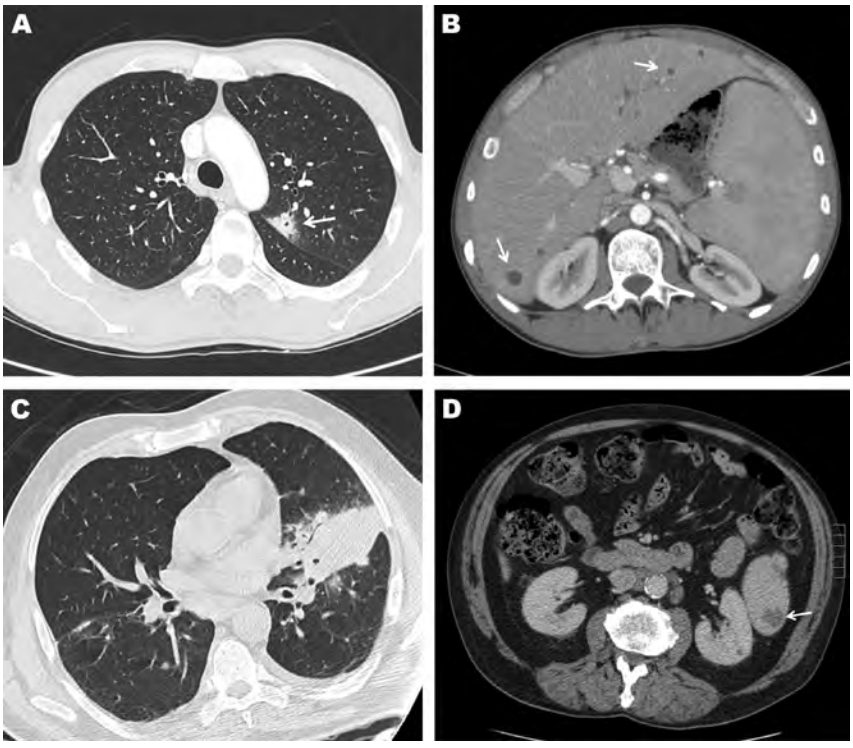

Figure. Computed tomography (CT) images of the chest and abdomen of case-patient 1 showing A) a subpleural nodular and cavitary lesion (arrow) in the left upper lobe of the lung and B) multiple small round liver abscesses, seen as multiple foci of ill-defined areas of hypoattenuation (arrows), and enlargement of the spleen. CT images of the chest and abdomen of case-patient 2 showing C) a focal area of parenchymal consolidation in the left lung associated with an ipsilateral mild pleural effusion and D) and a spleen abscess (arrow).

(TMP/SMX) (10/50 mg/kg/d) and oral doxycycline (200 m/d) for 3 weeks. The outcome was good.

Oral treatment with TMP/SMX and doxycycline (200 mg/d) was continued for 20 weeks. Treatment for hairy cell leukemia with cladribine was initiated after 10 weeks of antimicrobial drug treatment. Two years later, the patient showed complete remission of hairy cell leukemia and melioidosis.

Case-patient 2 was a 64-year-old man hospitalized in Paris for persistent fever 16 days after his return from Thailand. Two months earlier in Thailand, he had received treatment for hepatosplenic melioidosis with ceftazidime (120 mg/kg/d), TMP/SMX (10/50 mg/kg/d), and doxycycline (200 mg/d) for 15 days, and then oral amoxicillin/clavulanic acid (3 g/d) for 3 months. At admission, he had fever, chills, abdominal pain, and cough. Clinical examination showed a temperature of 40°C and left lung crackles. Chest and abdomen CT images showed a focus of lung consolidations (Figure, panels C and D), left pleural effusion, pericarditis, and spleen abscesses. Laboratory tests showed a leukocyte count of 1.05×10^9 cells/L, a monocyte count of 0.04×10^9 cells/L, a hemoglobin level of 7.9 g/dL, a platelet count of 62×10^9/L, and a serum ferritin level of 8,530 IU/L.

Blood cultures were positive for *B. pseudomallei*. The strain was sensitive to amoxicillin/clavulanic acid. Bone marrow aspiration and biopsy showed hemophagocytosis and interstitial infiltration of CD20-positive, monoclonal antibody DBA.44–positive, CD 103-positive, CD25-positive, CD11c-positive, and CD123-positive cells, leading to a diagnosis of hairy cell leukemia. The patient was given a 2-week course of intravenous TMP/SMX (50 mg/10 mg/kg/d), oral doxycycline (4 mg/kg/d), and intravenous ceftazidime (120 mg/kg/d), followed by a 6-month course of oral TMP/SMX (10 mg/50 mg/kg/d) and doxycycline (200 mg/d). The condition of the patient improved and pancytopenia resolved. Thus, he did not require any treatment for hairy cell leukemia. No relapse of melioidosis occurred.

Melioidosis is endemic to the Pacific region and Southeast Asia (*3*,*4*). Most cases reported in other regions are imported (*5*). In Thailand, where both patients had traveled, the number of cases increased from 11.5/100,000 inhabitants in 1997 to 21.3/100,000 in 2006 (*6*). The 2 main routes of transmission are transcutaneous and aerosols. Natural disasters, such as flooding, are a risk factor for melioidosis, as for case-patient 1.

This disease has an overall mortality rate of 50%. The clinical spectrum ranges from acute septicemia (mortality rate 80%) to the subacute form. *B. pseudomallei* is difficult to detect by culture of biologic samples, and serologic analysis or PCR for this bacteria are not routinely available. Therefore, a diagnosis of melioidosis can be easily missed.

Melioidosis occurs mainly in patients with underlying diseases such as diabetes (37%–60% of cases), chronic alcoholism (12%–39%), thalassemia, and chronic nephropathy, and in persons receiving long-term corticosteroid treatment (*7*). Reports of patients with melioidosis and hematologic malignancies or solid cancers are scarce (*4*,*5*,*7*). Hairy cell leukemia could now be included in this group of diseases.

Hairy cell leukemia is a rare chronic B-cell lymphoproliferative disorder characterized by pancytopenia; splenomegaly; and infiltration of the bone marrow, spleen, and liver by malignant B cells that have hair-like cytoplasmic projections (*8*,*9*). The incidence of hairy cell leukemia is

<1 case/100,000 population/year, and the disease accounts for ≈2%–3% of all leukemias in adults in the United States (*8*). Infections are a common complication for patients with this disease (*10*).

These 2 cases of imported melioidosis show that travelers with hematologic malignancies are at risk for such infections (*1*). Immunocompromised travelers might be first sentinels for ongoing endemic diseases. When travelers return with uncommon diseases, physicians should check for underlying diseases. Physicians providing care for patients with hairy cell leukemia should be aware of the risk for contracting melioidosis.

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Letters

Letters commenting on recent articles as well as letters reporting cases, outbreaks, or original research are welcome. Letters commenting on articles should contain no more than 300 words and 5 references; they are more likely to be published if submitted within 4 weeks of the original article's publication. Letters reporting cases, outbreaks, or original research should contain no more than 800 words and 10 references. They may have 1 Figure or Table and should not be divided into sections. All letters should contain material not previously published and include a word count.

Plague Epidemics and Lice, Democratic Republic of the Congo

To the Editor: Plague, a zoonotic disease caused by the gram-negative bacterium *Yersinia pestis*, is transmitted to humans by the bites of infected fleas (such as *Xenopsylla cheopis*), scratches from infected animals, and inhalation of aerosols or consumption of food contaminated with *Y. pestis* (*1*). Decades ago, Blanc and Baltazard proposed that human-to-human transmission of *Y. pestis* could be mediated by human ectoparasites, such as the human body louse (*2*). This hypothesis was further supported by experimental data from animal models (*3*).

To further test this hypothesis among humans, we conducted a field assessment in April 2010, in which we collected body and head lice from persons living in a highly plague-endemic area near the Rethy Health District, Province Orientale, Democratic Republic of the Congo. This health district has 157,000 inhabitants, and during 2004–2009 it had more suspected plague cases (1,624 cases of suspected plague, 39 deaths) than any other health district in the Democratic Republic of the Congo. In April 2010, we visited the dwellings of 10 patients for whom suspected cases of plague had been diagnosed during January–April 2010. All patients had symptoms typical of bubonic plague, and their illnesses were reported as suspected bubonic plague. However, because of the lack of laboratory facilities in Rethy, none of these diagnoses could be microbiologically confirmed.

A total of 154 body lice and 35 head lice were collected from clothes and hair of persons living in or near the patients' dwellings. Body lice were preserved in ethanol before

being sent to the laboratory. Total DNA was extracted by using an EZ1 automated extractor (QIAGEN, Courtaboeuf, France) and subjected to parallel real-time PCRs selective for the *Y. pestis pla* gene, the *Rickettsia prowazekii omp*B gene, a *Borrelia recurrentis* noncoding genomic fragment, and the *Bartonella quintana* internal transcribed spacer. *B. quintana* PCR primers and probe have been shown to be specific for *B. quintana* (*4*). Primers and probe sequences and experimental conditions have been reported (*4*). Negative controls contained PCR buffer without DNA, as described (*4*). Any amplification with a cycle threshold (C_t) <40 was regarded as positive.

Negative controls remained negative in all PCR-based experiments, which were not prone to in-laboratory contamination, and all samples were negative for *R. prowazekii* and *B. recurrentis*. Conversely, *B. quintana* was detected in 50 (32.5%) of the 154 body lice (C_t 18.62–38.45) and 6 (17.1%) of the 35 head lice (C_t 29.48–38.68). The *Y. pestis pla* gene was detected in 1 head louse (C_t 38), which was negative for the other pathogens, and in 2 body lice (C_t 37.36 and 36.97, respectively), which were positive for *B. quintana*.

B. quintana has been detected in head louse specimens collected in Ethiopia and Senegal (*5*–*7*) and in body louse specimens collected in Burundi, Rwanda, Zimbabwe (*8*), and Ethiopia (*5*); we add Democratic Republic of the Congo to the list. Body lice are acknowledged vectors for human-to-human transmission of *B. quintana* (*9*). Detection of *Y. pestis* in head and body lice has been reported (*2*). Detailed observations in south Morocco showed that body lice collected from blood culture–negative bubonic plague patients were negative for *Y. pestis*, whereas body lice collected from septicemic patients were positive according to guinea pig inoculation results (*2*). Further experiments in a rabbit experimental model demonstrated the possibility of direct louse-bite transmission of *Y. pestis* (*3*). A recent search for *Y. pestis* in head lice in Ethiopia found none (*5*).

Our detection of *B. quintana* and the plague agent *Y. pestis* in modern head and body lice is similar to findings of a paleomicrobiological investigation at a medieval plague site near Paris (*10*). There, high-throughput real-time PCR investigation of dental pulp collected from 14 teeth from 5 skeletons detected *B. quintana* DNA in teeth from 3 skeletons and *Y. pestis* DNA in teeth from 2, including 1 with co-infection. Altogether, these data suggest that transmission of *B. quintana* and *Y. pestis* has been ongoing for centuries in populations in which louse infestation is prevalent. This finding indicates that lice might play a role in transmission of *Y. pestis* and that preventing and controlling louse infestations might help limit the extension of plague epidemics in louse-infested populations.

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Armillifer armillatus Pentastomiasis in African Immigrant, Germany

To the Editor: Pentastomiasis is a parasitic zoonotic disease with an incremental number of reported human infections caused by larval stages (nymphs) of pentastomes (*1*–*3*). The vermiform parasites are in their own phylum and are related to branchiuran crustaceans (*2*). Most human infections with these parasites are caused by *Armillifer armillatus* (*2*), a parasite endemic to western and central Africa. Most cases are reported from the Congo region and Nigeria, and occasionally infections in African immigrants to Europe and North America have been reported (*4*,*5*). Imported cases to Germany have not been reported. *A. grandis*, a related parasite from central Africa, has been rarely found (*6*), but *A. moniliformis*, a pentastome species from Asia, has recently reemerged and caused a human infection after ≈40 years in Malaysia (*1*).

Adult *Armillifer* spp. inhabit the respiratory tract of large snakes (*Python* spp.). These dioecious parasites produce large amounts of ova that are shed into the environment by snake feces and secretions. When intermediate hosts, such as rodents or other small mammals, ingest ova, larvae hatch, migrate to the viscera, encyst, and molt several times (*3*). Humans become accidental intermediate hosts after uptake of environmental parasite ova or by consumption of contaminated snake meat. We report an infection with *A. armillatus* in an African immigrant to Germany that was diagnosed by histopathologic analysis and confirmed by PCR.

In 2005, a 23-year-old man from Togo who had immigrated to Germany 3 years earlier showed development of acute myeloid leukemia. He subsequently underwent stem cell transplantation, which was followed by graft versus host disease. The patient died of sudden intracerebral hemorrhage and leukencephalopathy. His medical history also included α-thalassemia and a heterocygotous sickle cell trait, chronic hemolytic anemia, splenomegaly, and cardiomyopathy. He had been treated for schistosomiasis and filariasis.

An autopsy specimen showed several living pentastome nymphs of ≈2 cm in size, which were found in the subscapular region of liver parenchyma. A presumptive diagnosis of visceral pentastomiasis caused by *A. armillatus* nymphs was made in accordance with the origin of the patient and the geographic distribution of the parasite.

Microscopic slides from patient specimens were retrieved from an archive and reanalyzed (Figure; online Technical Appendix Figure, wwwnc.cdc.gov/EID/article/19/3/12-1508-Techapp1.pdf). A pentastome-specific PCR targeting the 18S rRNA gene (*2*,*7*) was conducted after DNA extraction from formalin-fixed tissue on a remaining unstained microscope slide. The resulting 383-bp amplicon was sequenced, and BLAST analysis (www.ncbi.nlm.hih.gov/blast) confirmed 100% identity with *A. armillatus* (GenBank accession no. HM756289.1) and 99% homology with *A. agkistrodontis* (FJ607339.1) and *A. moniliformis* (HM048870.1).

Visceral pentastomiasis in humans is often asymptomatic and an incidental finding during surgery (*1*,*3*) or autopsy (*8*,*9*). In a large autopsy series from Malaysia, a pentastomiasis prevalence of 45.5% was found in adult Aborigines (*8*). In Nigeria, a rate of 33% was seen during autopsies of patients who had died of malignancies (*9*). However, a few severe and even lethal cases have been described for heavy *A. armillatus* and *A. grandis* infections in persons from Africa (*4*,*6*).

Diagnosis is achieved by gross pathologic and histopathologic analyses. Nymphs are found in the serosa around the liver and spleen, in liver parenchyma, mesenterium, intestine wall, and abdominal lymph nodes. The lungs or pleura are occasionally infected (*3*). Radiographic analysis may show typical C-shaped chest or abdominal calcifications (*10*). Species identification is performed by counting annulations (*A. armillatus* 18–22,

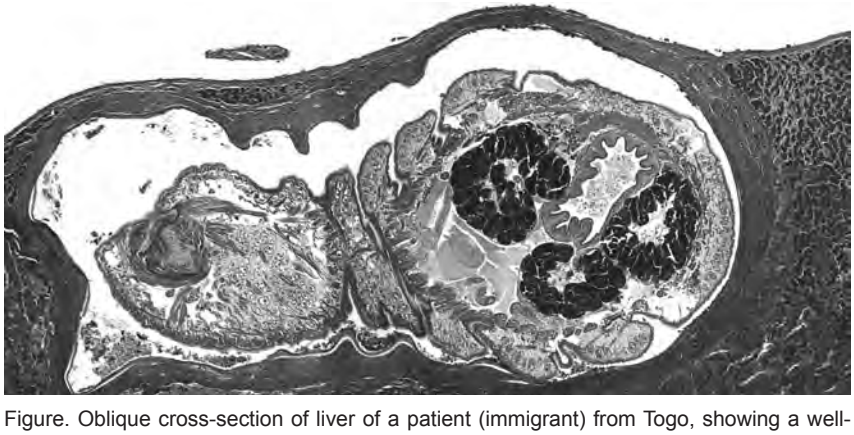

Figure. Oblique cross-section of liver of a patient (immigrant) from Togo, showing a well-preserved *Armillifer armillatus* nymph in a subcapsular location. The annulated parasite is encapsulated by its shed cuticle (exuvia) and dense fibrosis. Consistent with the viable type of a pentastomid lesion (*3*), no inflammatory infiltrate is visible. This image also shows internal structures of the pentastome, such as prominent bunches of acidophilic glands surrounding the intestine (Masson's trichrome stain, original magnification ×10). A color version of this figure is available online (wwwnc.cdc.gov/EID/article/19/3/12-1508-F1.htm).

A. grandis >25) and measuring the size of larval parasites (*3*). Recently, PCR has been used for diagnosis in veterinary infections (*2*,*7*).

For the patient in our study, molecular analysis identified human pentastomiasis by using a formalin-fixed microscope slide that had been stored for 7 years. A difference of 2 nt each was seen when the amplified nucleotide sequence was compared with database sequences of *A. agkistrodontis* and *A. moniliformis*. However, there is no database entry in GenBank for *A. grandis*, the geographically closest *Armillifer* species. Serologic assays have been developed for identification of *A. armillatus* (*2*), but no serum was available for retrospective analysis. In special settings, such as tropical snake farming and pet keeping, pentastomiasis may be a public health concern (*2*). However, most infections have been linked to consumption of undercooked snake meat or other snake products (*1*).

Most immigrants who were given a diagnosis of visceral pentastomiasis were from Nigeria or the Congo region, and diagnoses were made after death. Molecular analysis is particularly valuable when only autoptic paraffin-embedded patient material is available. For industrialized countries, where experience in morphologic identification of unusual parasite species is limited, molecular analysis is a valuable diagnostic tool. Our case-patient constitutes a record of imported *Armillifer* species pentastomiasis to Germany. Because of increasing international migration, more cases of pentastomiasis are likely to be seen.

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Mycobacterium kyorinense Infection

To the Editor: *Mycobacterium kyorinense* is a nonpigmented, slowly growing mycobacterium that was initially isolated in 2007 from a patient with pneumonia in Japan (*1*,*2*). The sequences of the 16S rRNA, *hsp65*, and *rpoB* genes of *M. kyorinense* were closely related to, but different from, those of the type strains of *M. celatum* and *M. branderi*, the 2 most phylogenetically related species (*1*). Biochemical tests, such as those for arylsulfatase activity, tellurite reduction, and heat-stable catalase, also distinguish *M. kyorinense* from *M. celatum* and *M. branderi*. In our initial report, in which this species was first recognized, we described 3 strains isolated from Japanese patients (*1*). Recently, 1 additional case was reported in Brazil (*3*). Here we describe 7 newly identified patients whose infection may have been caused by *M. kyorinense*.

In reviewing the characteristics of these 11 patients (10 from Japan and 1 from Brazil), we found no apparent contacts among them. Nine of the 11 patients had respiratory infections, 1 had lymphadenitis, and 1 had arthritis (Table). Of these, 9 patients fulfilled the criteria for infections of clinical significance (*4*) and were considered to harbor infection by *M. kyorinense*. Of the 9 patients with respiratory infections, 4 died as a result of the infection. These data suggest that *M. kyorinense* belongs to a class of nontuberculous mycobacteria that are pathogenic for humans and have substantial clinical effects.

Among the 10 patients for whom precise clinical records were available, 7 patients were treated with first-line tuberculosis drugs, mainly rifampin, isoniazid, and ethambutol, but these therapies were ineffective for all patients.

Table. Clinical characteristics of patients infected with *Mycobacterium kyorinense* and antimicrobial susceptibility of the organism*

Characteristic	Patient/strain no.										
	1†	2‡	3‡	4	5	6	7	8	9	10	11§
Year of diagnosis	2005	2006	2008	2008	2009	2009	2010	2010	2011	2011	2007
Age, y	89	64	70	81	50	67	72	48	66	60	26
Sex	M	F	M	M	M	M	M	F	M	M	M
Major infection site	Lung	Lung	Lung	Lung	Lymph node	Lung	Lung	Joint	Lung	Lung	Lung
Specimen	Sputum	Sputum	Sputum	BAL	Pus	BAL	Sputum	Pus	Sputum	Sputum	Sputum
Coexisting disease	COPD	Breast cancer	None	None	MDS	None	None	RA, SLE	None	COPD	NA
Country	Japan	Japan	Japan	Japan	Japan	Japan	Japan	Japan	Japan	Japan	Brazil
AM drug MIC, μg/mL											
STR	0.25	0.25	0.25	S	ND	0.25	0.125	0.5	0.25	0.5	S
EMB	4	4	2	R	128	2	1	2	4	4	S
KAN	0.5	1	0.25	S	ND	0.5	0.5	0.5	0.5	0.5	ND
INH	16	16	32	R	2	0.5	8	4	1	4	R
RIF	32	32	32	R	32	32	32	32	32	32	R
LVX	0.125	0.125	0.03	R	0.25	0.06	0.06	0.125	0.125	0.25	ND
CLR	0.03	0.03	0.03	ND	0.03	0.03	ND	ND	0.03	0.125	ND
AMK	0.5	0.5	0.5	ND	0.5	1	ND	0.5	0.5	0.5	S
Clinical efficacy of AM drug combination											
Efficacious	None	None	None	None	CLR, RIF, LVX, AMK	CLR, STR, MXF	None	LVX, EMB, CLR	RIF, CLR, LVX	CLR, LVX	NA
Nonefficacious	BIP	None	RFB, EMB, CLR	INH, EMB, RIF	None	RIF, EMB, CLR, RIF, AZM, LVX	CLR, RIF, EMB	INH, RIF, EMB	INH, RIF, EMB	RFB, EMB	NA
Outcome	Dead	Dead	Dead	Dead	Alive	Alive	Alive	Alive	Alive	Alive	Dead

*BAL, bronchoalveolar lavage; COPD, chronic obstructive pulmonary disease; MDS, myelodysplastic syndrome; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; NA, not available; AM, antimicrobial; STR, streptomycin; S, sensitive; ND, not done; EMB, ethambutol; R, resistant; KAN, kanamycin; INH, isoniazid; RIF, rifampin; LVX, levofloxacin; CLR, clarithromycin; AMK, amikacin; AM, antimicrobial; RFB, rifabutin; MXF, moxifloxacin; BIP, biapenem; AZM, azithromycin.
†Reported in (*1*,*2*).
‡Reported in (*1*).
§Reported in (*3*).
¶Strains 1–10 (except for 4): assayed by broth microdilution MIC for nontuberculosis mycobacteria (BrothMIC NTM; Kyokuto Pharmaceutical Industrial Co., Ltd., Tokyo, Japan). Strains 4 and 11: susceptibility test performed by using Vite Spectrum SR (Kyokuto Pharmaceutical Industrial Co., Ltd.) and BACTEC MGIT 960 Mycobacterial Detection System (Becton, Dickinson and Company, Franklin Lakes, NJ, USA), respectively; therefore, numeric MIC data were not available for these strains.

Six patients received a combination of antimicrobial drugs, including macrolides and fluoroquinolones, as first- or second-line chemotherapy, and infection was subdued without recurrence in 5 patients. In contrast, 4 patients with pneumonia who did not receive sufficient therapy with the latter regimen eventually died of infection (3 patients) or breast cancer (1 patient).

MICs of various antimicrobial drugs for the 9 strains of *M. kyorinense* were determined by the broth microdilution method as described (*1*). For most strains, the MICs of rifampin, ethambutol, and isoniazid were relatively high, and MICs of macrolides, aminoglycosides, and quinolones were relatively low. Notably, MICs of rifampin were remarkably high (>32 μg/mL) for all tested strains (Table).

Direct sequencing of the 16S rRNA gene, performed as previously described, revealed that 8 of the 9 available *M. kyorinense* isolates were identical across the entire sequenced interval (1,470 bp). The sole exception was the strain from Brazil, which showed a 4-bp substitution that the other strains did not (*3*). Although the other 8 strains had identical 16S rRNA sequences, all showed heterogeneity at 9 positions that had not been observed to be heterogeneous in the previous investigation (*1*). This observation might reflect the presence of 2 copies of the 16S rRNA gene, as has been occasionally reported for other mycobacterial species, including *M. celatum* (*5*). Direct sequencing of the entire *rpoB* gene demonstrated that all strains had identical sequences for this locus. The strains differed from the sequence of *M. tuberculosis* at 15 nt within codons 511–533. At the amino acid level, these changes were synonymous for the 2 species, with the exception of amino acid residue 531. This residue, Ser531 in the *M. tuberculosis* RpoB protein, was replaced by an Asp in *M.*

kyorinense. Notably, Ser531 is the most frequent location of substitutions in rifampin-resistant strains of *M. tuberculosis* (*6*).

Why *M. kyorinense* has been isolated almost exclusively in Japan is not clear. This tendency may be largely caused by a reporting bias in Japan. However, *M. kyorinense* may have a particular geographic distribution. In this context, it is noteworthy that the sole strain from Brazil characterized in the current study differed slightly in 16S rRNA sequences from the strains isolated in Japan.

It also is notable that the *M. kyorinense* strains isolated so far were invariably resistant to rifampin by in vitro susceptibility testing. Rifampin appeared to have been clinically ineffective in most patients, although definite efficacy of antimicrobial drugs cannot be evaluated by this retrospective type of study. Analysis of the *rpoB* gene sequence of *M. kyorinense* revealed the replacement of aa 531 when compared to the *rpoB* gene sequence of the *M. tuberculosis* protein. This finding suggests that *M. kyorinense* is inherently resistant to rifampin because of the structural features of its RpoB protein. Amino acid replacement at RpoB residue 531 also has been reported in other bacterial species resistant to rifampin, such as *M. celatum*, *Borrelia burgdorferi*, and *Spiroplasma citri* (*7–9*). In any case, understanding the intrinsic resistance of *M. kyorinense* to rifampin is critical for appropriately treating infection by this microorganism. On the basis of the results of our study, we recommend that a combination of fluoroquinolones and macrolides and/or aminoglycosides be used for the initial treatment of infection by *M. kyorinense* in most patients.

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The 16S rRNA and *rpoB* sequences of the *M. kyorinense* type strain KUM 060204 were deposited in GenBank with accession numbers AB370111 and JQ744020, respectively. The variant 16S rRNA sequences of *M. kyorinense* strains isolated from case-patients nos. 9 (KUM060200) and 11 (HF1629) were deposited as JN634643 and JQ717033, respectively.

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Recurring Influenza B Virus Infections in Seals

To the Editor: Until 1999, influenza B virus was considered to infect humans only. However, more recent data proved that harbor seals (*Phoca vitulina*) and gray seals (*Halichoerus grypus*) also can be infected (*1*). Since the identification of seals as a novel host, antibodies against human influenza B viruses have been detected in some additional otarid and phocid species in a few relatively small studies (*2*,*3*). It has been speculated that seals may be an animal reservoir for human influenza B viruses, although whether influenza B viruses continues to circulate among pinnipeds is unknown.

To investigate whether influenza B viruses had continued to circulate in seals, we analyzed serum samples from 615 seals (548 harbor seals [*Phoca vitulina*] and 67 gray seals [*Halichoerus grypus*]). The samples had been collected upon the animal's admission to the Seal Rehabilitation and Research Centre (SRRC) in Pieterburen, the Netherlands, from seals living in Dutch coastal waters during 2002–2012. We tested these samples for influenza B virus–specific antibodies with a previously described hemagglutination inhbition (HI) assay, using the following influenza B virus strains as antigens: B/Seal/Netherlands/1/1999, B/Jiangsu/010/2003, B/Yamanashi/166/1998, and B/Malaysia/2506/2004 (*4*). Influenza B virus–specific antibodies were not detected in serum specimens collected from seals during 2002–2009 and after 2011; however, in 2010, HI serum antibodies against influenza B viruses were detected in 9 of 21 samples, and in 2011, they were detected in 1 of 150 samples from both harbor seals (n = 6) and gray seals (n = 4) (Figure, panel A). Nine of these positive samples were collected from juvenile seals 6–12 months of age with severe respiratory disease, and 1 was collected from a pup of ≈4 weeks of age. In seals >6 months of age, maternal antibodies have declined to undetectable levels (*5*). Therefore, these 9 juvenile seals must have become infected from late 2009 through early 2010. This suggests that the infection was caused by the novel introduction of an influenza B virus in seals in the coastal waters of the Netherlands, either by seals or by another source. Because most serum samples were collected within 1 day of the animal's arrival at SRRC, seals must have been infected in the wild and not at the center.

Although the 9 positive samples found in 2010 represent 43% of the tested serum samples for that year, this finding does not reflect the proportion of seropositive seals in the population. Only a limited number of seals of the population, most with respiratory problems, are admitted to SRRC, and serum is not collected from all these animals.

Although the 9 seropositive seals, all >6 months of age, had been admitted to SRRC with severe respiratory signs, it should be noted that severe respiratory disease in seals has many other causes (*6*). Because no respiratory samples suitable for diagnostic purposes had been stored, the viral agent could not be determined. Consequently, whether the influenza B virus infection of these seals, as evidenced by serologic test

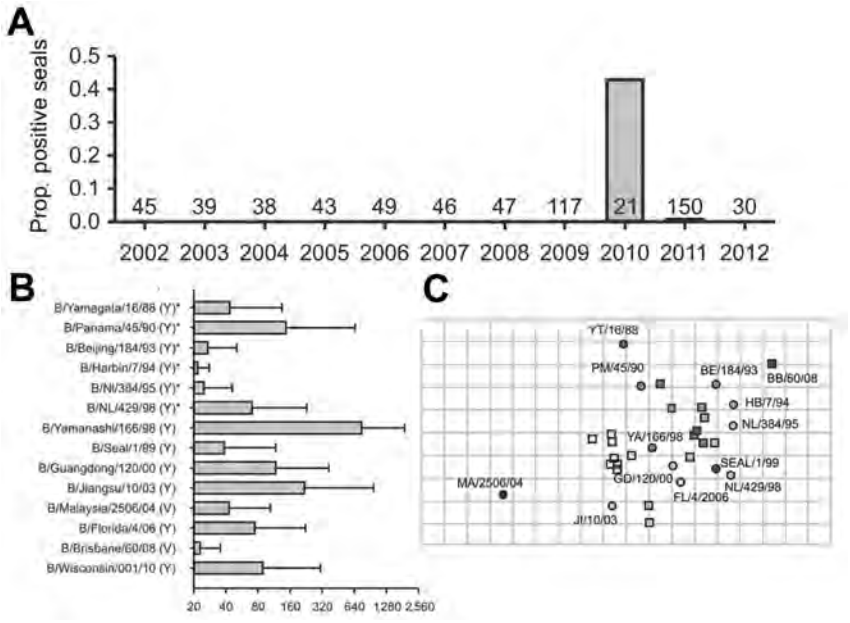

Figure. Serologic evidence of influenza B virus in seals, the Netherlands, 2002–2012. A) Proportion (prop.) of serum samples for each year shown that were positive for influenza B virus antibodies. The number above the year represents the serum samples tested for that year. B) Mean hemagglutination inhibition (HI) antibody titers (±SD) of tested positive serum samples against different influenza B strains belonging to either the Yamagata (Y) or Victoria lineage (V). Asterisk indicates that only 8 of 10 serum samples were tested against these strains because of the limited amount of serum available from 2 seals. C) Antigenic map of influenza B viruses. Indicated are the relative positions of strains (circles) and ferret antiserum samples (squares). Symbols of strains and homologs of ferret antiserum samples are identical, whereas serum samples of seals are indicated as unfilled squares. The spacing between grid lines is 1 antigenic unit, which corresponds to a 2-fold dilution of antiserum in the hemagglutination inhibition assay. Only serum samples and antigens that were positive against at least 3 influenza B virus antigens or serum samples were included in the antigenic map. YT, Yamagata; PM, Panama; BE, Beijing: BB, Brisbane; HB, Harbin; NL, Netherlands; YA, Yamanashi; GD, Guandong; MA, Malaysia; FL, Florida; JI, Jiangsu. A color version of this figure is available online (wwwnc.cdc.gov/eid/article/19/3/12-0965-F1.htm).

results, had been symptomatic could not be ascertained.

To further characterize the influenza B virus strain that most likely had infected the seropositive seals, we tested their serum samples for the presence of HI antibodies against additional human influenza B virus antigens of both B/Yamagata/16/88 and B/Victoria/2/87 lineages. These represent influenza B viruses that circulated in humans during the past 20 years. Highest mean antibody titers were measured against influenza B/Yamanashi/166/98 (mean HI titer 781, SD 168), whereas lower antibody titers were detected against all other viruses, including influenza B/Seal/Netherlands/1/99, which was isolated from seals in the Dutch coastal waters in 1999 (Figure, panel B). These results suggest that the seals had been infected with an influenza B virus similar to B/Yamanashi/166/98, which is antigenically different from B/Seal/Netherlands/1/1999.

On the basis of serum antibody titers against influenza B virus antigens tested and those of respective homologs of ferret antiserum samples, an antigenic map was prepared as described (*7*). This map, in which the antigenic distance between serum and antigen is inversely correlated with the HI titer, shows relative positions of the antigens and serum samples. Also in this map, tested seal serum samples appear to be most closely associated with influenza B/Yamanashi/166/98 **(**Figure, panel C). The obvious remaining question is whether the influenza B virus that had infected these seals in 2009–2010 is a drifted variant of influenza B/Seal/1/99 or the result of another introduction of a human influenza B virus several years ago. No evidence for transmission of influenza B virus from seals to humans was found when strains circulating in humans in the Netherlands were compared with those circulating in seals during the observation period (*8*,*9*). In conclusion, results of this study confirm that influenza B viruses continue to infect seals and support the notion that seals could serve as a reservoir of human influenza B viruses.

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Reemerging Schmallenberg Virus Infections, Germany, 2012

To the Editor: In 2011, Schmallenberg virus, a novel orthobunyavirus of the Simbu serogroup, emerged in Germany and the Netherlands and spread rapidly over large parts of central and western Europe (*1*–*5*). The infection primarily affects ruminants but affects camelids as well (*1*,*6*). So far, evidence has not shown that humans are susceptible to Schmallenberg virus infection (*7*). Although the infection in adult animals causes only mild symptoms (*1*) or remains clinically inapparent, in pregnant animals, transplacental transmission during a limited period can lead to the birth of severely malformed progeny (*1*,*2*). Acute infections of adult ruminants or malformed Schmallenberg virus–positive offspring have been detected on >5,000 farms in Austria, Belgium, Denmark, Finland, France, Ireland, Germany, Italy, Luxembourg, Norway, Poland, Spain, Sweden, Switzerland, the Netherlands, and the United Kingdom. Also, a high proportion of adult ruminants were seropositive for antigens of the virus in the core region affected by Schmallenberg virus in the Netherlands, Germany, and Belgium (*2*,*4*,*5*). Schmallenberg virus caused the first known outbreak of an infection with a virus of the Simbu serogroup in Europe. Schmallenberg virus infections are notifiable in Germany. Biting midges seem to play a key role in the transmission of the infection (*8*), and this transmission led to seasonal spread of the infection in summer and autumn 2011.

We report the recurrence of Schmallenberg virus infection in adult cattle, sheep, and a goat in Germany in 2012. Veterinary authorities at the county or town level report the animal holdings where laboratory-confirmed Schmallenberg virus infections are found to the central national database for notifiable animal diseases (Tierseuchennachrichtensystem), which is maintained by the Friedrich-Loeffler-Institut; the reports are made online. This database was analyzed for reported holdings with Schmallenberg virus infections that had been detected in adult animals from June 1, 2012 through October 31, 2012, and confirmed by PCR (*9*) or virus isolation. In total, 82 infections were reported in adult cattle, 8 in adult sheep, and 1 in a goat (Figure). Forty-five of the cattle holdings and 4 sheep holdings submitted samples for testing because the affected animals had shown clinical signs. One case was detected in a sheep flock, and 5 cases were detected in cattle in trade examinations. For the remaining cases, no specific reason for testing was reported.

Although some cases were reported from the region in western and northern Germany where the epidemic had its center in 2011 (Figure, panel A), several new infections occurred in regions in southern Germany where no cases or only few cases of Schmallenberg virus infection had been detected before (Figure, panel B). This phenomenon may have occurred because of a high level of protective immunity at the population level in the region affected before transmission resumed in 2012, although a substantial proportion of the animals at the margin of the affected area remained susceptible. Schmallenberg virus that has overwintered in these areas may thus be transmitted to naive animals and has apparently spread to regions in southern Germany that were not affected or were less affected by the previous Schmallenberg virus epidemic. Schmallenberg virus could also be introduced into neighboring countries through infected arthropods. Although the respective reports may not have been formally published, indications were that Schmallenberg virus had spread at least to Austria, Ireland, Finland, Norway, Poland, Sweden, and Switzerland by summer/autumn 2012.

Schmallenberg virus infection is often mild or clinically inapparent in adult animals and leads only to a short viremic period of ≈4–5 days (*1*). Because a substantial proportion of new infections in adult animals are likely not recognized, the new cases reported in Germany starting in June

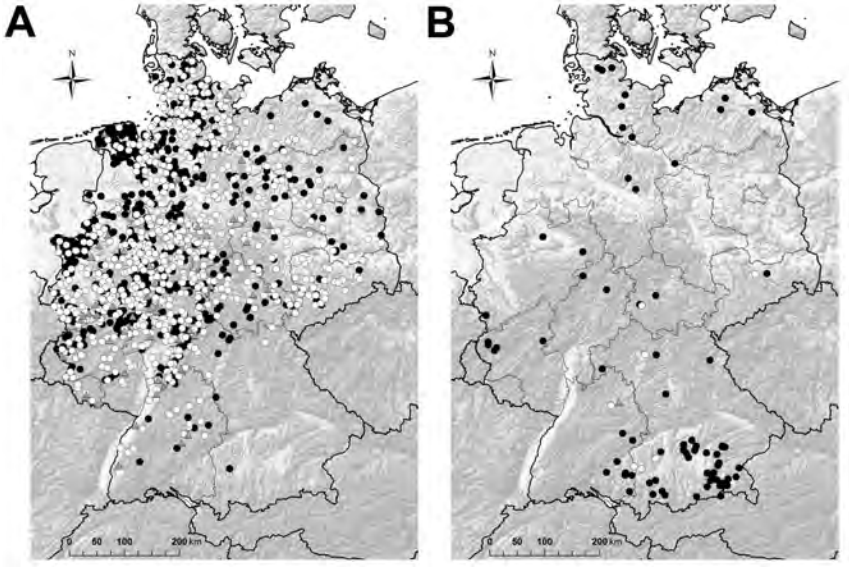

Figure. PCR-confirmed cases of Schmallenberg virus-infections in Germany in A) cattle (black circles, 791), sheep (white circles, 860), and goat holdings (triangles, 47) from August 1, 2011, through May 31, 2012, and B) cattle (black circles, 82), sheep (open circles, 8), and goat holdings (triangle, 1) from June 1, 2012, through October, 31, 2012. A color version of this figure is available online (wwwnc.cdc.gov/eiod/article/10/3/12-1324-F1.htm).

2012 probably represent only the so-called tip of the iceberg. Nevertheless, PCR analysis to detect Schmallenberg virus in samples from animals with clinical signs is a valuable method for identifying first cases in areas where Schmallenberg virus infections have not previously been found.

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Peritoneal Tuberculosis in a Pregnant Woman from Haiti, United States

To the Editor: A 29-year-old woman at 23 weeks' gestation during her first pregnancy came to our hospital's obstetrics clinic after 6 days of vaginal bleeding and abdominal pain. She had not experienced fever, sweats, weight loss, contractions, or other symptoms. She was otherwise healthy; she was taking no medications, but was taking iron and multivitamin supplements. She had legally immigrated to the United States from Haiti 8 months previously and had no known tuberculosis contacts. Physical examination disclosed brown vaginal discharge and a closed cervix. Obstetric ultrasound was normal, and vaginal swab samples were negative for *Neisseria gonorrhea* and *Chlamydia trachomatis*.

Over the ensuing 2 weeks, her vaginal bleeding and abdominal pain worsened. She was admitted to the hospital. Physical examination revealed vaginal bleeding, but her condition was otherwise unchanged. Routine laboratory studies were normal. Repeat obstetric ultrasound showed a viable fetus, ascites, and a 15 × 15 × 3–cm rind of echogenic material anterior to the uterus. This abnormality was in the upper abdomen, an area not imaged on her previous ultrasound. Abdominal magnetic resonance imaging revealed moderate ascites and a 21 × 14 × 3–cm omental mass of intermediate intensity on T1 and T2 sequences; there was no lymphadenopathy (Figure). A tiny left pleural effusion was seen on chest radiograph. Routine HIV and tuberculin skin test results had been negative 4 months previously, and pre-immigration examination results and chest radiograph had been normal.

Fine-needle aspiration of the omental mass was nondiagnostic. The patient's vaginal bleeding and abdominal pain persisted, and her cervix dilated. She had an oral temperature of 38.9° Celsius. Exploratory laparotomy demonstrated a friable omental mass with implants on the small bowel; a partial omentectomy was performed at 26 weeks' gestation. During this procedure, the patient gave birth to a male infant.

Multiple granulomata, some containing acid-fast bacilli, were identified upon histologic examination of the omentum (Technical Appendix Figure, wwwnc.cdc.gov/EID/article/19/3/12-1109-Techapp1.pdf). Transcription-mediated amplification of the specimen was positive for *Mycobacterium tuberculosis* rRNA; cultures later grew *M. tuberculosis* susceptible to all first-line antituberculosis medications. Sputum smears and cultures were not performed. The patient's treatment began with isoniazid, rifampin, ethambutol, and pyrazinamide; her fevers and abdominal pain

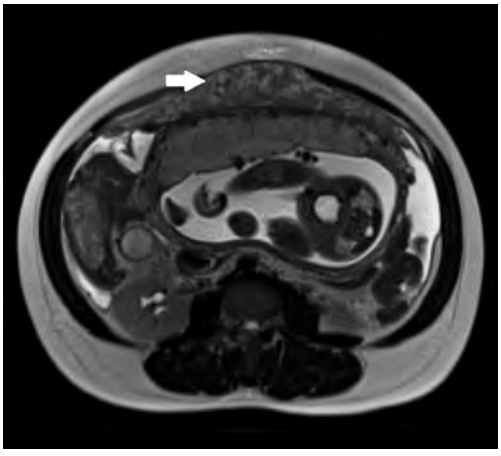

Figure. T2-weighted magnetic resonance imaging sequence of the abdomen of a pregnant woman from Haiti. An omental mass of intermediate intensity (white arrow) is shown anterior to the uterus.

resolved. Her son was admitted to the neonatal intensive care unit and was placed on antimycobacterial therapy. He also recovered and was discharged after 135 days.

This case highlights several issues related to tuberculosis epidemiology and diagnosis. Although pulmonary disease is the most common manifestation of tuberculosis overall, extrapulmonary tuberculosis accounts for a significant and increasing proportion of cases in the United States (*1*). Pregnancy is associated with greater likelihood of extrapulmonary disease; extrapulmonary infection accounts for 13% of all cases worldwide (*2*) but 50% of cases in pregnancy, according to a recent study (*3*).

The frequency of peritoneal tuberculosis in pregnancy is unknown; few cases have been reported in the literature (*4*–*7*), although we know of 3 additional cases from Haiti (online Technical Appendix Table). However, cases are likely underdiagnosed or diagnosed late in the course of illness. Underdiagnosis and delayed diagnosis may be caused by the nonspecific nature of symptoms, commonly abdominal pain and ascites, which can be attributed to pregnancy itself or obstetrical complications. These erroneous explanations for symptoms are reflected in this patient, whose symptoms were initially attributed to abruption and who was not diagnosed with tuberculosis until >3 weeks after seeking medical assistance. Such delays in diagnosis are typical of peritoneal tuberculosis and are associated with increased death rates (*8*). In many cases, clinical features cannot distinguish peritoneal tuberculosis from malignancy, necessitating more extensive evaluation (*7*).

Failure to diagnose peritoneal tuberculosis, in pregnancy or otherwise, might also stem from the insensitivity of noninvasive diagnostic testing. Paracentesis with acid-fast staining detects only a minority of cases (*8*). The sensitivity of mycobacterial cultures of ascites fluid varies, and culture results are often not available for weeks (*8*). Ascites fluid adenosine deaminase has shown promise as a reliable, minimally invasive diagnostic test in resource-poor countries, but was insensitive in a United States study (*9*). In addition, although tuberculin skin testing and interferon gamma release assay performance are not affected by pregnancy (*10*), neither can distinguish active from latent infection. Without diagnostic clinical features or sensitive noninvasive tests, the diagnosis of peritoneal tuberculosis might only be confirmed through laparoscopy or laparotomy, as in our case. Such invasive testing methods and facilities, equipment, and personnel might not be readily available in resource-poor settings.

This case also illustrates the ongoing threat of tuberculosis in countries of all income levels. It is not clear where our patient contracted tuberculosis; she was most likely exposed in Haiti, but transmission within her Haitian community in the United States, or from another source, is also possible. Regardless, as in her case, a majority of tuberculosis cases within the United States occur in foreign-born persons. Given the ease and frequency of travel, lapses in tuberculosis control in any locale are likely to have effects more broadly. Wherever they work, clinicians must maintain vigilance for tuberculosis in all of its protean forms.

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Microsporidial Keratoconjunctivitis Outbreak among Athletes from Hong Kong Who Visited Singapore, 2012

To the Editor: An international outbreak of microsporidial keratoconjunctivitis related to soil contact in a Singapore sport venue during April was reported to Hong Kong Department of Health, People's Republic of China, in May 2012. Microsporidia are obligate intracellular, unicellular, eukaryotic, parasitic protists related to fungi (*1*). Fourteen species of microsporidia have been detected in humans (*1*). Several species of microsporidia, such as *Vittaforma corneae*, can cause keratoconjunctivitis (*1*). An increasing incidence of microsporidial keratitis in Singapore that is strongly correlated with exposure to soil was reported during 2004–2007 (*2*). In a case series of 22 patients during 2006–2008 in Singapore, soil or mud were reported as predominant ocular contaminants that were contacted by athletes during sporting activities, such as playing rugby in muddy fields (*3*).

The outbreak reported in May 2012 affected 34 (41%) of 82 rugby players from Hong Kong who had participated in a rugby tournament in Singapore during April 21–22, 2012. In addition to the affected athletes from Hong Kong, there were 89, 15, 13, and 9 affected players, respectively, from Singapore, Malaysia, Australia, and United Arab Emirates (*4*). We conducted a retrospective cohort study among players from Hong Kong to identify potential risk and preventive factors for microsporidial keratoconjunctivitis.

The rugby tournament involved ≈1,600 boys and girls from 16 rugby clubs in Singapore, Hong Kong, Malaysia, Australia, and the United Arab Emirates. We invited 82 boys (8–16 years of age) from 2 Hong Kong rugby clubs that participated in the tournament to participate in telephone interviews during May 18–25, 2012. Using a standardized questionnaire, we collected information describing demographics and potential risk and preventive factors. We defined a case-patient as any player who had eye redness and 1 of the following ocular signs or symptoms since April 21: pain, discharge, swelling, or itchiness.

We interviewed 73 (89%) of the 82 players: 34 (47%) met the case definition. The median age of case-patients was 13 years (range 9–16 years); these figures were not different from those of the cohort (median age 13 years, range 8–16 years).

Onset of the reported 34 cases ranged from April 26 through May 22, peaking on May 7 (Figure). The distribution of onset of cases over time indicates a point-source outbreak and reflects a wide range of incubation periods. Symptom onset occurred a median of 15 days (range 5–31 days) after opening day of the tournament.

Except for 2 players who had histories of asthma, all players reported good past health. Ocular signs and symptoms of the 34 case-patients were redness (100%), pain (53%), itchiness (53%), discharge (47%), and swelling (41%). Corneal scraping samples from 3 players were positive for *V. corneae* by PCR.

Heavy rainfall on playing fields was reported on April 21, when all the players from Hong Kong participated in the games and were exposed to soil and muddy water. Some players washed their eyes after exposure to the dirt in the field. We identified the following as preventive factors for keratoconjunctivitis: individual eye washing by bottled or tap water (relative risk [RR] 0.38, 95% CI 0.23–0.62), bottled water (RR 0.44, 95% CI 0.25–0.76) and tap water (RR 0.50, 95% CI 0.27–0.92). Group eye washing with water from a hose held by a trainer was not preventive. It is possible that individual eye washing by the players was more thorough.

The 47% attack rate among the players from Hong Kong is higher than the overall 10% (160/1,600) attack rate for tournament participants. Rugby players from Hong Kong might have been less aware of the risk of contracting microsporidial keratoconjunctivitis through soil or muddy water exposure than were players from other locations.

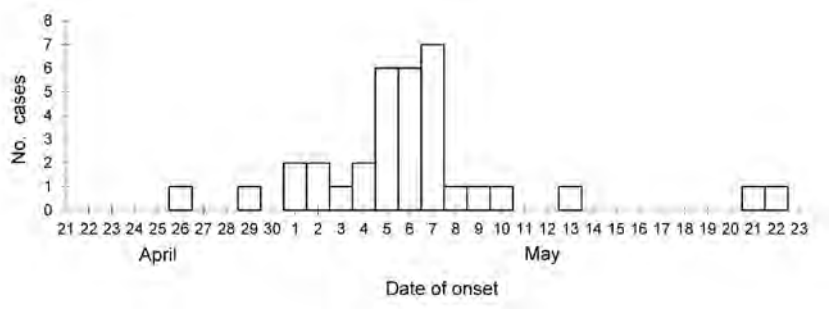

Figure. Onset dates of 34 cases of microsporidial keratoconjunctivitis among rugby players from Hong Kong, People's Republic of China, who were exposed to contaminated soil and mud during a tournament in Singapore, April 21–22, 2012. Three cases (onset May 4, 5, and 7) were diagnosed in players by positive PCR testing; all other cases were diagnosed by the presence of eye redness and 1 of the following ocular signs or symptoms since April 21: pain, discharge, swelling, or itchiness.

This outbreak provided an opportunity to study the incubation period of *V. corneae* keratoconjunctivitis in otherwise healthy persons. Time from soil exposure to development of ocular symptoms of microsporidial keratitis has been reported to be 2–21 days (median 14 days) (*2*) and 5–14 days (mean 6.8 days) (*3*). The incubation period during this outbreak was 5–31 days (median 15 days).

An investigation by the Singapore Ministry of Health of this outbreak revealed that microsporidial spores are probably ubiquitous in soil in Singapore (*4*). All interviewed rugby players from Hong Kong were exposed to soil or muddy water in Singapore before the outbreak, but we could not demonstrate the dose–response relationship because of the long incubation period and difficulty quantifying exposure to soil and muddy water.

This was an uncommon microsporidial keratoconjunctivitis outbreak. Advice about hygiene should be given to athletes who are exposed to dirt and mud on playing fields to minimize their risk for infection. Instructions should be given for safe and thorough washing of eyes, especially after dirt/mud exposures on waterlogged playing fields. Clinicians and public health professionals must consider microsporidial keratoconjunctivitis as a differential diagnosis for conditions of field athletes who exhibit eye redness accompanied by eye pain, discharge, swelling, or itchiness after exposure to soil or mud.

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Mycobacterium fortuitum Endocarditis Associated with Cardiac Surgery, Serbia

To the Editor: *Mycobacterium fortuitum* is a member of the group of rapidly growing nontuberculous mycobacteria. It is a well-known causative agent of skin and soft tissue infections, postsurgical wound infections, and other health care–associated infections (*1*). Only sporadic cases of endocarditis caused by this bacterium have been reported (*2–4*). We describe a cardiac surgery–related outbreak of endocarditis caused by *M. fortuitum* in 3 children.

Over a 3-week period during 2009, eight children consecutively underwent surgery for correction of ventricular septal defect (VSD) by insertion of a bovine pericardial patch at the University Children's Hospital in Belgrade, Serbia. None of them had previous cardiac surgery.

The same patch, SJM Pericardial Patch with EnCap Technology (St. Jude Medical, St. Paul, MN, USA), was used as a source for smaller, tailored patches for all patients. Sterile scissors and forceps were used to tailor a piece of the patch needed for a corresponding VSD closure. During repeated performances of this procedure and between surgeries, the patch had been continuously stored in 2% propylene oxide (PO) provided by the manufacturer. Each tailored piece of the patch had been immersed into freshly prepared sterile saline for 6 min before defect patching. The postoperative course had been uneventful for all patients, and they were discharged 7 days after the procedure. However, 3 patients were readmitted to the hospital because of prolonged fever and increasing fatigue. Patients 1, 2, and 3 (Table) had been the fourth, sixth, and eighth patients undergoing VSD repair, respectively. Diagnosis of infective endocarditis in these patients was established by transthoracic echocardiography findings and blood cultures positive for acid-fast bacteria (Table). Acid-fast bacteria also were recovered from the patch and vegetation taken during reoperation in patient 3 (Table). The isolates were identified as *M. fortuitum* by the GenoType Mycobacterium CM assay (Hain Lifescience, Nehren, Germany) (*5*). Empiric treatment with vancomycin and ceftriaxone was switched to amikacin, ciprofloxacin, and imipenem. After 6 weeks of treatment, the patients were discharged, and all were asymptomatic 12 months later.

The cultural characteristics and susceptibility patterns of all the isolates obtained were indistinguishable. To explore their possible clonal relatedness, we genotyped 3 *M. fortuitum* strains isolated from blood cultures (1 isolate per patient) and 2 *M. fortuitum* isolates recovered from samples taken during reoperation in 1 of the patients. The enterobacterial repetitive intergenic consensus PCR was used (*6*), and all isolates produced identical patterns.

Nosocomially acquired *M. fortuitum* endocarditis has been reported but only sporadically in adults, and these cases usually were fatal (*3*,*4*,*7*). In contrast, we describe 3 related cases of *M. fortuitum* endocarditis in children who recovered. The relatedness of the cases is strongly supported by the following. First, epidemiologic links are obvious because the 3 patients underwent surgery in the same operating room, and the same patch was used in all of them. Second, *M. fortuitum* strains isolated from the 3 patients were phenotypically and genotypically identical.

Repeated use of the same patch in multiple surgeries strongly suggests the contaminated patch was the source of *M. fortuitum* infection in the 3 patients. This possibility could not be corroborated by bacteriologic examination of the patch because the remaining unusable fragments had been discarded after the surgeries (i.e., ≈3 months before the outbreak became evident). Although contamination of the patch during manufacture is possible (*8*), it seems more reasonable to assume that the contamination occurred intraoperatively. The common factor in nosocomially acquired *M. fortuitum* infections is presumed to be exposure to a liquid contaminated with this organism (*1*,*9*). The patch was not exposed to solutions other than the PO in which it had been stored and the sterile saline used during the rinsing procedure. Because only a piece of the patch tailored for a particular patient was exposed to a saline freshly prepared for each surgery, contamination of the PO by *M. fortuitum* presumably led to contamination of the patch. Liquid PO is used as a chemical sterilant for bioprostheses intended for single use. However, multiple use of the same patch implied repeated exposure of the PO solution to the environment and prolonged storage at 4°C between surgeries. Because PO effectiveness is markedly reduced at temperatures <16°C (*10*), the specific circumstances could have compromised the sterilizing capacity of the PO solution and enabled

Table. Characteristics of patients in an outbreak of *Mycobacterium fortuitum* endocarditis, Serbia*

Characteristic	Patient 1	Patient 2	Patient 3
Age, y/sex	12.0/F	2.0/F	0.5/M
Comorbidity	No	Down syndrome	No
Time between surgery and readmission, d	86	97	76
Position of vegetation	Septal cusp of tricuspid valve	Septal cusp of tricuspid valve and VSD patch	Septal cusp of tricuspid valve and VSD patch
Hemodynamic consequence	Moderate tricuspid valve regurgitation	Moderate tricuspid valve regurgitation	VSD patch dehiscence
No. blood cultures collected, aerobic/anaerobic	3/3	10/4	6/3
No. blood cultures positive for acid-fast bacteria, aerobic/anaerobic	2/0	5/0	2/0
Time of collection of first positive/last positive blood culture, d after readmission	8/11	1/21	4/16
Outcome	Resolved after antimicrobial drug treatment	Resolved after antimicrobial drug treatment	Resolved after antimicrobial drug treatment and reoperation

*VSD, ventricular septal defect.

contamination by ubiquitous *M. fortuitum*.

We are well aware that the patch was intended for single use only and that application of the same patch in multiple patients is not a practice in industrialized countries. However, it is a practice in some resource-limited countries. The outbreak of *M. fortuitum* endocarditis we describe is a clear warning that such practice is associated with high risk and thus should be discontinued.

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Cryptococcus gattii, Florida, USA, 2011

To the Editor: Cryptococcosis is a systemic mycosis most commonly caused by 2 species of encapsulated yeast: *Cryptococcus neoformans* and *C. gattii*. *C. gattii* is a globally emerging pathogen. In the United States, an outbreak of *C. gattii* infection caused by molecular type VGII has been ongoing since 2004, primarily in the Pacific Northwest (*1*). In addition, sporadic cases caused by molecular types VGI and VGIII have been reported in other areas, including North Carolina, Rhode Island, New Mexico, Michigan, Georgia, and Montana (*2*). We report a case of disseminated *C. gattii* VGIIb infection in the United States outside of the Pacific Northwest in an otherwise healthy Florida native who had no known travel to *C. gattii*–endemic areas.

In May 2011, a 50-year-old man sought care for 6 months of progressive pain, swelling, and deformity of the left thigh and stiffness of his left knee. His only recent trauma was a minor left lower extremity injury 2 years earlier when a horse rolled on him. However, he had no fracture, and the injury eventually healed without medical care. He also reported occasional productive coughing and smoking 1 pack of cigarettes per day for 30 years. The patient was born and raised in Pasco County, Florida, and had not traveled outside of Florida in 20 years. He reported working on a dairy farm and having regular exposure to horses and pigs. Imaging showed a possible fracture of his left femur at the same site as the horse-related injury 2 years earlier. Computed tomographic scan of the chest demonstrated mediastinal lymphadenopathy and multiple pulmonary nodules bilaterally.

The man underwent open biopsy and fixation of the left femur fracture. Arthrocentesis was performed on his

left knee. The bone and joint fluid were full of India ink–positive encapsulated budding yeast. The serum cryptococcal antigen was 1:4,096 (reference value, negative). An HIV antibody test result was negative, and CD4 count was 800 cells/mL (reference 500–2,600 cells/mL). A lumbar puncture showed normal opening pressure, 27 leukocytes/mL (reference 0–5 cells/mL) (89% lymphocytes [reference 40%–80%]), protein 464 mg/dL (reference 15–45 mg/dL), and glucose 21 mg/dL (reference 40–70 mg/dL). The cerebrospinal fluid (CSF) cryptococcal antigen was 1:4,096 (reference, negative). Magnetic resonance imaging of the brain indicated mild enhancement of the lining of the lateral ventricles and mild dilatation.

C. gattii was isolated from the femur wound (superficial and deep) and CSF. Phenotypic testing was performed at ARUP Laboratories (Salt Lake City, UT, USA). In addition, the isolate was identified by multilocus sequence typing analysis as *C. gattii* type VGIIb by the Centers for Disease Control and Prevention (Atlanta, GA, USA) (*3*) (Figure).

The patient was treated with liposomal amphotericin B and 5-flucytosine for 4 weeks for disseminated *C. gattii* infection with musculoskeletal, central nervous system, and pulmonary involvement. Repeat lumbar puncture revealed a normal opening pressure. CSF studies were not performed on this specimen. The patient gradually improved and was discharged on oral voriconazole (to be continued for 1 year) after 4 weeks of hospitalization. By July 2011, the patient was walking with crutches and had no symptoms other than persistent swelling and pain of his left leg. As of July 2012, he had fully recovered except for some residual pain and weakness in his left leg.

In addition to its newfound endemicity in the US Pacific Northwest, *C. gattii* is known to be endemic to Australia, Papua New Guinea, South and Southeast Asia, and some parts of Mexico and southern California (*4*). Its genetic diversity, the global distribution of isolates, and a broad range of hosts contribute to its success as a pathogen. *C. gattii* can be subdivided into at least 4 molecular types: VGI, VGII, VGIII, and VGIV (*5*). Most isolates identified from the Pacific Northwest outbreak are molecular type VGII, primarily comprising 3 distinct clonal subtype lineages: VGIIa, VGIIb, and VGIIc (*6*,*7*).

The case reported here involved *C. gattii* (VGIIb) outside the Pacific Northwest or other regions to which it is known to be endemic. Although the source of this patient's infection remains unknown, his previous horse-related injury is intriguing as a possible source (*8*). All 4 isolates from horses in the Centers for Disease Control and Prevention's collection are molecular type VGIIb (S.R. Lockhart, unpub. data). Other infections have been reported to seed the body and proliferate in areas of prior injury (*9*); this patient could have inhaled the cryptococcal yeast during exposure to horses, which then disseminated and seeded his prior injury site.

Clinically, infection caused by *C. gattii* outbreak strains (VGIIa/b/c) is characterized primarily by pulmonary complaints and pneumonia, with or without meningitis (*10*); other strains, such as VGI, occur as CNS disease (*10*). The patient reported here showed mainly musculoskeletal complaints, although involvement of the CNS and pulmonary systems was later found. Continued surveillance for *C. gattii* outside the Pacific Northwest will help shed more light on the spectrum of clinical manifestations. In the United States, *C. gattii* is likely to be seen increasingly outside the Pacific Northwest and other regions to which it is endemic.

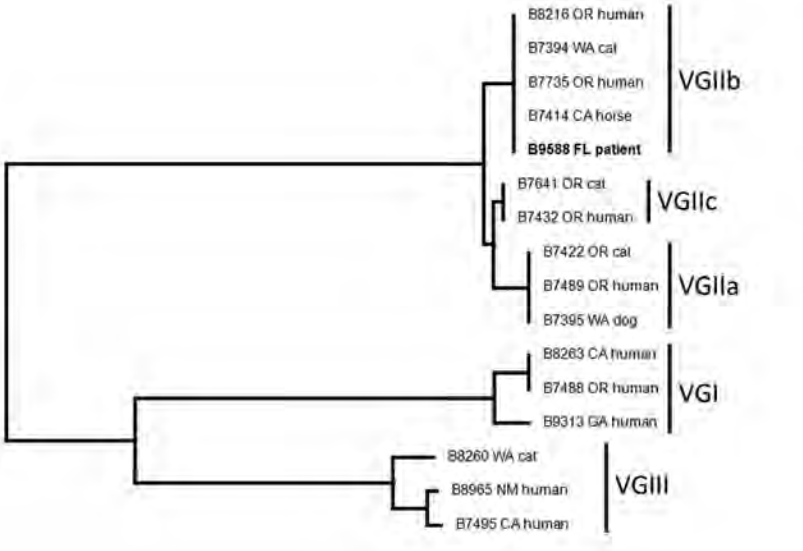

Figure. Neighbor-joining dendrogram of FL isolate (B9588, in **boldface**) with other US isolates showing that the FL isolate is identical to the VGIIb isolates from the US Pacific Northwest. The dendrogram was constructed by using multilocus sequence typing (*3*). FL, Florida; OR, Oregon; WA, Washington; CA, California; GA, Georgia; NM, New Mexico.

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Characterization of *Mycobacterium orygis*

To the Editor: We thank Gey van Pittius and colleagues for their addition to the markers that identify *Mycobacterium orygis* as a distinct subspecies in the *M. tuberculosis* complex (*1*). Its isolation from a wild buffalo broadens the host range of *M. orygis*. Gey van Pittius and colleagues raise 3 issues: the utility of the *gyrB*^{oryx} single-nucleotide polymorphism (SNP) being equally specific as the reported SNP in *Rv2042*^{38}, the presence of genomic regions RD701 and RD702 in *M. orygis*, and the addition of the sequence type (ST) 701 spoligotype to *M. orygis*–specific spoligotypes.

We agree that use of the *gyrB*^{oryx} mutation is more practical for routine daily use because this gene helps identify several subspecies of the *M. tuberculosis* complex. However, use of the partial *Rv2042* sequencing is similarly practical because it can be combined with sequencing of the adjacent *pncA* gene, which enables identification of several *M. tuberculosis* complex species and some subspecies (i.e., *M. orygis*, *M. bovis*, *M. canettii*) (*2*), to identify the CAS genotype of *M. tuberculosis* (J. van Ingen, unpub. data) and, to some degree, assess susceptibility to pyrazinamide (*3*).

With the added data, we can conclude that *M. orygis* is an *M. tuberculosis* complex subspecies defined by the presence of genomic regions RD1, RD2, RD4, RD5a, RD6, RD13–RD16, RD701, and RD702, by the C-to-G SNP in *mmpL6*^{551}, and by the deletion of regions RD3, RD5b, RD7-RD12, RDoryx_1, RDoryx_4, and RDoryx_wag22. Subspecies-specific SNPs are present in *gyrB* and *Rv2042*. Spoligotypes ST587, ST701, and closely related types are characteristic of *M. orygis*, and this subspecies yields 17–20 copies of insertion sequence *6110* and a distinct 24-locus variable number tandem repeats pattern (*4*,*5*). Given the rapid progress in genome sequencing, additional markers specific for the different subspecies will further enrich this panel of differences.

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Mycobacterium tuberculosis Beijing Type Mutation Frequency

To the Editor: A striking finding in the study by de Steenwinkel et al. (*1*) is the high frequency of mutation to rifampin resistance by 2 *Mycobacterium tuberculosis* Beijing strains, which might play a role in the association between the Beijing strains and multidrug-resistant tuberculosis. Earlier reported frequency of mutation to rifampin resistance by *M. tuberculosis* has been 10^{-8} CFU (*2*,*3*), including the Beijing genotype (*3*,*4*). Of note, the Beijing 2002–1585 strain, for which frequency of mutation to rifampin resistance is 10^{-3} CFU (1 mutant/1,000 CFU), showed a moderate frequency of 10^{-8} CFU in another study (*4*). We think that a mutation frequency increase of 100,000× is remarkably high. In contrast, rifampin-resistant mutants of the Beijing 1585 strain did not emerge in low-density cultures (5×10^5 CFU/mL) used for time-kill kinetics experiments, although frequency of mutation to rifampin resistance was determined to be 10^{-3} CFU.

Mutation frequency is determined by fluctuation assays. To exclude preexisting mutants, which would bias the mutation frequency by so-called jackpots, a series of low-inoculum cultures is typically used (*5*). However, for unknown reasons, de Steenwinkel et al. used only 1 high-density culture of 10^{10} CFU of each strain to determine mutation frequency. This strategy is not recommended because mutations can occur early or late, resulting in substantial mutation frequency fluctuation between test episodes. A strain with known mutation rates should preferably be included to rule out possible technical errors.

We propose the following explanations for the remarkable results: 1) the rifampin concentration for selecting mutants might have been too low, enabling growth of some colonies of drug-susceptible bacteria; 2) rifampin mutants arose early or preexisted in the cultivation of Beijing strains 1585 and 1607, producing jackpots; or 3) the 2 Beijing isolates might contain rifampin-resistant subpopulations (heteroresistance). The capacity of the Beijing strain to develop and, especially, transmit multidrug-resistant tuberculosis remains to be further analyzed.

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In Response: We explain the differing frequencies of mutation to rifampin resistance mentioned by Werngren (*1*). First, the strains of *Mycobacterium tuberculosis* that we tested differed from those previously tested (*2*). Second, we used different rifampin concentrations in subculture plates. For Beijing strain 2002–1585, Bergval et al. (*3*) found a mutation frequency of $4–24 \times 10^{-8}$ at a subculture concentration of 8 mg/L, whereas we found a mutation frequency of $3–4 \times 10^{-3}$ at a subculture concentration of 1 mg/L and a lower mutation frequency at 2 mg/L. Thus, the concentration of drugs in subculture plates is crucial to mutation frequency assays. Absent a subculture concentration standard, we applied rifampin at 1 mg/L (*4*) because bacteria growing at this concentration are considered resistant to rifampin. Our mutation frequency and time-kill kinetics assay results are not contradictory

because in the time-kill kinetics assays, the subculture rifampin concentration was 4 mg/L.

We performed no classical fluctuation assays. We compared the Beijing genotype with the East African/Indian genotype to learn how M. tuberculosis strains differed in their capacity to withstand antituberculosis drug treatment. For reference strain H37Rv, mutation frequency was 1.5 × 10-6, higher than that found with higher subculture concentrations.

With regard to the 3 other issues, our drug-susceptibility testing of mutants showed a stable rifampin-resistant phenotype. We agree that these bacteria might represent preexisting mutants selected during drug exposure in a certain drug concentration window. By using different concentrations in subculture plates in our mutation frequency assay, we detected such preexisting mutants. Heteroresistance probably does not explain our observations because in our time-kill kinetics experiments, the whole mycobacterial population decreased over time in a drug concentration-dependent way, and regrowth of a drug-resistant subpopulation was not observed.

By not sticking to the fixed test conditions as used in the classical drug-susceptibility assays, research leads to highly interesting findings. One can conclude that serendipity flourishes with variation.

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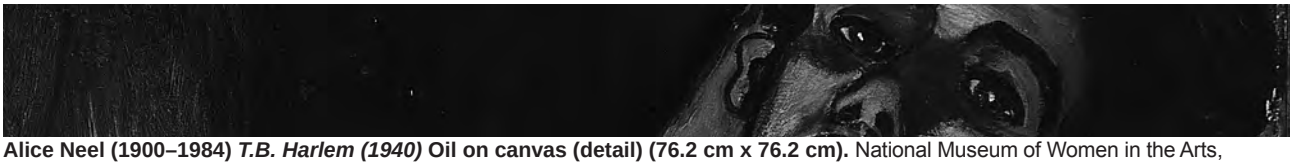
Correction: Vol. 18, No. 8

The name of author Arina Zanuzdana was misspelled in the article Vaccination of Health Care Workers to Protect Patients at Increased Risk for Acute Respiratory Disease. The article has been corrected online (http://wwwnc.cdc.gov/eid/article/18/8/11-1355_intro.htm).



Alice Neel (1900–1984) ***T.B. Harlem (1940)*** **Oil on canvas (detail) (76.2 cm x 76.2 cm).** National Museum of Women in the Arts, Washington, D.C. Gift of Wallace and Wilhelmina Holladay; copyright The Estate of Alice Neel, Courtesy David Zwirner, New York

Captain Consumption and the Collector of Souls

Polyxeni Potter

"Whenever I think of writing a biography, I think of … the color red in our living room. My mother said I could not have remembered it because I was only about 6 months old," Alice Neel said of her precocious recollection of red. Yet she was more than 70 years old before her art began to sell. In the meantime, she took a great interest in those around her: "I paint my time using people as evidence." "I love to paint people torn by all the things that they are torn by today in the rat race in New York." And as she created portrait after portrait, she came to see herself as a "painter of people" and "collector of souls."[1] "The road that I pursued, and the road that I think keeps you an artist, is that no matter what happens to you, you still keep on painting."

A native of Delaware County, Pennsylvania, Neel attended art school in Philadelphia before moving to New York. "I don't know what you expect to do with your life," her mother warned, "You're only a girl." A bohemian from the start, Neel resisted movements and labels. Though she came of age when the art scene was changing, she formed her own style, a mixture of figurative, expressionist, and abstract elements. "I am not against abstraction. What I can't stand is that the abstractionists pushed all the other pushcarts off the street." Some of her favorite painters, Morris Louis and Clyfford Still, were abstractionists. "I hate the New Realism. I hate equating a person and a room and a chair. Compositionally, a room, a chair, a table, and a person are all the same for me, but a person is human and psychological."

"All during the 40s and 50s, New York was nothing but Abstract Expressionism and nobody painted people or anything like that …. They couldn't even let people painters get a foot in the door. But during that time I couldn't give up what I was interested in for what was the fashion, so I kept right on painting." Neel's choice, portraiture, was a declining genre, but she did not see it that way. "When portraits are good art, they reflect the culture, the time, and many other things."

Neel painted full-body portraits of unconventional, controversial, or little-known persons around her, who were often asked to pose nude. "Every person is a new universe unique with its own laws." She dwelled on the character and circumstances of her subjects and her engagement with them. "I always loved the working class and the most wretched, but then I also loved the most effete and the most elegant." Her portrait of Andy Warhol gained great notoriety. During a career that stretched more than 5 decades and produced 3,000 works, she was called "radical" and worse.

Neel had a turbulent life. She married Cuban artist Carlos Enriquez and moved to Havana, where they lived "painting night and day." She lost one child, age 11 months, to diphtheria, another soon afterward to the failed marriage. She became seriously ill, physically and emotionally. "I didn't see life as *Picnic in the Park*. I wasn't happy like Renoir." She had money problems well into her old age and often could not afford painting supplies. Aside from a rare sale, from 1933 to 1943, her income came from New Deal art projects. The Federal Arts Project of the Works Progress Administration provided a steady income and time to work. In return, she had to deliver a finished oil painting once every 4 to 6 weeks, depending on the size of the canvas. "This was fabulous," she said later, "as most of the artists had nothing in those days and in fact there were free lunches for artists in the village …. All the artists were on the project. If there had been no such cultural projects there might well have been a revolution." Eventually, project funding dried up, and she had to depend on public assistance. Her work began to receive some attention and she a degree of notoriety, but real recognition did not come until the 1970s. In the interim, she was investigated by FBI for her political activities. The files described her as a "romantic bohemian type communist."

Neel remembered her rural small-town beginnings as "benighted, unenlightened, and obscured by darkness." As an adult, she lived in urban New York, first in Greenwich Village, then East or Spanish Harlem, where she accomplished her mission as "collector of souls" by finding and painting a fantastic array of colorful characters and capturing their individuality and uniqueness as well as their

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[1]A reference to Chichikov, the protagonist of Nicolai Cogol's *Dead Souls*, who collected the souls of dead serfs.

precarious social and economic reality. Many of these underprivileged "souls" lived in the same building where she had her studio.

In one of her best portraits, *T.B. Harlem*, on this month's cover, Neel painted Carlos Negrón, the brother of José Negrón, a Puerto Rican musician friend of Neel's and father of one of her sons. The 24-year-old Carlos, in whose face she saw "the face of Puerto Rico," had advanced tuberculosis. Neel knew Carlos well. She had also painted his wife and children, who lived in overcrowded quarters nearby. But this portrait is not just the image of a friend or a statement about social ills and contagion. It is also a historical record of tuberculosis and its treatment in 1940.

The shrunken Carlos is fully aware of his predicament. From his hospital bed, consumed by this horrible illness, he faces the reader. Tuberculous pallor lightens his brow, cheeks and shoulders. His eyes are feverish, his pouting lips register exasperation.

Everyone thought that introduction of antimicrobial drugs in the 1940s would end tuberculosis. But for patients in advanced stages of the disease, radical treatments were not abandoned. During surgical procedures frequently done under local anesthetic, several ribs were removed, allowing soft tissues of the chest to collapse on the lung. Carlos' skeletal fingers draw attention to the stark bandage against his chest, roughly covering a large wound. He has undergone thoracoplasty to reduce the size of abnormal space in the thoracic cavity created by the collapsed lung. The procedure itself caused serious deformity from the pull of muscles on the operated side and compensatory cervical scoliosis on the opposite side. His distended belly and ravaged frame form an S curve against the hospital bed.

In the Life and Death of Mr. Badman (1680), John Bunyan speculated on his lead character's demise, "Pray, of what disease did Mr. Badman die?" "I cannot properly say that he died of one disease, for there were many that had consented, and laid their heads together to bring him to his end. He was dropsical, he was consumptive, he was surfeited, was gouty, and some say, he had a tang of the pox in his bowels. Yet the captain of all these men of death that came against him to take him away was the consumption, for it was that that brought him down to the grave."

John Bunyan's understanding of consumption and its connection with virtue reflected his life and times. Better information did not come about until much later, in 1882, when Robert Koch made his famous presentation at the Berlin Physiological Society: "In the future, the fight against this terrible plague of mankind will deal no longer with an undetermined something, but with a tangible parasite, whose living conditions are for the most part known and can be investigated further." But progress was slow. Effective chemotherapy against the disease would come too late to help the mutilated Carlos and so many others.

Despite a steady decline in Europe and the United States in the mid-20th century, tuberculosis is still a leading cause of death in the world. Reemergence in the United States in the 1980s and 1990s and continued transmission around the world have been attributed to many factors, among them antimicrobial drug resistance; HIV/AIDS; increased homelessness; and lack of effective treatment programs, as brought home in Neel's *T.B. Harlem*.

New York City has been a center for both tuberculosis and its control. This fact did not escape the attention of Alice Neel. What she felt so acutely and tried to convey in *T.B. Harlem*—poverty, malnutrition, overcrowded living conditions—are social determinants, which in the case of tuberculosis, overcame progress in diagnosis and treatment. Bunyan's "captain consumption" rules until new diagnostics and therapies are aligned with measures to address the social inequities at the heart of reemergence, acknowledging the egalitarian nature of this lethal airborne disease.

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Article Title

Increasing *Pneumocystis* Pneumonia, England, UK, 2000–2010

CME Questions

1. You are a public health official consulting with the World Health Organization regarding trends in *Pneumocystis jirovecii* pneumonia in England over the past decade. Based on findings from the database study by Dr. Maini and colleagues, which of the following statements about changes in incidence of *P. jirovecii* pneumonia in England from 2000–2010 would be most likely to appear in your report?

A. Between 2000 and 2010, laboratory-confirmed cases increased by an average of 3% per year
B. Between 2000 and 2010, death registrations increased by an average of 2% per year
C. Between 2000 and 2010, Hospital Episode Statistics (HES) cases increased by an average of 9% per year
D. Most of the increase in HES cases occurred in the first half of the decade

2. Based on findings from the database study by Dr. Maini and colleagues, which of the following statements about changes in risk factors associated with *P. jirovecii* pneumonia in England from 2000–2010 is most likely correct?

A. Rates of *P. jirovecii* pneumonia in adults with diagnosed HIV infection increased from 2000-2010
B. Patients with pre-existing renal disease were identified as a new risk group
C. The largest proportion of cases was patients who had undergone transplantation
D. A total of 17.5% of cases were associated with pre-existing lung disease

3. Which of the following statements about the clinical and public health implications of findings from the database study by Dr. Maini and colleagues is most likely correct?

A. The diagnosis of potentially preventable *P. jirovecii* pneumonia in non-HIV infected persons did not increase during the study period.
B. Earlier diagnosis of HIV was the only potential explanation for the significant reduction during the study period in *P. jirovecii* pneumonia infection among HIV-infected persons
C. There is no reason to suspect that *P. jirovecii* pneumonia ascertainment increased over the study period
D. The findings support enhanced *P. jirovecii* pneumonia surveillance for patients with chronic lung disease, systemic inflammatory diseases, and solid tumors

Activity Evaluation

1. The activity supported the learning objectives.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
2. The material was organized clearly for learning to occur.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
3. The content learned from this activity will impact my practice.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
4. The activity was presented objectively and free of commercial bias.				
Strongly Disagree				Strongly Agree
1	2	3	4	5

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Article Title

Tuberculosis and HIV Co-infection, California, USA, 1993–2008

CME Questions

1. You are a public health consultant to a California HMO, and you are asked to prepare a report regarding tuberculosis (TB)/HIV co-infection. Based on the epidemiological study by Dr. Metcalfe and colleagues, which of the following statements about rates of HIV co-infection among persons with TB and changes in TB incidence among persons with HIV is most likely to appear in your report?

A. About 3% of patients with TB had HIV co-infection
B. Among persons with HIV, overall TB incidence decreased from 437/100,000 in 1993 to 126/100,000 in 2008
C. Rates of TB co-infection increased from 1993 to 2008among blacks with HIV
D. Rates of TB co-infection increased from 1993 to 2008 among Hispanics with HIV

2. Based on the study by Dr. Metcalfe and colleagues, which of the following statements about characteristics of patients with TB/HIV co-infection in the modern era versus those in the pre-highly active antiretroviral therapy (HAART era is most likely correct?

A. Compared with the pre-HAART era, patients with TB HIV in the modern era were less likely to be foreign-born
B. Compared with the pre-HAART era, patients with TB HIV in the modern era were less likely to be Hispanic
C. Compared with the pre-HAART era, patients with TB HIV in the modern era were younger
D. Pyrazinamide-monoresistant TB occurred in 8% of patients with TB/HIV in the modern era compared with 2% in the pre-HAART era

3. Based on the study by Dr. Metcalfe and colleagues, which of the following statements about mortality and other characteristics of TB/HIV in California would most likely be correct?

A. Mortality of TB/HIV co-infection decreased from 30% in the pre-HAART era to 14% in the modern era
B. Mortality of TB/HIV is currently 50% higher than in TB patients without HIV
C. Mortality of TB/HIV is currently higher in men than in women
D. Risk factors for mortality in TB/HIV included younger age and men who have sex with men vs heterosexual HIV risk group

Activity Evaluation

1. The activity supported the learning objectives.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
2. The material was organized clearly for learning to occur.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
3. The content learned from this activity will impact my practice.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
4. The activity was presented objectively and free of commercial bias.				
Strongly Disagree				Strongly Agree
1	2	3	4	5

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Article Title

Clinical and Therapeutic Features of Pulmonary Nontuberculous Mycobacterial Disease, Rio de Janeiro, Brazil, 1993–2011

CME Questions

1. You are seeing a 55-year-old man with a 4-month history of cough, night sweats, and weight loss. You suspect that he has pulmonary tuberculosis, but you also consider whether he has pulmonary nontuberculous mycobacterial (PNTM) disease. What should you consider regarding PNTM disease?

A. Immunosuppression affects the risk of pulmonary tuberculosis but not PNTM disease
B. The highest prevalence of PNTM disease in the United States is among men between the ages of 20 and 35 years
C. The prevalence of PNTM disease is higher in the United States compared with Brazil
D. Pulmonary infection with *Mycobacterium kansasii* has a clinical presentation very similar to that of pulmonary tuberculosis

2. Which of the following statements regarding the clinical presentation of PNTM disease among patients in the current study is most accurate?

A. Nearly all patients had significant comorbid illnesses
B. Most patients reported a history of smoking
C. Systemic symptoms were more common than respiratory symptoms
D. Less than 10% of patients had received prior treatment for tuberculosis

3. You order sputum cultures for this patient. Which of the following organisms were most common in cases of PNTM disease in the current study?

A. *M. abscessus* and *M. fortuitum*
B. *M. abscessus* and *M. avium* complex (MAC)
C. *M. kansasii* and MAC
D. *M. kansasii* and *M. fortuitum*

4. On further testing, the patient does appear to have PNTM disease. Which of the following organisms was associated with the LOWEST cure rate in the current study?

A. *M. fortuitum*
B. *M. abscessus*
C. MAC
D. *M. kansasii*

Activity Evaluation

1. The activity supported the learning objectives.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
2. The material was organized clearly for learning to occur.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
3. The content learned from this activity will impact my practice.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
4. The activity was presented objectively and free of commercial bias.				
Strongly Disagree				Strongly Agree
1	2	3	4	5

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Title Page. Give complete information about each author (i.e., full name, graduate degree(s), affiliation, and the name of the institution in which the work was done). Clearly identify the corresponding author and provide that author's mailing address (include phone number, fax number, and email address). Include separate word counts for abstract and text.

Keywords. Use terms as listed in the National Library of Medicine Medical Subject Headings index (www.ncbi.nlm.nih.gov/mesh).

Text. Double-space everything, including the title page, abstract, references, tables, and figure legends. Indent paragraphs; leave no extra space between paragraphs. After a period, leave only one space before beginning the next sentence. Use 12-point Times New Roman font and format with ragged right margins (left align). Italicize (rather than underline) scientific names when needed.

Biographical Sketch. Include a short biographical sketch of the first author—both authors if only two. Include affiliations and the author's primary research interests.

References. Follow Uniform Requirements (www.icmje.org/index.html). Do not use endnotes for references. Place reference numbers in parentheses, not superscripts. Number citations in order of appearance (including in text, figures, and tables). Cite personal communications, unpublished data, and manuscripts in preparation or submitted for publication in parentheses in text. Consult List of Journals Indexed in Index Medicus for accepted journal abbreviations; if a journal is not listed, spell out the journal title. List the first six authors followed by "et al." Do not cite references in the abstract.

Tables. Provide tables within the manuscript file, not as separate files. Use the MS Word table tool, no columns, tabs, spaces, or other programs. Footnote any use of boldface. Tables should be no wider than 17 cm. Condense or divide larger tables. Extensive tables may be made available online only.

Figures. Submit figures in black and white. If you wish to have color figures online, submit both in black and white and in color with corresponding legends. Submit editable figures as separate files (e.g., Microsoft Excel, PowerPoint). Photographs should be submitted as high-resolution (600 dpi) .jpeg or .tif files. Do not embed figures in the manuscript file. Use Arial 10 pt. or 12 pt. font for lettering so that figures, symbols, lettering, and numbering can remain legible when reduced to print size. Place figure keys within the figure. Figure legends should be placed at the end of the manuscript file.

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Types of Articles

Perspectives. Articles should not exceed 3,500 words and 40 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. Articles should provide insightful analysis and commentary about new and reemerging infectious diseases and related issues. Perspectives may address factors known to influence the emergence of diseases, including microbial adaptation and change, human demographics and behavior, technology and industry, economic development and land use, international travel and commerce, and the breakdown of public health measures.

Synopses. Articles should not exceed 3,500 words and 40 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. This section comprises concise reviews of infectious diseases or closely related topics. Preference is given to reviews of new and emerging diseases; however, timely updates of other diseases or topics are also welcome.

Research. Articles should not exceed 3,500 words and 40 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. Report laboratory and epidemiologic results within a public health perspective. Explain the value of the research in public health terms and place the findings in a larger perspective (i.e., "Here is what we found, and here is what the findings mean").

Policy and Historical Reviews. Articles should not exceed 3,500 words and 40 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. Articles in this section include public health policy or historical reports that are based on research and analysis of emerging disease issues.

Dispatches. Articles should be no more than 1,200 words and need not be divided into sections. If subheadings are used, they should be general, e.g., "The Study" and "Conclusions." Provide a brief abstract (50 words); references (not to exceed 15); figures or illustrations (not to exceed 2); tables (not to exceed 2); and biographical sketch. Dispatches are updates on infectious disease trends and research that include descriptions of new methods for detecting, characterizing, or subtyping new or reemerging pathogens. Developments in antimicrobial drugs, vaccines, or infectious disease prevention or elimination programs are appropriate. Case reports are also welcome.

Photo Quiz. The photo quiz (1,200 words) highlights a person who made notable contributions to public health and medicine. Provide a photo of the subject, a brief clue to the person's identity, and five possible answers, followed by an essay describing the person's life and his or her significance to public health, science, and infectious disease.

Commentaries. Thoughtful discussions (500–1,000 words) of current topics. Commentaries may contain references but no abstract, figures, or tables. Include biographical sketch.

Etymologia. Etymologia (100 words, 5 references). We welcome thoroughly researched derivations of emerging disease terms. Historical and other context could be included.

Another Dimension. Thoughtful essays, short stories, or poems on philosophical issues related to science, medical practice, and human health. Topics may include science and the human condition, the unanticipated side of epidemic investigations, or how people perceive and cope with infection and illness. This section is intended to evoke compassion for human suffering and to expand the science reader's literary scope. Manuscripts are selected for publication as much for their content (the experiences they describe) as for their literary merit. Include biographical sketch.

Letters. Letters commenting on recent articles as well as letters reporting cases, outbreaks, or original research, are welcome. Letters commenting on articles should contain no more than 300 words and 5 references; they are more likely to be published if submitted within 4 weeks of the original article's publication. Letters reporting cases, outbreaks, or original research should contain no more than 800 words and 10 references. They may have 1 figure or table and should not be divided into sections. No biographical sketch is needed.

Books, Other Media. Reviews (250–500 words) of new books or other media on emerging disease issues are welcome. Title, author(s), publisher, number of pages, and other pertinent details should be included.

Conference Summaries. Summaries of emerging infectious disease conference activities (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.

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