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September 2010

Respiratory Infections

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On the Cover

Thomas Hart Benton (1889–1975)
Interior of a Farm House (1936)
Tempera on board (45.7 cm × 76.2 cm)

Courtesy of The John and Mable Ringling Museum of Art, The State Art Museum of Florida, a division of Florida State University, USA

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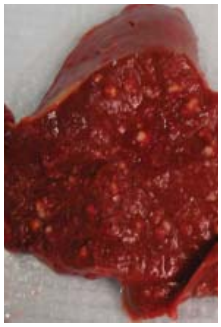

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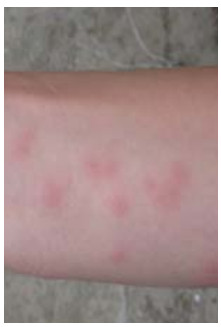

EMERGING INFECTIOUS DISEASES
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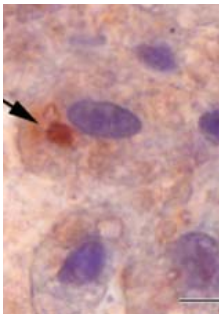
Dispatches



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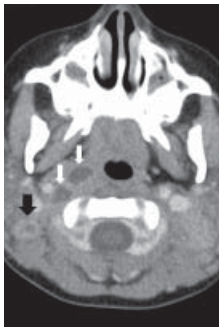

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Etymologia

Recurrent *Granulibacter bethesdensis* Infections and Chronic Granulomatous Disease

David E. Greenberg, Adam R. Shoffner, Adrian M. Zelazny, Michael E. Fenster, Kol A. Zarember, Frida Stock, Li Ding, Kimberly R. Marshall-Batty, Richard L. Wasserman, David F. Welch, Kishore Kanakabandi, Dan E. Sturdevant, Kimmo Virtaneva, Stephen F. Porcella, Patrick R. Murray, Harry L. Malech, and Steven M. Holland

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Learning Objectives

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

- Identify infectious organisms in cases of chronic granulomatous disease (CGD).
- Distinguish the clinical presentation of *Granulibacter bethesdensis* infection in CGD.
- Diagnose *G. bethesdensis* infection in CGD effectively.
- Plan effective treatment for *G. bethesdensis* infection in CGD.

Editor

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Chronic granulomatous disease (CGD) is characterized by frequent infections, most of which are curable. *Granulibacter bethesdensis* is an emerging pathogen in patients with CGD that causes fever and necrotizing lymphadenitis. However, unlike typical CGD organisms, this organism can cause relapse after clinical quiescence. To better define whether infections were newly acquired or recrudesced, we use comparative bacterial genomic hybridization to characterize 11 isolates obtained from 5 patients with CGD from North and Central America. Genomic typing showed that 3 patients had recurrent infection months to years after apparent clinical cure. Two patients were infected with the same strain as previously isolated, and 1 was infected with a genetically distinct strain. This organism is multidrug resistant, and therapy required surgery and combination antimicrobial drugs, including long-term ceftriaxone. *G. bethesdensis* causes necrotizing lymphadenitis in CGD, which may recur or relapse.

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Chronic granulomatous disease (CGD) is a rare genetic disease caused by mutations in any of the 4 structural genes of the NADPH oxidase system and leads to defective production by phagocytes of superoxide and downstream oxygen metabolites (*1*). Infections in patients with CGD are caused by a narrow spectrum of pathogens, including *Staphylococcus aureus*, *Serratia marcescens*, *Burkholderia cepacia* complex, *Nocardia* spp., and *Aspergillus* spp. (*2–4*). Although lymphadenitis is commonly encountered, a pathogen is isolated in only ≈60% of cases (*5*).

Most human bacterial infections, even those that are severe, are transient and curable. Bacteria such as *Mycobacterium tuberculosis* are unique human pathogens in part because of their ability to persist in a dormant state and reactivate later. The recurrent infections observed in patients with CGD, even when caused by the same species of organism, are the result of reinfection rather than relapse (*3*,*6*). *Granulibacter bethesdensis* is a recently described gram-negative bacterium in the family *Acetobacteraceae*; it has been isolated from 6 patients with CGD from North and Central America and Spain (*7–10*). The initial case was in an adult who had prolonged fever, necrotizing lymphadenitis, and multiple disease recurrences culminating in cure 2 years after seeking treatment. Persons with subsequent cases in the Americas had shorter periods before diagnosis and more rapid responses to therapy. A fatal case reported in Spain involved a patient with CGD in whom *G. bethesdensis* was the only pathogen identified. Given the increasing cases of this emerging pathogen, we present in greater detail the clinical course of these patients and molecular epidemiologic evidence to support the recurrent infections we have diagnosed for some of these patients.

Five patients were followed up at the National Institutes of Health (NIH) Clinical Center (Bethesda, MD, USA) under protocol 93-I-0119. Patients 2, 3, and 5 had been actively followed up at NIH for at least 8 years before receiving a diagnosis of *G. bethesdensis* infection. Patient 1 had been sent to NIH for evaluation of his lymphadenopathy and *Granulibacter* infection was diagnosed shortly thereafter. Patient 4 was referred to NIH for treatment and follow-up after his *Granulibacter* infection was diagnosed at an outside hospital (by R.L.W. and D.F.W.).

The *G. bethesdensis* high-density microarray platform, DNA microarray hybridization, and comparative genomic hybridization analysis used for typing of the *G. bethesdensis* isolates have been described (*9*). Bacterial DNA was isolated by using the NucliSens Kit (bioMérieux, Durham, NC, USA), and 16S rRNA genes from the 5 patient isolates were sequenced and analyzed as described (*8*). DNA was isolated from human tissue by using the Maxwell 16 Tissue DNA Purification Kit (Promega, Madison, WI, USA) according to the manufacturer's protocol. DNA concentrations were measured by using a UV spectrophotometer (NanoDrop, Wilmington, DE, USA).

The 16S rRNA and methanol dehydrogenase subunit 1 (GeneID YP_744165.1) genes of *G. bethesdensis* were analyzed by using a PCR and primer sequences 16S-forward: 5′-TCGGGTGGGCACTCTAAAGG-3′, 16S-reverse: 5′-GCATCACTGCCTAGCTTCCC-3′, MDH-forward: 5′-CCGCAATACGGTCAATTCG-3′, and MDH-reverse: 5′-GCCGATCTTCCAGGTTTCTTC-3′. Each reaction mixture contained 47 μL of Platinum Blue PCR SuperMix (Invitrogen, Carlsbad, CA, USA) and 1.5 μL of each primer at a final concentration of 0.75 μmol/L, and the PCR was performed in a thermocycler (Eppendorf, Hauppauge, NY, USA). The PCR amplification conditions were 94°C for 10 min; 40 cycles at 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s; and a final extension at 72°C for 5 min. PCR fragments were visualized by electrophoresis on a 2% agarose gel and showed the expected sizes of 137 bp (16SrRNA) and 63 bp (methanol dehydrogenase subunit 1).

Case Reports

Patient 1

A 39-year-old man from the United States with X-linked CGD had persistent fever, chills, weight loss, and increased levels of inflammatory markers starting in April 2003, as described (*5*). Computed tomography (CT) scans showed necrotic mediastinal and cervical nodes (online Appendix Table, www.cdc.gov/eid/content/16/9/1341-appT.htm). Resection samples of cervical nodes grew *G. bethesdensis*. Therapy with meropenem and doxycycline resulted in resolution of the lesions. However, the patient had recurrences of necrotizing cervical and axillary lymphadenitis over the next 2 years, and *G. bethesdensis* was isolated on 3 separate occasions. Treatment with ceftriaxone and doxycycline for 1 year resolved his lymphadenitis. He has had no further recurrence but has had persistent chronic fatigue since onset of infection.

Patient 2

A 36-year-old man from the United States with X-linked CGD had cough and fever in November 2005. He had lymphadenopathy of the supraclavicular, paratracheal, subcarinal, perihilar, internal mammary, perigastric, retroperitoneal, iliac, and inguinal lymph nodes and multiple splenic lesions (Figure1, panel A; online Appendix Table). The erythrocyte sedimentation rate (ESR) was 50 mm/h. Empirical treatment with itraconazole and linezolid did not prevent increased abdominal distension and continued fever.

A CT scan in December 2005 showed enlarging left internal mammary lymphadenopathy, ascites, and enlarging splenic lesions (Figure 2, panel A). Leukocyte count

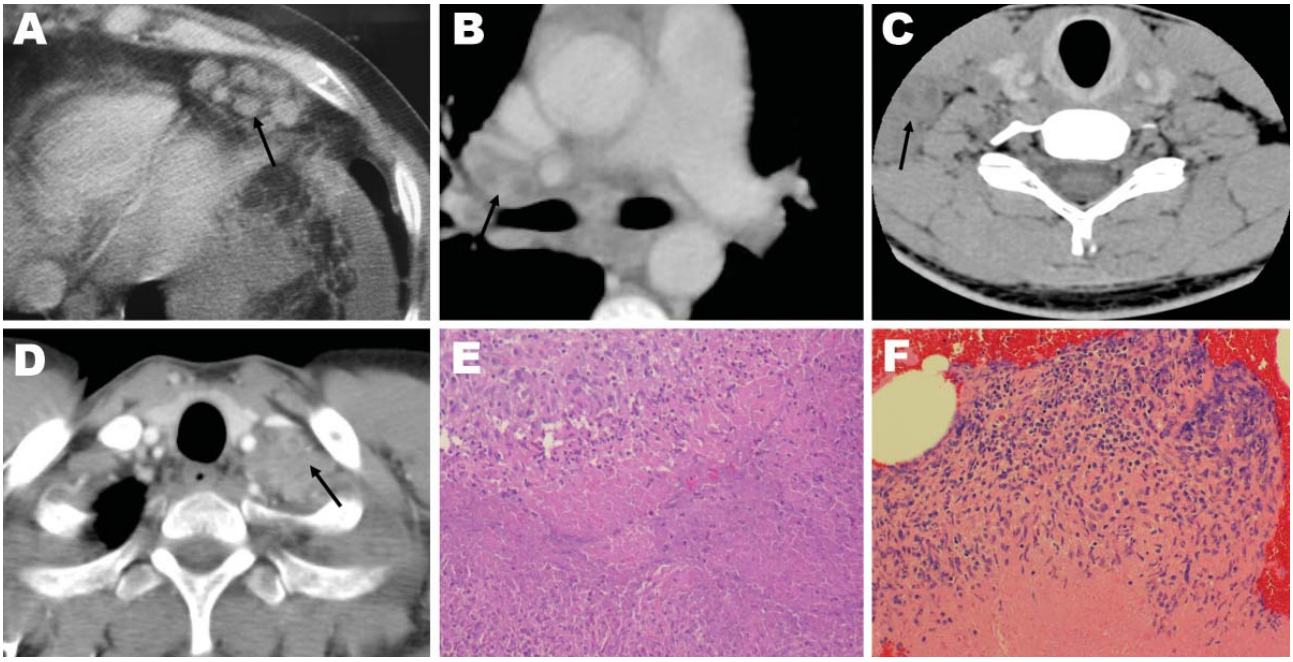

Figure 1. Radiologic and histologic findings for patients with *Granulibacter bethesdensis* infections. A) Contrast-enhanced computed tomography (CT) image for patient 2, showing enlarged epigastric nodes (arrow) in the abdomen. B) Contrast-enhanced CT image for patient 3, showing lymphadenopathy (arrow). The heterogeneity of the lymph node suggests necrosis. C) Noncontrast CT image of the spine for patient 4, showing enlarged cervical lymph nodes (arrow). D) Contrast-enhanced CT image for patient 5, showing a left supraclavicular mass. Arrow highlights an area suggestive of necrosis. E) Hematoxylin and eosin–stained section from patient 4, showing necrotizing granulomatous inflammation in the excised node (original magnification ×10). F) Hematoxylin and eosin–stained section from patient 2, showing necrotizing granulomas composed of epithelioid histiocytes, lymphocytes, and plasma cells from a perigastric node biopsy specimen (original magnification ×10).

was 13.5×10^3 cells/μL with 79% neutrophils. Ascitic fluid was cloudy with 3,100 leukocytes/μL (45% lymphocytes, 27% neutrophils) and 2,813 erythrocytes/μL. Gram stain showed no organisms. Treatment with meropenem and trimethoprim/sulfamethoxazole was begun. A CT-guided biopsy specimen of perigastric lymph nodes showed necrotizing granulomatous inflammation (Figure 1, panel F), but results of fungal and acid-fast staining were negative. Multiple blood cultures were negative. On hospital day 4, fever continued. Itraconazole was stopped, and voriconazole and tobramycin were started. Fever abated shortly thereafter and the patient recovered.

A gram-negative rod was isolated from ascitic fluid on Middlebrook 7H11 agar after 15 days of incubation; 16S rDNA sequencing confirmed that the isolate was *G. bethesdensis*. The patient was treated with ceftriaxone for 6 weeks and showed a positive response over the next year and resolution of his lesions (Figure 2, panel B). He continued to take trimethoprim/sulfamethoxazole and was treated with γ-interferon. In September 2007, sweats, chills, and splenic lesions developed. After CT-guided biopsy, he was treated with cefpodoxime and doxycycline; treatment with trimethoprim/sulfamethoxazole and γ-interferon continued.

One colony of a gram-negative rod was seen on Middlebrook 7H11 medium after 10 days of incubation; 16S rDNA sequencing confirmed that the isolate was *G. bethesdensis*. Despite antimicrobial drug therapy, the splenic lesions became more numerous and necessitated a splenectomy in April 2008 (Figure 2, panels C, D). A portion of splenic tissue was used for PCR detection of *Granulibacter*-specific 16S rRNA and methanol dehydrogenase genes (Figure 3). PCR products of the expected sizes were identified from splenic tissue. The culture from the splenectomy showed a positive result 6 days later on mycobacterial media. The patient was treated with tigecycline for 3 months and has had no further relapses.

Patient 3

A 13-year-old boy from the United States with X-linked CGD was admitted for persistent fever and diffuse thoracic lymphadenopathy in January 2006. He had been followed up for *Staphylococcus epidermidis* submandibular abscesses since November 2004. He had enlarged nodes, many of which appeared necrotic, in the superior mediastinum, pretracheal region, aortopulmonary window, right hilum and subcarinal areas (Figure 1, panel B; on-

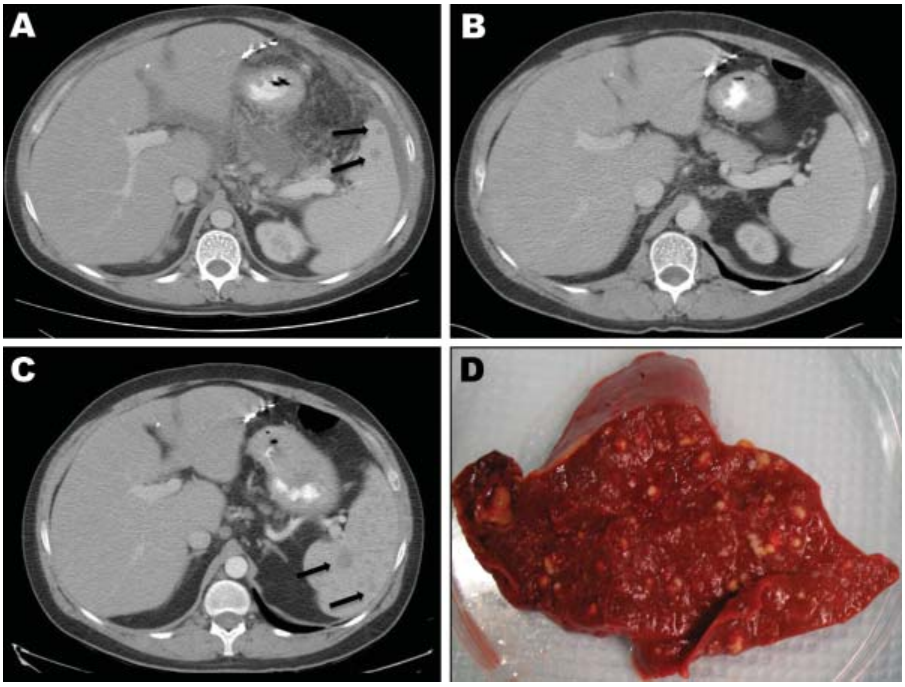

Figure 2. Radiologic and pathologic findings of *Granulibacter bethesdensis* infection in patient 2, a 36-year-old man from the United States with X-linked chronic granulomatous disease. A) Contrast-enhanced computed tomography (CT) image at initial examination (December 2005), showing multiple lucencies in the spleen (arrows) and edema and stranding in the omentum and mesentery. B) Contrast-enhanced CT image (September 2006), showing resolution of splenic lesions after prolonged antimicrobial drug therapy. C) Contrast-enhanced CT image (April 2008), showing multiple lucencies in the spleen consistent with abscesses. D) Gross view of the sectioned spleen after splenectomy (April 2008), showing numerous abscesses.

line Appendix Table). A transbronchial biopsy specimen of the subcarinal and right paratracheal nodes grew 1 colony each of *Candida glabrata*, *S. epidermidis*, *Streptococcus mitis* group, and a gram-negative rod. The gram-negative rod grew after 4 days of incubation on buffered-charcoal yeast extract (BCYE) agar. Routine biochemical tests did not identify the organism, but 16S rDNA sequencing confirmed that the isolate was *G. bethesdensis*.

The patient was initially treated with meropenem and voriconazole. When a repeat CT scan 2 weeks later showed enlarged mediastinal lymph nodes, meropenem was discontinued and ceftriaxone, tobramycin, and doxycycline were initiated. A CT scan 1 week later showed smaller lymph nodes. The patient stopped taking tobramycin after renal toxicity developed in March 2006, and he received ceftriaxone until May 2006. His lymphadenopathy resolved and he received doxycycline until March 2007. In December 2008, new fevers and subcarinal lymphadenopathy developed. A fine-needle aspiration specimen yielded a gram-negative rod on fungal media (IMA agar) after 15 days of incubation; 16S rDNA sequencing confirmed that the isolate was *G. bethesdensis*. He responded clinically and radiographically to ceftriaxone. He was then switched to cefdinir in January 2009 after gallstones developed and continued to receive this therapy until July 2009. The patient has had no further relapses.

Patient 4

A 17-year-old boy from Panama with X-linked CGD had cervical lymphadenopathy for 2 months in June 2005. He had been treated with trimethoprim/sulfamethoxazole until the age of 15 when a chest wall infection with an unidentified basidiomycete developed. Since then, he had responded well to itraconazole and trimethoprim/sulfamethoxazole prophylaxis until lymphadenopathy developed. Cervical and mediastinal lymphadenopathy biopsy specimens (Figure 1, panel C) showed necrotizing granulomatous inflammation. One week after surgery, painful hard, 1-cm, right anterior cervical and 2-cm, right supraclavicular lymph nodes developed. Treatment with levofloxacin was begun, and another lymph node was excised and cultured. Necrotizing granulomata were seen again (Figure 1, panel E).

Pinpoint colonies were seen on sheep blood agar after 6 days of incubation and were subcultured in BCYE medium. The gram-negative rod could not be identified biochemically; 16S rDNA sequencing confirmed that the isolate was *G. bethesdensis*. While receiving levofloxacin, adenopathy increased in the patient. Five weeks later, another cervical lymph node was removed. After 4 days, the culture grew *G. bethesdensis* as pinpoint colonies on sheep blood agar. The patient was treated with doxycycline and responded well clinically without further recurrences.

Patient 5

The 37-year-old brother of patient 2, who also had X-linked CGD, had fever, chills, and chest pain for 2 weeks in October 2008. New left lower lobe nodules and left infrahilar adenopathy prompted bronchoalveolar lavage, which showed macrophages and scattered neutrophils. Cultures were negative, but the patient was treated with levofloxa-

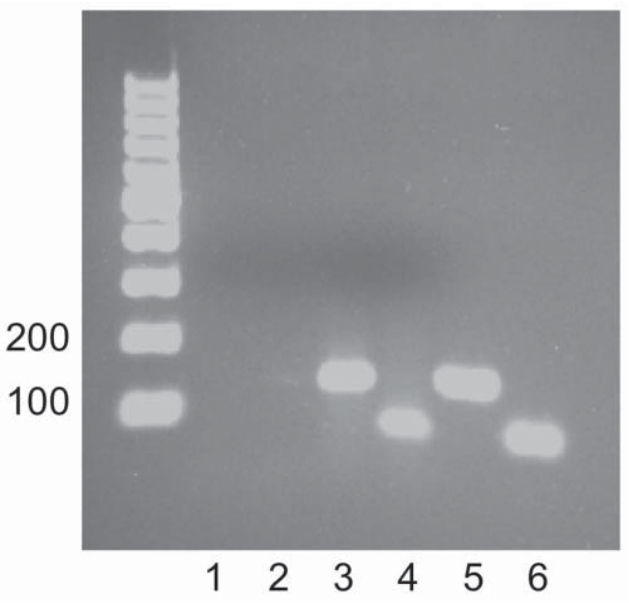

Figure 3. Detection of *Granulibacter* sp. DNA by PCR of the spleen of a patient with chronic granulomatous disease. Lanes 1 and 2, no template controls for each primer set; lane 3, 400 ng of spleen DNA amplifying *Granulibacter* sp. 16S rRNA gene; lane 4, 400 ng of spleen DNA amplifying the *Granulibacter* sp. methanol dehydrogenase gene; lane 5, 100 ng DNA from the *G. bethesdensis* type strain amplifying the 16S rRNA gene (positive control); lane 6, 100 ng DNA from the *G. bethesdensis* type strain amplifying the methanol dehydrogenase gene (positive control). Left lane, molecular mass ladder. Expected PCR product sizes were 137 bp for the 16S rRNA gene and 63 bp for the methanol dehydrogenase gene. Values on the left are in bp.

cin, linezolid and posaconazole and continued receiving trimethoprim/sulfamethoxazole. Fever and pulmonary nodules resolved but adenopathy persisted. Treatment with antimicrobial drugs was stopped in December 2008. Fever and chills returned in February 2009. His ESR was 78 mm/h. Magnetic resonance imaging of the chest showed multiple enlarged anterior mediastinal and bilateral hilar lymph nodes. Bronchoscopy with transbronchial needle aspiration yielded *S. epidermidis* and α-hemolytic streptococci in liquid media. The patient was then treated with voriconazole and ceftriaxone.

Despite this therapy, left supraclavicular lymphadenopathy and splenic and liver lesions developed in April 2009 (Figure 1, panel D; online Appendix Table). A lymph node biopsy specimen in May 2009 showed necrotizing granulomatous inflammation and grew *G. bethesdensis* after 7 days of incubation on BCYE and mycobacterial media. Ototoxicity developed while he was being treated with ceftriaxone, gentamicin, and vancomycin. He was then treated with ceftriaxone, doxycycline, and trimethoprim/sulfamethoxazole for 4 months and showed clinical and radiographic improvement. He was treated with cefdinir in September 2009 and has continued treatment with doxycycline and trimethoprim/sulfamethoxazole and has not had any new relapses. Patients 2 and 5 live apart but see each other a few times per year and had been together during the illness of patient 2.

All patients had fever, lymphadenopathy, and increased ESRs for weeks or months (online Appendix Table). The chronicity of the prodrome is distinct from the more typical clinical appearance of staphylococcal lymphadenitis in CGD, which is frequently cervical and of shorter duration before being observed. All biopsy tissues showed necrotizing granulomatous inflammation but staining did not detect any organisms. However, organisms are not usually abundant in persons with CGD lymphadenitis and stains are often not diagnostic. All samples grew *G. bethesdensis* within 3 weeks on a variety of media (Middlebrook 7H11, BCYE, and fungal media), but growth was sparse. PCR amplification identified specific *G. bethesdensis* bands in excised spleen, which indicated that molecular diagnosis can be made from fresh tissue, a useful consideration for an organism that is slow growing and difficult to identify (Figure 3).

Those patients who received ceftriaxone had good clinical responses. However, patients appeared to have responded to other agents, including meropenem, aminoglycosides, doxycycline, and trimethoprim/sulfamethoxazole in various combinations. Patients 1 and 2 had recurrences over prolonged periods, which indicated a capacity for *G. bethesdensis* to survive in a clinically latent state.

The 16S rDNA sequences for all patients showed 100% matches to the 16S rDNA sequence derived from the isolate from patient 1, NIHCGD1, the type strain of *G. bethesdensis* (GenBank accession no. AY788950). This finding indicated that these isolates are all appropriately designated *G. bethesdensis* sensu stricto. The isolate reported by Lopez et al. (*10*) from Spain showed only a 99.7% match for the 16S rDNA sequence, which suggested regional variability or another *Granulibacter* sp.

We had shown that all isolates from patient 1 over a 2-year period were identical (*9*). DNA samples from 3 isolates from patient 2 obtained over a 3-year period were hybridized to the previously reported *Granulibacter* microarray chip (*9*). Hybridization patterns of the 3 isolates were essentially identical, which indicated that the same organism was responsible for disease in patient 2 from his initial hospitalization through his splenectomy (Figure 4). This comprehensive analysis of genomic stability over several years indicates that *G. bethesdensis* can potentially achieve clinical latency over prolonged periods in the human host, even without causing signs or symptoms.

In contrast to DNA from patient 2, DNA from the second isolate from patient 3 was compared with that from his original isolate by using the *Granulibacter* microarray

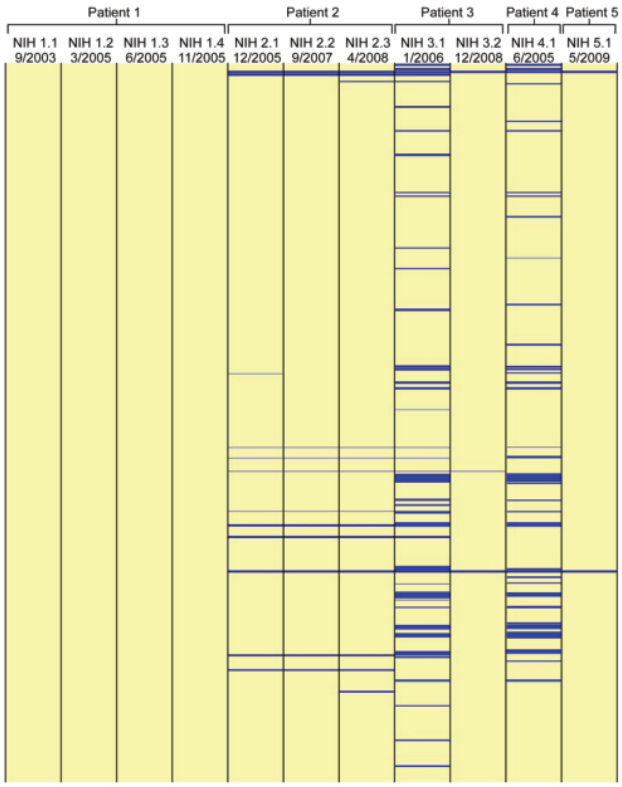

Figure 4. Physical gene representation of DNA hybridization of 11 isolates of *Granulibacter bethesdensis* by DNA–DNA hybridization microarray-based investigation of gene distributions among *G. bethesdensis* isolates. Every open reading frame in the *G. bethesdensis* type strain is represented. Hybridization is shown in yellow, and absence of hybridization is shown in blue. Four sequential isolates (NIH 1.1, NIH 1.2, NIH 1.3, and NIH 1.4) from patient 1 are shown from left to right and group with an identical hybridization pattern. Three sequential isolates (NIH 2.1, NIH 2.2, and NIH 2.3) from patient 2 are shown from left to right and demonstrate virtually identical patterns of hybridization. Two isolates (NIH 3.1 and NIH 3.2) from patient 3 have different hybridization patterns. One isolate (NIH 4.1) from patient 4 has a unique hybridization pattern. One isolate (NIH 5.1) from patient 5 is distinct from that of his brother (patient 2). Dates that each infected tissue sample were obtained are listed. NIH, National Institutes of Health.

chip. The hybridization pattern of his second isolate was distinct from his first isolate at multiple loci of the genome, which indicated reinfection with a different strain of *G. bethesdensis* (Figure 4). The *G. bethesdensis* isolate from patient 5 was distinct from that of his brother (patient 2) at multiple loci, as determined by using the *Granulibacter* microarray chip (Figure 4).

Conclusions

Five patients with CGD from different parts of North and Central America had strikingly similar syndromes of prolonged fever and necrotizing lymphadenitis, all of which were caused by *G. bethesdensis*. Unlike most bacteria, *G. bethesdensis* can cause acute infections, relapses after apparently effective therapy, and reinfections, which indicate a lack of sterilizing and protective immunity in persons with CGD. These features are uncommon but serious. Tuberculosis, for example, causes acute disease, relapsing disease after prolonged latency and reinfection after definitive treatment. Sterilizing immunity has been difficult to prove (*11*,*12*). In contrast to most other CGD pathogens, most disseminated *G. bethesdensis* infections were chronic but not fatal. Although none of the 5 patients reported here died from their *G. bethesdensis* infections, a child from Spain died from infection with a *G. bethesdensis* isolate that was slightly different in its 16S rDNA sequence from the reference strain (*10*). Similar to our experience with humans, infection with *G. bethesdensis* in mice with CGD leads to a chronic persistent infection with a paucity of symptoms (*7*).

The *Granulibacter* microarray has enabled us to definitively identify genetic variability in *G. bethesdensis* through comparative genomic hybridization. Although all patient isolates were phenotypically *Granulibacter* spp. and 100% identical on the basis of full-length 16S rDNA sequencing, most of the hybridization patterns of patient isolates were different and unique according to comparative genomic hybridization, which indicated that these isolates are potentially distinct strains (*9*). This capacity proved critical for identifying persistence (the 4 isolates from patient 1 and the 3 isolates of patient 2) and reinfection (patient 3). Therefore, for patients 1 and 2, *Granulibacter* spp. most likely resided within the host in clinically latent reservoirs.

The ability to recur months to years after apparent clinical improvement is unlike other bacterial pathogens in persons with CGD. Use of comparative genomic hybridization to type our strains was critical in differentiating relapse from reinfection and in reinforcing our approach to treat these persons with these infections with long courses of antimicrobial drugs. Patients 1 and 2 may have had recurring environmental exposure to a source of nearly genetically identical *G. bethesdensis*, but this suggestion cannot be proven because we have not identified the environmental source for any of the isolates obtained. Patient 3 may have had a mixed infection at his initial hospitalization, followed by clearance of 1 isolate of *Granulibacter* spp. and persistence of the other. The microarray comparative genomic hybridization data demonstrate that for patients 2 and 5, brothers with splenic disease, each had different isolates.

Isolation of *G. bethesdensis* is time-consuming, growth is consistently sparse, and initial definitive identification requires sequencing the 16S rRNA gene. In the clinical laboratory, *G. bethesdensis* was most frequently isolated on special media and not on routine media normally used for cultivating gram-negative bacteria. Therefore, develop-

ment of a molecular test for *G. bethesdensis* is imperative and will improve the speed and sensitivity of diagnosis. PCR of fresh tissue with 2 highly specific gene probes accurately detected *Granulibacter* spp. DNA in the spleen of patient 2. Development of PCR technology for fixed specimens is ongoing.

Members of the family *Acetobacteraceae* have been isolated from various tropical fruits, fermented foods, flowers, and soil (*13*). Although *G. bethesdensis* has thus far been isolated only from patients with CGD, other members of the family *Acetobacteraceae* appear to be emerging as pathogens in non-CGD hosts. Within the past 5 years, infections with other *Acetobacteraceae* spp. in 6 patients have been described. *Acetobacter cibinongensis* was found in the blood of a patient with end-stage renal disease and fever (*14*). *Acetobacter indonesiensis* was found in the sputum of a lung transplant patient (*15*). *Asaia bogorensis* has been reported as a cause of bacteremia in 2 intravenous drug users (*16*,*17*) and as a cause of peritonitis in a patient receiving peritoneal dialysis (*18*). *Asaia lannaensis* was described as a cause of bacteremia in a bone marrow transplant patient (*19*). It is unclear whether the increasing recognition of these opportunistic pathogens has been caused by improved microbiologic detection or by a yet unrecognized shift in our interactions with the microbial world. Why *G. bethesdensis* appears to cause clinical disease in the CGD host specifically is the subject of ongoing laboratory studies.

G. bethesdensis is resistant to antimicrobial drugs in vitro and apparently in vivo. Susceptibility testing is difficult because of the inherent slow growth of this pathogen and the lack of established susceptibility criteria. This organism shows extensive resistance to most cephalosporins, penicillins (including carbapenems), and quinolones. However, ceftriaxone (MIC 8 μg/mL), aminoglycosides (e.g., gentamicin, MIC 8 μg/mL), doxycycline, and trimethoprim/sulfamethoxazole show activity in vitro. Our patients were treated with various combinations of these drugs; all showed initial resolution of disease. Ceftriaxone is currently our preferred treatment.

The reasons underlying the emergence of new pathogens are complex. The changing environments in which we and our microbial companions live make predicting when, where, and why a new pathogen will emerge difficult. Typically, we are best at recognizing clinical syndromes and working back toward the etiology. Given the difficulty of growing and identifying *Granulibacter* spp., other cases of this infection in patients with CGD and those without CGD may have been overlooked. To address this issue, we now concentrate specimens, particularly from lymph nodes of patients with CGD, by centrifugation, place the specimens on BCYE and Middlebrook 7H11 agar, and incubate cultures for a minimum of 2 weeks.

Clinical management of CGD has long been confounded by the relatively high rate of failure of isolating a pathogen from involved sites. This failure has been attributed to many features of CGD, including exuberant inflammation. In some cases, clinicians attribute culture-negative lesions to the inflammatory diathesis of CGD. However, the finding of a difficult-to-grow and difficult-to-identify pathogen makes this assumption unlikely, particularly in the setting of necrotizing lymphadenopathy. Identification of a fastidious, slow-growing bacterium that causes a discrete, chronic infection reminds us that new syndromes remain to be identified, even within well-described and well-characterized diseases.

NIH has filed an international patent application with regard to *G. bethesdensis*. Co-inventors include D.E.G., A.M.Z., P.R.M., and S.M.H.

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Worldwide Diversity of *Klebsiella pneumoniae* That Produces β-Lactamase bla_{KPC-2} Gene[1]

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Klebsiella pneumoniae isolates that produce carbapenemases (KPCs) are rapidly disseminating worldwide. To determine their genetic background, we investigated 16 bla_{KPC-2}-harboring *K. pneumoniae* isolates from 5 countries. The isolates were multidrug resistant, possessed the bla_{KPC-2} gene, and differed by additional β-lactamase content. They harbored a naturally chromosome-encoded *bla* gene (bla_{SHV-1} [12.5%], bla_{SHV-11} [68.7%], or $bla_{OKP-A/B}$ [18.8%]) and several acquired and plasmid-encoded genes (bla_{TEM-1} [81.3%], $bla_{CTX-M-2}$ [31.3%], $bla_{CTX-M-12}$ [12.5%], $bla_{CTX-M-15}$ [18.7%], and bla_{OXA-9} [37.5%]). The bla_{KPC-2} gene was always associated with 1 of the Tn*4401* isoforms (a, b, or c). Tn*4401* was inserted on different-sized plasmids that belonged to different incompatibility groups. Several bla_{KPC}-containing *K. pneumoniae* clones were found: 9 different pulsotypes with 1 major (sequence type 258) and 7 minor distinct allelic profiles. Different clones harboring different plasmids but having identical genetic structure, Tn*4401*, could be at the origin of the worldwide spread of this emerging resistance gene.

Resistance of *Klebsiella pneumoniae* to carbapenems is mainly associated with acquired carbapenem-hydrolyzing β-lactamases (*1*). These β-lactamases can be metallo β-lactamases (IMP, VIM), expanded-spectrum oxacillinases (OXA-48), or Ambler class A enzymes (NMCA, IMI, SME, GES, and KPC) (*1*–*4*). The most common class A carbapenemases in *K. pneumoniae* are the *K. pneumoniae* carbapenemases (KPCs) (*4*). KPCs in carbapenem-resistant *K. pneumoniae* strains were first reported in 2001 in North Carolina (*5*), and until 2005, the geographic distribution of these enzymes in *Enterobacteriaceae*, including *K. pneumoniae*, was limited to the eastern part of the United States (*5*,*6*). Now, KPC-producing *K. pneumoniae* isolates are frequently identified among nosocomial pathogens (*7*). Recently, dissemination of a single major clone of KPC-producing *K. pneumoniae* (sequence type [ST] 258) in the eastern United States has been suggested (*8*). KPCs have been observed more rarely among other gram-negative rods such as *Pseudomonas* spp. (*9*,*10*).

Outside the United States, KPC-producing *K. pneumoniae* are also being reported more often. The first case of KPC-producing *K. pneumoniae* infection was reported in 2005 in France and had a US origin (*11*). The first outbreak of KPC-producing *K. pneumoniae* outside the United States was in Israel (*12*). In South America, dissemination of KPC-producing *K. pneumoniae* was initially reported in 2006 in Colombia (*13*) and then in Brazil and Argentina (*14*,*15*). KPC enzymes have also been identified in *P. aeruginosa* isolates from Colombia (*16*). In the People's Republic of China, KPC enzymes in several enterobacterial species are being increasingly reported (*17*). Finally, in Europe a few cases of KPC-producing *K. pneumoniae* infection have been described, but in Greece, outbreaks have occurred (*18*). In Europe, different variants of KPCs (KPC-2 and KPC-3) have been described; some patients

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carrying KPC-positive isolates had been transferred from the United States, Israel, or Greece (*19*–*21*).

Reports of this β-lactamase being found in novel locations are increasing worldwide, probably signaling active spread. The genetic element carrying the bla_{KPC-2} gene, Tn*4401*, was recently elucidated (*22*). Three isoforms of this Tn*3*-like transposon (a, b, and c) are known. Several other genetic environments of bla_{KPC} gene have been described; other insertion sequences have been found upstream of the bla_{KPC} gene (*23*,*24*). Nevertheless, the downstream sequences of the bla_{KPC} gene matched perfectly with Tn*4401*, which suggests that these insertion sequences have been inserted into Tn*4401*.

Insertion sequences may play major roles in the evolution of Tn*4401*, but little information is available about the bacterial strains and the plasmids that may explain this rapid spread. Our goal, therefore, was to characterize the genetic background of several bla_{KPC-2}- harboring *K. pneumoniae* isolates from various geographic origins.

Materials and Methods

Bacterial Strains

K. pneumoniae isolates used in this study and their origin are listed in Table 1 (*11*,*13*,*16*,*21*,*25*). Electrocompetent *Escherichia coli* DH10B (Invitrogen, Eragny, France) was used as a recipient in electroporation experiments. *E. coli* J53Az^R, which is resistant to sodium azide, was used for conjugation experiments. *E. coli* 50192 was used as a reference strain for plasmid extraction (*22*).

Antibiograms and MIC Determinations

Antibiograms were created by using the disk-diffusion method on Mueller-Hinton agar (Bio-Rad Laboratories, Marnes-La-Coquette, France), and susceptibility break points were determined as previously described and interpreted as recommended by the Clinical and Laboratory Standards Institute (*22*,*26*). All plates were incubated at 37°C for 18 h. MICs of β-lactams were determined by using the Etest technique (bioMérieux, Marcy l'Etoile, France).

Electroporation and Plasmid Extraction

Direct transfer of resistance into azide-resistant *E. coli* J53 was attempted as reported (*22*). Plasmids were introduced by electroporation into *E. coli* DH10B (*22*) by using a Gene Pulser II (Bio-Rad Laboratories).

Plasmid DNA was extracted by using a QIAGEN Plasmid Maxi Kit (QIAGEN, Courtaboeuf, France) and analyzed by agarose gel electrophoresis (Invitrogen, Paris, France). Natural plasmids were extracted by using the Kieser extraction method (*27*) and subsequently analyzed by electrophoresis on a 0.7% agarose gel.

Hybridization

DNA–DNA hybridization was performed as described by Sambrook et al. (*28*) with Southern transfer of an agarose gel containing Kieser method–extracted total DNA. The probe consisted of a 796-bp PCR-generated fragment from recombinant plasmid pRYC-1 (*22*) and was internal to the bla_{KPC-2} gene. Labeling of the probe and detection of signal were conducted by using an ECL nonradioactive labeling and detection kit according to the

Table 1. Geographic origin and structure of Tn*4401* and other β-lactamases of *Klebsiella pneumoniae* isolates*

Isolate no.	Isolate type	Origin	PCR result								
				Tn*4401*				Other β-lactamases			
			KPC-2	TnpA	ISKPN7	ISKPN6	Deletion, bp	SHV	TEM	CTX-M	OXA
1	YC	USA	+	+	+	+	–100	SHV-11	TEM-1	–	OXA-9
2	GR	Greece	+	+	+	+	–100	SHV-11	TEM-1	–	OXA-9
3	K271	Sweden	+	+	+	+	–100	SHV-11	TEM-1	–	OXA-9
4	KN2303	Colombia	+	+	+	+	None	SHV-11	–	–	–
5	KN633	Colombia	+	+	+	+	None	OKP-A	TEM-1	CTX-M-12	–
6	INC H1521-6	Colombia	+	+	+	+	None	SHV-1	TEM-1	CTX-M-15	–
7	INC H1516-6	Colombia	+	+	+	+	None	SHV-1	TEM-1	CTX-M-15	–
8	HPTU 27635	Colombia	+	+	+	+	None	OKP-B	–	–	–
9	HPTU 2020532	Colombia	+	+	+	+	None	OKP-A	TEM-1	CTX-M-12	–
10	A28006	Brazil	+	+	+	+	None	SHV-11	TEM-1	CTX-M-2	–
11	A28008	Brazil	+	+	+	+	None	SHV-11	TEM-1	CTX-M-2	–
12	A28009	Brazil	+	+	+	+	None	SHV-11	TEM-1	CTX-M-2	–
13	A28011	Brazil	+	+	+	+	None	SHV-11	TEM-1	CTX-M-2	OXA-9
14	A33504	Brazil	+	+	+	+	None	SHV-11	TEM-1	CTX-M-2	OXA-9
15	475	Israel	+	+	+	+	–200	SHV-11	–	CTX-M-15	–
16	588	Israel	+	+	+	+	–200	SHV-11	TEM-1	–	OXA-9

*KPC, *K. pneumoniae* carbapenemase.

manufacturer's instructions (Amersham Biosciences, Orsay, France).

PCR Amplification and Sequencing

The bla_{CTX-M}-, bla_{SHV}-, bla_{TEM}-, and $bla_{OXA-1/9}$-like genes were searched for and characterized as described (*21*). PCR experiments were performed on an ABI 2700 thermocycler (Applied Biosystems, Les Ulis, France) by using laboratory-designed primers (Table 2). PCR products were then analyzed on agarose gel and sequenced.

Both strands of the PCR products were sequenced by using laboratory-designed primers with an automated sequencer (ABI PRISM 3100; Applied Biosystems). The nucleotide and the deduced protein sequences were analyzed by using software from the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov).

Isoelectrofocusing

Crude β-lactamase extracts, obtained as described (*21*) from 10-mL cultures of clinical isolates and their *E. coli* transconjugants or electroporants were subjected to analytical isoelectrofocusing on an ampholine-containing polyacrylamide gel, pH 3.5–9.5 (Ampholine PAG plate; GE Healthcare, Orsay, France) for 90 min at 1,500 volts, 50 milliamps, and 30 watts. The focused β-lactamases were detected by overlaying the gel with 1 mmol nitrocefin (Oxoid, Dardilly, France). Isoelectric points were determined and compared with those of known β-lactamases (*22*).

Table 2. Primers used for PCR of *Klebsiella pneumoniae* isolates producing β-lactamase bla_{KPC-2} gene*

Primer name	Primer no.†	Sequence, 5′ → 3′
KpcA	1	CTGTCTTGTCTCTCATGGCC
KpcB	2	CCTCGCTGTGCTTGTCATCC
4281	3	GGCACGGCAAATGACTA
4714	4	GAAGATGCCAAGGTCAATGC
EcoRIout	5	CACCCGACCTGGACGAACTA
3′YCEnd	6	GCATCAAACGGAAGCAAAAG
3781L	7	CACAGCGGCAGCAAGAAAGC
3098U	8	TGACCCTGAGCGGCGAAAGC
905L	9	GCGACCGGTCAGTTCCTTCT
816U	10	CACCTACACCACGACGAACC
141R-6	11	TCACCGGCCCTCACCTTTGG
5′endYC	12	CTTAGCAAATGTGGTGAACG
Pre-SHV-5 U	–	GGTCAGCGCGAGAAGCATCC
Pre-SHV-5 L	–	AAATAGCGTTCATCGTCAAT
Pre-TEM 1	–	GTATCCGCTCATGAGACAATA
Pre-TEM 2	–	TCTAAAGTATATATGAGTAAA CTTGGTCTG
OXA-9 A	–	TTCGTTTCCGCCACTCTCCC
OXA-9 B	–	ACGAGAATATCCTCTCGTGC
CTX-M A	–	CGCTTTGCGATGTGCAG
CTX-M B	–	ACC GCG ATA TCG TTG GT

*Primers from (*21*).
†Numbers correspond to those in Figure 1, panel A. –, primer not shown in Figure 1, panel A.

Pulsed-field Gel Electrophoresis

Pulsed-field gel electrophoresis (PFGE) was performed by using *Xba*I (GE Healthcare) as described (*29*). *Xba*I-macrorestriction patterns were interpreted according to the recommendations of Tenover et al. (*30*).

Multilocus Sequence Typing

Multilocus sequence typing (MLST) with 7 housekeeping genes (*rpoB*, *gapA*, *mdh*, *pgi*, *phoE*, *infB*, *and tonB*) was performed according to Diancourt et al. (*31*). Allele sequences and STs were verified at http://pubmlst.org/kpneumoniae. A different allele number was given to each distinct sequence within a locus, and a distinct ST number was attributed to each distinct combination of alleles.

Replicon and Transposon Typing

PCR-based replicon typing of the main plasmid incompatibility groups reported for *Enterobacteriaceae* was performed as described (*32*). Genetic structures surrounding the bla_{KPC-2} gene were determined according to the Tn*4401* PCR-mapping scheme as described (*22*).

Results

Pulsotypes

Molecular typing by PFGE identified 9 major pulsotypes among the isolates (Table 3). The first pulsotype (pulsotype A) corresponded to the strains from the United States and Greece. We found 4 different pulsotypes (B–E) among strains from Colombia, which suggested polyclonal diffusion inside this country. We also identified 2 different clones among strains from Brazil (pulsotypes F and G) and from Israel (pulsotypes H and I). These results indicate much heterogeneity among KPC-producing isolates from various geographic regions.

MLST of the 16 isolates resulted in 8 distinct allelic profiles: ST 258 (allelic profile 3–3-1–1-1–1-79) corresponding to isolates *K. pneumoniae* YC (United States), *K. pneumoniae* GR (Greece), and *K. pneumoniae* K271 (Greece); ST 14 (allelic profile 1–6-1–1-1–1-1) corresponding to isolates *K. pneumoniae* INC H1521–6 and *K. pneumoniae* INC H1516–6 (Colombia); ST 11 (allelic profile 3–3-1–1-1–1-4) corresponding to isolates from Brazil; ST 277 (allelic profile 3–1-1–1-1–1-43) corresponding to isolate *K. pneumoniae* 475 (Israel); novel ST 337 (allelic profile 2–1-11–1-1–1-13) corresponding to isolate *K. pneumoniae* KN2303 (Colombia); ST 338 (allelic profile 17–19–22–39–34–21–52) corresponding to isolates *K. pneumoniae* KN633 and *K. pneumoniae* HPTU 2020532 (Colombia); ST 339 (allelic profile 18–15–25–24–11–13–51) corresponding to isolate *K. pneumoniae* HPTU 27635 (Colombia); and ST 340 (allelic profile 3–3-1–1-1–1-18) corresponding to isolate *K. pneumoniae* 588 (Israel). The

Table 3. Plasmid analysis, pulsotype, and sequence type of *Klebsiella pneumoniae* isolates from 5 countries*

Isolate no.	Isolate name	Plasmids		PFGE type	MLST							
		Size, kb	Inc		gap	infB	mdh	pgi	phoE	rpo	tonB	ST
1	YC	80	FiiAS	A	3	3	1	1	1	1	79	258
2	GR	80	FiiAS	A	3	3	1	1	1	1	79	258
3	K271	80	FiiAS	A	3	3	1	1	1	1	79	258
4	KN2303	75, 35	N	B	2	1	11	1	1	1	13	337
5	KN633	12	ND	C	17	19	22†	39	34†	21	52	338
6	INC H1521-6	75	L/M	D	1	6	1	1	1	1	1	14
7	INC H1516-6	75	L/M	D	1	6	1	1	1	1	1	14
8	HPTU 27635	35	L/M	E	18	15	25†	24	11†	13	51	339
9	HPTU 2020532	12	ND	C	17	19	22†	39	34†	21	52	338
10	A28006	12	L/M	F	3	3	1	1	1	1	4	11
11	A28008	12	L/M	F	3	3	1	1	1	1	4	11
12	A28009	12	L/M	F	3	3	1	1	1	1	4	11
13	A28011	12	L/M	F	3	3	1	1	1	1	4	11
14	A33504	50	ND	G	3	3	1	1	1	1	4	11
15	475	80	N	H	3	1	1	1	1	1	43	277
16	588	70	N	I	3	3	1	1	1	1	18	340

*Countries shown in Table 1. PFGE, pulsed-field gel electrophoresis; MLST, multilocus sequence type; ST, sequence type; ND, could not be determined with the Inc primers tested.
†Alleles are variants of previously described alleles (Figure 2).

analysis of STs by eBURST (http://pubmlst.org) showed that ST 11 and ST 340 are single-locus variants of ST 258 and that ST 277 is a double-locus variant of ST 258. These results matched perfectly with PFGE results. One isolate from Brazil (*K. pneumoniae* A33504) showed a different pattern by PFGE but the same ST (ST 11) as other isolates from the same origin, which suggests a strong genetic relatedness.

Antimicrobial Drug Susceptibility

All isolates were resistant to penicillins and cephalosporins but showed varying levels of susceptibility to carbapenems (Table 4). Resistance to other drug classes varied among the isolates. For aminoglycosides, 2 clones (A and I) were susceptible to gentamicin only, 1 clone (H) was susceptible to amikacin only, and 3 clones (C, D, and G) were resistant to all tested aminoglycosides. Six clones (A, C, D, F, G, and I) showed resistance to fluoroquinolones. Percentages of nonsusceptible isolates to the non–β-lactam drugs were as follows: gentamicin, 75%; amikacin, 81.3%; ciprofloxacin, 81.5%; trimethoprim/sulfamethoxazole, 81.5%; and tetracycline, 87.5%. Two isolates were also resistant to colistin (*K. pneumoniae* GR and *K. pneumoniae* K271); each was from Greece, where this drug is often used (*33*).

β-Lactamase Genes

Positive results of CTX-M–, TEM-, SHV-, and OXA-specific PCRs are indicated in Table 1. All isolates possessed the bla_{KPC-2} gene and a naturally chromosome-encoded *bla* gene: bla_{SHV-1} (12.5%), bla_{SHV-11} (68.7%), or $bla_{OKP-A/B}$ (18.8%). The bla_{OKP} genes are 1 of the 3 families of the chromosomal β-lactamase genes found in *K. pneumoniae* (*34*) with bla_{SHV} and bla_{LEN} and share 88% similarity with bla_{SHV-1}. *K. pneumoniae* isolates also harbored several acquired and plasmid-encoded genes: bla_{TEM-1} (81.3%), $bla_{CTX-M-2}$ (31.3%), $bla_{CTX-M-12}$ (12.5%), $bla_{CTX-M-15}$ (18.7%), and bla_{OXA-9} (37.5%).

Characterization Results for Tn*4401*

Primer couples specific for the different genes found on Tn*4401* (Table 2; Figure 1, panel A) obtained similar-sized fragments for all strains, which suggests that the strains have a similar genetic organization. For only 1 primer pair, hybridizing in IS*Kpn7* and bla_{KPC} gene (primers 7 and 8 in Figure 1, panel A), located upstream of the bla_{KPC} gene,

Table 4. MICs of carbapenems for clinical *Klebsiella pneumoniae* isolates expressing KPC-2 β-lactamase*

Isolate type	Carbapenem MIC, mg/L		
	Imipenem	Meropenem	Ertapenem
YC	4	2	24
GR	12	6	12
K271	4	4	16
KN2303	>32	>32	>32
KN633	>32	4	>32
INC H1521-6	6	3	8
INC H1516-6	4	4	32
HPTU 27635	4	2	12
HPTU 2020532	16	16	24
A28006	16	32	24
A28008	24	16	32
A28009	>32	>32	>32
A28011	>32	>32	>32
A33504	>32	>32	>32
475	16	>32	>32
588	24	16	32

*KPC, *K. pneumoniae* carbapenemase.

an ≈100-bp (*K. pneumoniae* YC, *K. pneumoniae* GR, and *K. pneumoniae* 271) or 200-bp (*K. pneumoniae* 475 and *K. pneumoniae* 588) shorter fragment was observed, compared with the Tn*4401*b structure, thus indicating that the 3 isoforms of Tn*4401* were present in this collection of isolates (Figure 1, panel B).

To investigate the flanking sequences of Tn*4401*, we used PCR primers located in the Tn*4401* structure and in the flanking sequences derived from *K. pneumoniae* YC (*22*). PCR products of expected size were obtained for *K. pneumoniae* GR and *K. pneumoniae* K271 isolates only. For all other strains, no PCR product could be obtained, suggesting that the Tn*4401* insertion site might differ from that found in *K. pneumoniae* YC.

Genetic Support for bla_{KPC} in the Isolates

The carbapenem-resistant *K. pneumoniae* isolates contained several plasmids of different sizes, ranging from <5 kb to >170 kb (Figure 2, left panel). At least 1 plasmid hybridized with an internal probe for bla_{KPC-2} gene in each isolate, ranging from 13 kb to 80 kb (Figure 2, right panel; Table 3). We observed 2 hybridization signals (35 kb and 75 kb) for *K. pneumoniae* KN2303, as described (*22*). Plasmid location of the bla_{KPC} genes was confirmed by electroporation of these plasmids into *E. coli* DH10B, but no transformant could be obtained for *K. pneumoniae* 2020532. The *E. coli* transformants had a β-lactam resistance pattern that corresponded to the expression of a bla_{KPC}-like gene. Electroporation of 4 plasmids harboring the bla_{KPC}-like gene into *E. coli* DH10B conferred resistance to at least an aminoglycoside molecule; pINC-H1521–6, pA33504, and p588 conferred resistance to all aminoglycosides except gentamicin, and electroporation of p475 into *E. coli* DH10B led to resistance to all aminoglycosides tested. No other antimicrobial drug resistance marker was cotransferred; the transformants remained susceptible to nalidixic acid, levofloxacin, ciprofloxacin, rifampin, tetracycline, trimethoprim/sulfamethoxazole, and colistin.

Mating-out assays showed that the ≈75–80-kb plasmids harboring bla_{KPC-2} from *K. pneumoniae* YC, GR, K271, and KN2303 were self-transferable to *E. coli*. The smaller plasmid from *K. pneumoniae* KN633 was not transferred to *E. coli*.

Origin of Replication

PCR-based replicon typing of the major plasmid incompatibility groups showed that the bla_{KPC-2}-positive plasmids belonged to at least 3 incompatibility groups (IncFIIAS, IncN, and IncL/M) (Table 3). The plasmids of *K. pneumoniae* KN633, HPTU-2020532 from Colombia and *K. pneumoniae* A33504 from Brazil gave negative results with the Inc primers tested and could not be classified into a major plasmid incompatibility group.

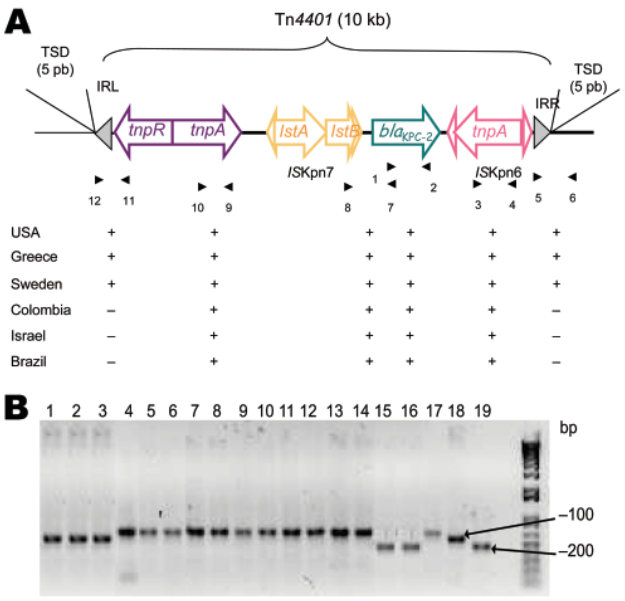

Figure 1. A) Schematic representation of Tn*4401* isoforms on plamids of *Klebsiella pneumoniae* isolates that produce *K. pneumoniae* carbapenemases (KPCs). Genes and their corresponding transcription orientations are indicated by horizontal arrows. Gray triangles represent the inverted repeats left (IRL) and right (IRR) of Tn*4401*. Small and empty triangles represent the inverted repeats of IS*Kpn6* and IS*Kpn7*. Target site duplications (TSD) are indicated above the sequence. Primers listed in Table 2 are shown below, with results of PCRs for each isolate. B) PCR results with primers 7 and 8 (Table 2). Lane 1, *K. pneumoniae* YC (*11*); lane 2, *K. pneumoniae* GR (*21*); lane 3, *K. pneumoniae* K271 (*25*); lane 4, *K. pneumoniae* KN2303 (*13*); lane 5, *K. pneumoniae* KN633 (*13*); lane 6, *K. pneumoniae* INC H1521-6; lane 7, *K. pneumoniae* INC H1516-6; lane 8, *K. pneumoniae* HPTU 27635; lane 9, *K. pneumoniae* HPTU 2020532; lane 10, *K. pneumoniae* A28006 (*16*); lane 11, *K. pneumoniae* A28008 (*16*); lane 12, *K. pneumoniae* A28009 (*16*); lane 13, *K. pneumoniae* A28011 (*16*); lane 14, *K. pneumoniae* A33504 (*16*); lane 15, *K. pneumoniae* 475; lane 16, *K. pneumoniae* 588; lane 17, PCR products of expected size with no deletion; lane 18, PCR products of expected size with 100-bp deletion; lane 19, PCR products of expected size with 200-bp deletion.

Discussion

Rapid spread of KPC-producing *K. pneumoniae* is a major clinical and public health concern. These broad-spectrum β-lactamases are increasing in new locations worldwide, indicating an ongoing process. Recently, a novel Tn*3*-based transposon, Tn*4401*, was identified in nonclonally related KPC-producing *K. pneumoniae* and *P. aeruginosa* isolates (*22*). This transposon is in most recently described isolates (*20*,*35*,*36*), although a recently characterized novel variant from China had another insertion sequence inserted upstream of bla_{KPC} gene (*24*). Identification of Tn*4401* inserted at different loci, on different plasmids, and flanked by different 5-bp target site duplications indicates a frequent and dynamic process of transposition.

It has been suggested that this novel transposon is at the origin of bla_{KPC}-like gene acquisition and dissemination (*22*). Sixteen *K. pneumoniae* isolates that express the bla_{KPC} gene from 5 countries were characterized here.

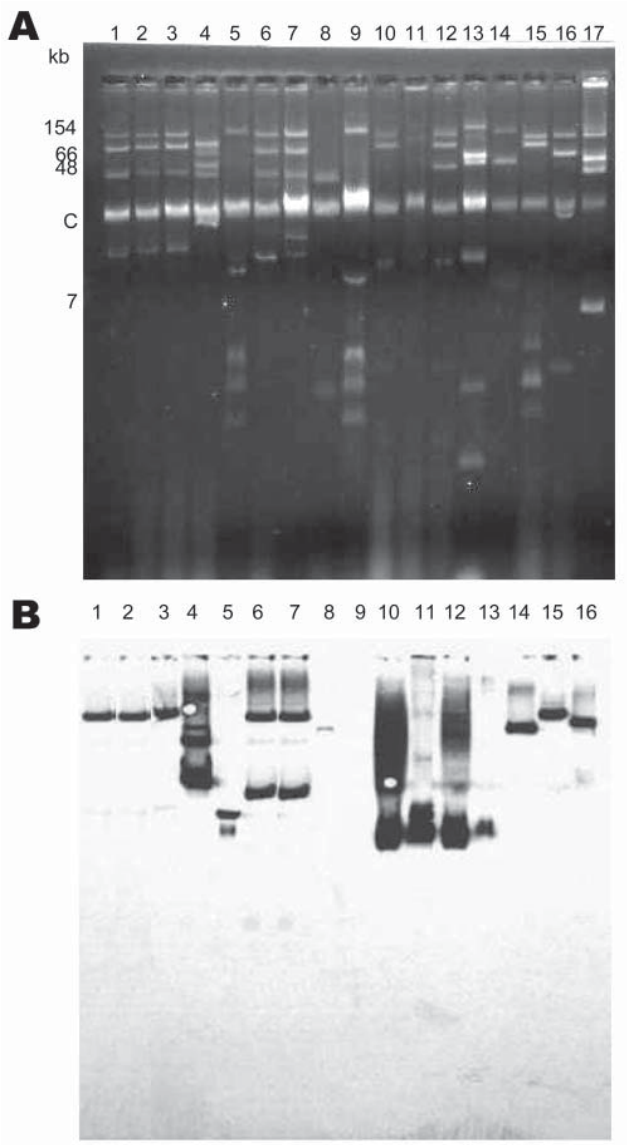

Figure 2. A) Plasmid extractions of culture of clinical *Klebsiella pneumoniae* isolates that produce β-lactamase bla_{KPC-2} gene. B) Southern hybridization of transferred plasmid extraction, conducted with an internal probe for bla_{KPC-2}. Lane 1, *K. pneumoniae* YC (*11*); lane 2, *K. pneumoniae* GR (*21*); lane 3, *K. pneumoniae* K271 (*25*); lane 4, *K. pneumoniae* KN2303 (*13*); lane 5, *K. pneumoniae* KN633 (*13*); lane 6, *K. pneumoniae* INC H1521-6; lane 7, *K. pneumoniae* INC H1516-6; lane 8, *K. pneumoniae* HPTU 27635; lane 9, *K. pneumoniae* HPTU 2020532; lane 10, *K. pneumoniae* A28006 (*16*); lane 11, *K. pneumoniae* A28008 (*16*); lane 12, *K. pneumoniae* A28009 (*16*); lane 13, *K. pneumoniae* A28011 (*16*); lane 14, *K. pneumoniae* A33504 (*16*); lane 15, *K. pneumoniae* 475; lane 16, *K. pneumoniae* 588; and lane 17, *Escherichia coli* 50192 harboring 4 plasmids (7, 48, 66, and 154 kb).

PFGE and MLST showed that several clones are currently spreading in different geographic locations. In Colombia, 3 pulsotypes could be identified. Overall, among the 16 isolates, 1 major ST (258) and its derivative ST 11 seemed to predominate. In a recent study that gathered isolates from 10 US states, ST 258 accounted for 70% of isolates, according to a database of KPC-producing *K. pneumoniae* PFGE results maintained by the Centers for Disease Control and Prevention (*8*). This ST has also been identified for KPC-producing *K. pneumoniae* in Sweden (in isolates imported from Greece and Israel) and more recently in Poland (*36*,*37*). These findings suggest possible international dissemination of KPC-producing ST 258. Apparently, the *K. pneumoniae* clone that contains the extended-spectrum β-lactamase (ESBL) determinant CTX-M-15 belongs to ST 11 (*38*).

KPC-producing *K. pneumoniae* contained diverse β-lactamases. All except 2 isolates harbored at least another β-lactamase; bla_{TEM-1} and a bla_{CTX-M}-type ESBLs were expressed by >80% and 62.5% of isolates, respectively. KPC producers have already been associated with other β-lactamase genes, such as the widespread ESBL gene $bla_{CTX-M-15}$ (*17*). SHV ESBLs have been found among isolates, as has been described for strains from the United States (*39*) and Norway (*36*). These additional β-lactamases are likely to complicate phenotype-based identification of KPC producers. Three isolates harbored the chromosome-encoded $bla_{OKP-A/B}$ genes and belonged to phylogenetic group KpII, which accounts for <10% of *K. pneumoniae* strains (*34*). Coexpression of OKP enzymes and ESBLs has rarely been reported.

Isolates also demonstrated diversity in their molecular features. In this study, the KPC-2 genes were encoded on a broad variety of plasmids, as shown by previous studies (*22*,*35*). These plasmids differed in size and incompatibility groups. Similar plasmids were observed among isolates with the same ST, whereas different plasmids were also associated with similar STs. Therefore, epidemiologic investigation of KPC producers should be performed at different molecular levels.

Tn*4401* was present in all tested strains. The overall structure of Tn*4401* seemed to be conserved, except for the 100-bp to 200-bp deletion. Of the 16 isolates, 11 encoded the full-length Tn*4401*b isoform, 3 encoded the Tn*4401*a isoform containing a 100-bp deletion (ST 258), and 2 encoded the Tn*4401*c isoform containing a 200-bp deletion upstream of the bla_{KPC} gene. These types of transposons tend to evolve by capturing various insertion sequences, as illustrated for the *vanA*-containing Tn*1546* transposon (*40*). For Tn*4401*, three descriptions have been published in which different insertion sequences were present upstream of bla_{KPC-2} (*22*–*24*). None of these atypical structures were found in our strains. Observation of Tn*4401* on different

plasmids further supports the hypothesis that this transposon contributes to the mobilization and dissemination of the bla_{KPC} genes.

Our analysis of several *K. pneumoniae* isolates from 5 geographic origins indicates the spread of different clones that were harboring different plasmids but with an identical genetic structure, Tn*4401*, that sustained a bla_{KPC} gene acquisition, which could likely be at the origin of the worldwide spread of this emerging resistance gene. Finally, taken together, our findings and those of recent studies report a major KPC-producing clone with ST 258, even if novel ST types could also be evidenced, especially from Colombia. Our data suggest that KPC genes benefit all molecular ingredients (transposon location, self-transferable plasmids, efficient STs) by facilitating their rapid spread to *K. pneumoniae* and other bacterial species.

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Cercarial Dermatitis Transmitted by Exotic Marine Snail

Sara V. Brant, Andrew N. Cohen, David James, Lucia Hui, Albert Hom, and Eric S. Loker

Cercarial dermatitis (swimmer's itch) is caused by the penetration of human skin by cercariae of schistosome parasites that develop in and are released from snail hosts. Cercarial dermatitis is frequently acquired in freshwater habitats, and less commonly in marine or estuarine waters. To investigate reports of a dermatitis outbreak in San Francisco Bay, California, we surveyed local snails for schistosome infections during 2005–2008. We found schistosomes only in *Haminoea japonica*, an Asian snail first reported in San Francisco Bay in 1999. Genetic markers place this schistosome within a large clade of avian schistosomes but do not match any species for which there are genetic data. It is the second known schistosome species to cause dermatitis in western North American coastal waters; these species are transmitted by exotic snails. Introduction of exotic hosts can support unexpected emergence of an unknown parasite with serious medical or veterinary implications.

One consequence of introduction of exotic species is possible establishment of new host–parasite associations, potentially resulting in emergence of new diseases (*1*–*3*). Exotic parasites can be introduced into new locations along with their exotic host species (*1*,*4*), sometimes causing extinction of indigenous parasites (*5*). Newly introduced parasites can extend their host ranges into related indigenous host species (*6*,*7*), or exotic hosts may play new roles in the transmission of indigenous parasites (*8*). Parasites newly supported by these exotic hosts can assume considerable human or animal roles as emerging disease agents (*9*).

San Francisco Bay has been the site of numerous well-documented introductions of exotic species (*10*–*12*). We document an outbreak of human cercarial dermatitis in San Francisco Bay that was related to the recent introduction of an exotic snail, the Japanese bubble snail *Haminoea japonica* Pilsbury 1895 (Cephalaspidea: Haminoeidae), which serves as the intermediate host of a schistosome that is responsible for the now annual dermatitis outbreaks.

Cercarial dermatitis (swimmer's itch) is caused by penetration of human skin with cercariae of schistosome parasites; the condition is common and recurrent in freshwater habitats worldwide. Adult schistosomes typically live in mesenteric blood vessels of birds or mammals and produce eggs that pass from the host in feces. The eggs then hatch and release miracidia, which penetrate and develop in an appropriate species of an intermediate snail host. Snail infections culminate in asexual production of numerous cercariae, which are regularly released into the water where they seek to penetrate the skin of a definitive vertebrate host. Penner reported an association between human dermatitis and a marine schistosome (*13*). He established that *Littorina keenae* Rosewater 1978 (Hypsogastropoda: Littorinidae) syn. *L. planaxis* snails collected along the rocky shores of southern California released schistosome cercariae that caused dermatitis in experimentally exposed human volunteers. Documented cases (*1*,*14*–*21*) have been attributed to species of *Austrobilharzia* Johnston 1917, the adults of which most commonly infect gulls and shorebirds (*14*).

A cercarial dermatitis outbreak in San Francisco Bay was reported in 1954 (*1*). The Bureau of Vector Control of the California Department of Health Services identified the cercariae as *Austrobilharzia variglandis* (Miller and Northrup 1926) collected from the eastern Atlantic mudsnail *Ilyanassa obsoleta* (Say 1822) (Hypsogastropoda: Nassariidae) syn. *Nassarius obsoletus* at Robert Crown Memorial Beach in 1955 and 1956 (*1*). *I. obsoleta* snails were accidentally introduced into San Francisco Bay in commercial shipments of Atlantic oysters and were observed in the bay in 1907 (*10*,*11*). *A. variglandis* schistosomes had been identified as the cause of cercarial dermatitis in coastal waters in the northeastern United States (*14*).

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After the 1954 outbreak, no additional cases of cercarial dermatitis were reported in San Francisco Bay or elsewhere on the Pacific Coast until the summer of 2001 when 36 cases of cercarial dermatitis were reported at Crescent Beach in Surrey, British Columbia, Canada; 44 cases were reported in 2002 (*20*). The agent again appeared to *A. variglandis* schistosomes carried by *I. obsoleta* snails, which are extremely abundant at Crescent Beach. The snail was accidentally introduced into British Columbia in the oyster trade and was first observed in the region in 1952 (*20*).

In June 2005, cercarial dermatitis appeared again in San Francisco Bay. The Alameda County Department of Environmental Health received ≈90 reports of skin irritation that occurred after water contact at Robert Crown Memorial Beach where the 1954 outbreak had occurred (The Swimmer Itch Hotline, www.acgov.org/aceh/recreational/beaches.htm). Although it was initially assumed that *A. variglandis* schistosomes, carried by *I. obsoleta* snails, were the causative agent, preliminary investigations suggested that the intermediate host involved was another exotic species, *Haminoea japonica*, from Asia. This snail was observed in San Francisco Bay in 1999 (*22*) and on the eastern shore of the bay, near Robert Crown Memorial Beach, in the summer of 2003, where it has become extremely abundant (A.N. Cohen, unpub. data). We describe the morphologic and genetic features of cercariae obtained from *H. japonica* snails and discuss possible transmission pathways of this parasite in San Francisco Bay.

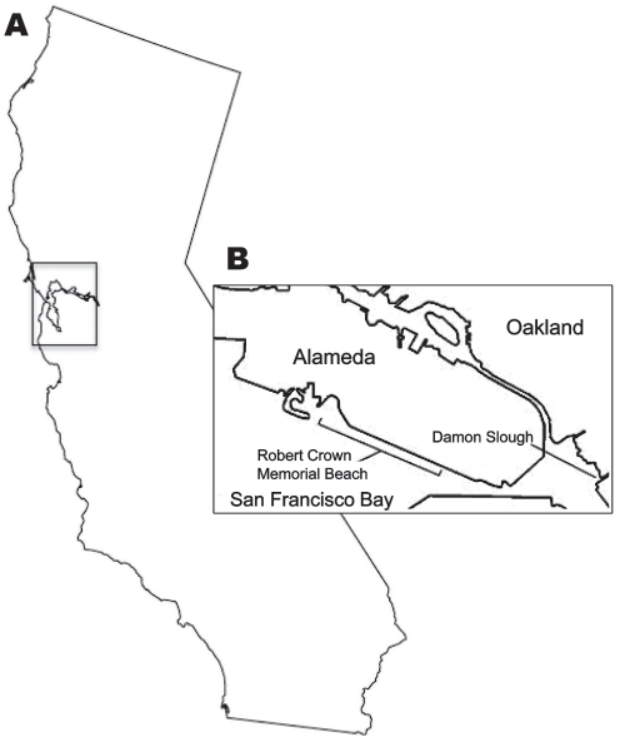

Figure 1. San Francisco Bay area, California, USA (A), and locations where *Haminoea japonica* snails were obtained (B).

Methods

Specimen Collection

This study was conducted under the University of New Mexico Institutional Animal Care and Use Committee Protocol 07UNM011, Animal Welfare Assurance # A4023-01. Samples of *H. japonica* snails were collected by hand at low tide from 4 locations (Figure 1): Crab Cove at the northern end of Robert Crown Memorial Beach (37°46′4.18″N, 122°16′39.55″W) in 2005–2008; the southern end of this beach (37°45′8.81″N, 122°14′57.33″W) in 2006 and 2008; Damon Slough (37°45′14.12″N, 122°12′21.6″W) in 2007; and Lake Merritt, a tidal lagoon connected to San Francisco Bay (37°48′22.25″N, 122°15′22.81″W) in 2008. All gastropod species observed at Robert Crown Memorial Beach were collected and examined for cercariae. *H. japonica* (Figure 2), *Philine* sp. (Cephalaspidea: Philinidae), and *I. obsoleta* snails (all exotic) were collected on tide flat sediments. The exotic oyster drill *Urosalpinx cinerea* (Say 1822) (Sorbeoconcha: Muricidae) and the native periwinkle *Littorina* spp. (*L. scutulata* Gould 1849 or *L. plena* Gould 1849) were collected from exposed bulwarks and other hard substrates. In addition, we examined native *H. virescens* Sowerby 1833 snails collected in August 2005 from Friday Harbor, San Juan Island, Washington.

Snails were isolated singly or in groups of 5 in plastic containers in saline water (20–35 parts per thousand [ppt]) and placed in natural light to induce cercarial shedding. For each species, a subset of the larger snails was then dissected. Cercariae were photographed, and ethanol-preserved specimens were measured and compared with published descriptions of schistosome cercariae.

Gulls are common hosts for schistosomes transmitted by marine snails. We examined 29 gulls of 4 species collected at the Oakland International Airport, ≈7.5 miles southeast of Robert Crown Memorial Beach, as part of the airport's Wildlife Management Program. For each bird, the mesenteric veins were examined, the intestine was opened, the muscosa was removed, and a sample from the intestinal wall was placed between 2 glass slides and examined for schistosome adults or eggs in the villi (*23*). Feces were screened for eggs.

Sequencing and Phylogenetic Analysis

DNA was extracted from fresh or ethanol-preserved cercariae, amplified by PCR (Takara Ex Taq; Takara Biomedicals, Otsu, Japan), and sequenced by using published primers (*24*,*25*). PCR products were purified on Montage

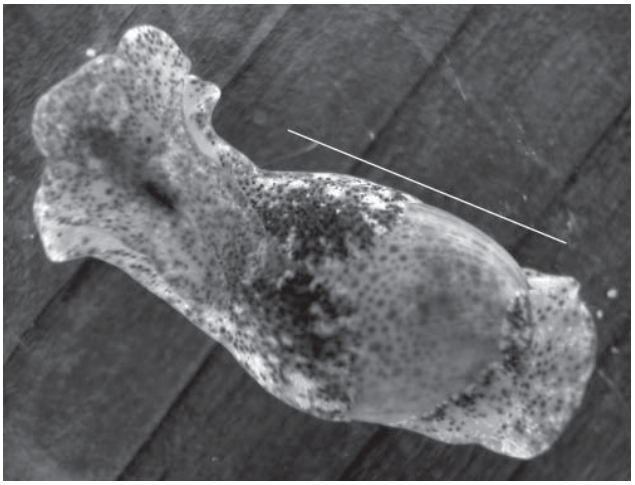

Figure 2. *Haminoea japonica* snail. Scale bar = 11 μm.

Microcon columns (Millipore, Billerica, MA, USA). Sequencing reactions were performed by using the BigDye Direct Sequencing Kit Version 3.1 (Applied Biosystems, Foster City, CA, USA).

Phylogenetic analyses of schistosomes obtained from *H. japonica* snails were performed for 2 datasets. The first dataset combined 18S and 28S rRNA sequence data to place our samples of *H. japonica* within the larger context of the family *Schistosomatidae* (*24*). The second dataset, which was composed of part of the internal transcribed spacer region 2, focused on taxa within the schistosome BTGD clade, which includes species of *Bilharziella*, *Trichobilharzia*, *Gigantobilharzia*, and *Dendritobilharzia* (sensu 24). Phylogenetic analyses with maximum parsimony (MP), maximum likelihood (ML), and minimum evolution (ME) were conducted by using PAUP* version 4.0b10 (*26*) and Bayesian inference (BI) by using MRBAYES 3 (*27*). The jModeltest (*28*) was used to determine the most appropriate nucleotide substitution model for ML and ME analyses.

Parsimony trees were reconstructed by using heuristic searches (300 replicates). Optimal ME and ML trees were constructed from heuristic searches (300 replicates for ME, 10 replicates for ML). Nodal support was estimated by bootstrap (200 replicates) analysis and determined for the MP and ME trees by using heuristic searches. For the ML dataset, the model selected (Akaike information model) was generalized time reversible + proportion invariant + Γ. For BI of the 18S–28S rRNA dataset, the parameters were unlinked: Nst = 6 rates = gamma ngammacat = 4. For both datasets, 4 chains were run simultaneously for 5×10^5 generations; the first 5,000 trees with preasymptotic likelihood scores were discarded as burning, and the retained trees were used to generate 50% majority-rule consensus trees and posterior probabilities.

Life Cycle

To obtain adult worms for species identification, we experimentally exposed young parakeets and *Gallus gallus* L. chicks for 30 min to schistosome cercariae from *H. japonica* snails obtained from San Francisco Bay (Figure 3). Bird hosts were selected according to the method of Leigh (*23*), who described adult worms derived from cercariae from *H. antillarum guadalupensis* Sowerby snails in Florida. In experiment 1, eight chicks were exposed by standing each chick in salt water (35 ppt) containing cercariae. In experiment 2, six parakeets were exposed by applying cercariae to their bare abdomens. In experiment 3, eight parakeets were exposed by standing each bird in salt water (35 ppt) containing cercariae.

Results

Specimens Collected

We identified 1 species of schistosome in *H. japonica* snails that had a prevalence of 1.2% as determined by observation of shedding and 8.7% as determined by dissection (Table 1). No schistosomes were found in other snails examined, including *Littorina* spp. and *I. obsoleta*, taxa that are known to host schistosomes in California (*1*,*13*). No other trematodes were found in *H. japonica* snails from San Francisco Bay or *H. virescens* snails from Washington State. *A. variglandis* was the only schistosome found in the gulls; 55% had adult worms in their mesenteric veins (Table 1).

Cercariae, most of which were collected by dissection, lay in contact with the surface film of water, where they were mostly inactive except for occasional tail twitching. The cercariae were apharyngeate with pigmented eyespots, a lightly spined body, dorsal and ventral fin folds on the full length of the tail furcae, 5 pairs of flame cells, and 3 pairs of penetration glands (Figure 3). With respect to these behavioral and morphologic features, and on the basis of size, the cercariae most closely resemble those of *Gigantobilharzia huttoni* (Leigh 1953), the only other schistosome previously collected from haminoeid snails (*23*,*29*) (Table 2). These cercariae differ from those of *G. huttoni* in having fewer pairs of penetration glands (3 pairs instead of 5–6 pairs), but this trait is difficult to discern accurately. In contrast, *A. variglandis* cercariae have 6 pairs of penetration glands and 6 pairs of flame cells, are larger, and have different proportions than cercariae we found (Table 2). Specimens of the schistosome obtained from *H. japonica* snails were deposited in the Parasite Division of the Museum of Southwestern Biology at the University of New Mexico (MSB185).

Sequencing and Phylogenetic Analysis

Schistosome taxa used in the phylogenetic analyses are shown in the online Appendix Table (www.cdc.goc/EID/

Table 1. Hosts examined for avian schistosomes at 2 locations, United States*

Animal, location, and date	Species	No. screened	No. positive	No. dissected	No. positive
Snails					
San Francisco Bay, California					
2005 Jun	*Ilynassa obsoleta*	96	0	48	0
2005 Jul	*Haminoea japonica*	672	0	300	8
	I. obsoleta	270	0	50	0
	Urosalpinx cinerea	220	0	0	0
	Littorina sp.	275	0	50	0
2006 May	*H. japonica*	400	0	100	0
2006 Jun	*H. japonica*	300	0	150	0
2006 Nov	*H. japonica*	222	0	100	0
2007 Jun	*H. japonica*	930	0	350	11
2007 Jul	*H. japonica*	655	0	266	37
	Philine sp.			38	0
2008 Aug	*H. japonica*	1,100	50	180	70
	I. obsoleta	400	0	100	0
	Littorina sp.	200	0	100	0
	Philine sp.	100	0		
San Juan Island, Washington					
2005 Aug	*H. virescens*	717	0	215	0
Birds					
San Francisco Bay, California					
2007 Aug	*Larus californicus*	NA	NA	4	2
	L. occidentalis	NA	NA	10	4
	L. delawarensis	NA	NA	1	1
2008 Jan	*L. occidentalis*	NA	NA	5	3
	L. glaucescens	NA	NA	1	0
2008 Mar	*L. occidentalis*	NA	NA	7	5
	L. glaucescens	NA	NA	1	1

*NA, not applicable.

content/16/9/1357-appT.htm). Cercariae from *H. japonica* were distinct from all other available schistosomes (GenBank accession nos. GQ920617–21). These cercariae belong to the BTGD clade, which contains only freshwater schistosomes (Figure 4). Our internal transcribed spacer region 2 dataset includes all reported schistosomes from GenBank that belong to the BTGD clade (Figure 5). Only an ML tree is shown in Figure 5. However, MP, ME, and BI analyses yielded near identical topologies with differences at the tips and at nodes where there is no clade support.

Life Cycle

Three experiments with birds were conducted to obtain adult worms. However, all birds experimentally exposed were negative for schistosome infection.

Discussion

Cercarial morphology and molecular genetic data for the schistosome from *H. japonica* indicate that these cercariae are not *A. variglandis*, a schistosome previously reported in San Francisco Bay, and the only species previously implicated in dermatitis outbreaks on the Pacific Coast. We obtained *A. variglandis* schistosomes from gulls but not from snails in the San Francisco Bay area. The *H. japonica*–transmitted schistosome is the second species reported to cause dermatitis after introduction of an exotic snail in California coastal waters. We found this schistosome in an opisthobranch snail in western North America. The only other schistosome known to be obtained from an opisthobranch snail is *G. huttoni* from *H. a. guadalupensis* snails obtained in Florida (*23*,*29*,*32*). Except for schistosomes collected from 2 species of *Siphonaria* (Pulmonata: Siphonariidae) snails (*33*,*34*), all other marine schistosomes have been obtained from caenogastropodid snails.

Cercariae from *H. japonica* closely resemble those of *G. huttoni*, for which sequence data are not available. DNA sequence data for cercariae from *H. japonica* did not match with those of any known schistosome species, including the congener *G. huronensis* Najim 1950. Cercariae from *H. japonica* did not group with other marine schistosomes, but belong to the BTGD clade that, until now, included only freshwater species that use pulmonate snails as intermediate hosts (Figure 5). The only other known marine schistosomes belong to species of the genera *Austrobilharzia* and *Ornithobilharzia* Ohdner 1912, which are distantly related to the BTGD clade (Figure 4). We exposed parakeets and chicks to cercariae from *H. japonica* snails but were unable to obtain adult worms for comparison with described species.

Schistosomes from *H. japonica* and those of *G. huttoni* are probably closely related because they are found in haminoeid snails and have morphologically similar cercariae. However, they differ from all other species in the genus *Gigantobilharzia* Ohdner 1910 in habitat (salt water rather than fresh water) and snail host (opisthobranchid rather than pulmonate). When appropriate genetic material becomes available, analyses may show that *G. huttoni* schistosomes and those from *H. japonica* snails should be placed in a separate genus.

Gigantobilharzia spp. have been reported in several gull species (*35*). Gulls are common at Robert Crown Memorial Beach, often resting on the beach flats at low tides and sometimes foraging in tide pools that contain *H. japonica* snails (A.N. Cohen, unpub. data). Gulls are thus a likely host for schistosomes from *H. japonica*, although we did not find them in gulls at the Oakland Airport near Robert Crown Memorial Beach. Leigh (*36*) and Kinsella et al. (*37*) found fragments of adult worms that they identified as *Gigantobilharzia* sp., and which closely resembled *G. huttoni* worms, in pelicans in Florida. Small flocks of brown pelicans and, rarely, white pelicans have been observed in shallow water off Robert Crown Memorial Beach, although not on the beach or in tide pools (A.N. Cohen, unpub. data). Pelicans are thus another possible host for schistosomes from *H. japonica*.

Other birds commonly observed at Robert Crown Memorial Beach include shorebirds that are most common in winter when water temperatures are probably less conducive to cercarial emergence (surface temperatures near this beach are typically 16°C–20°C in summer and 8°C–12°C in winter). Cormorants, grebes, and ducks are found in nearshore waters, and mallard ducks sometimes forage in tide pools. Larger wading birds (snowy and great egrets, and occasionally herons), oystercatchers, and several species of terns are sometimes seen in small numbers foraging on the beach or in shallows. Marine schistosomes have been reported in gulls, ducks, terns, herons, cormorants, and turnstones (*14*,*17*,*19*,*23*), and *Gigantobilharzia* spp. have been reported in grebes and cattle egrets (*23*,*35*). Thus, various

Table 2. Characteristics of selected schistosome cercariae from 5 locations, United States*

Characteristic	Schistosome					
Species	Unidentified	*Gigantobilharzia huttoni*	*G. huronensis*	*Austrobilharzia variglandis*	*A. variglandis*	*A. variglandis*
Number	14	30	50	?	10	232
Fixative	Alcohol	Formalin	Formalin	Bouin solution or hot corrosive sublimate fluid	Formalin	Formalin
Host species	*Haminoea japonica*	*H. antillarum guadalupensis*	*Physa gyrina*	*Ilyanassa obsoleta*	*I. obsoleta*	*I. obsoleta*
Collection location	San Francisco Bay, CA	Virginia Key, Miami, FL	Ann Arbor, MI	Quamquissett Harbor, MA	Rhode Island	San Francisco Bay, CA
Body length	188 ± 4.8 (160–216)	152 ± 1.4 (135–164)	240 (195–270)	262	237 (220–250)	258 (241–275)
Body width	58 ± 1.3 (50–64)	35 ± 0.7 (48–64)	72.5 (63.7–105)	77	72.5 (69.0–79.0)	–
Tail stem length	175 ± 3.3 (155–197)	154 ± 1.2 (140–166)	268.5 (255–300)	157	228 (200–236)	286 (264–315)
Tail stem width	24 ± 0.7 (21–29)	19 ± 0.3 (16–22)	30 (22.5–45)	–	22–36	–
Furca length	78 ± 2.6 (60–92)	79 ± 0.8 (72–86)	146.8 (102.5–172.5)	105	126 (120–140)	190 (161–216)
Furca width	13 ± 0.6 (10–16)	10 ± 0.4 (6–13)	15	–	12.0–16.5	–
Oral sucker length	57 ± 1.9 (49–70)	63 ± 0.6 (54–70)	–	–	72 (66–85)	–
Oral sucker width	46 ± 0.7 (43–51)	53 ± 0.7 (48–64)	–	–	43 (40–45)	–
Ventral sucker to posterior end	54 ± 3.3 (35–76)	44 ± 0.1 (35–51)	–	–	–	–
Body length:tail stem length	1.1	0.99	0.89	1.7	1	0.9
Tail stem length:furca length	2.2	1.9	1.8	1.5	1.8	1.5
Pairs of flame cells†	4 + 1	4 + 1	4 + 1	5 + 1	5 + 1	–
Pairs of penetration glands	3	5–6	5	6	6	–
Reference	This report	(*24*,*29*)	(*30*)	(*31*)	(*14*)	(*1*)

*Values are mean ± SE in microns (range), or range only, unless otherwise indicated. For Miller and Northup (*31*), the tail stem and furca lengths were estimated values based on information in the text. For Grodhaus and Keh (*1*), the ranges were mean values for 7 lots of cercariae obtained from 7 snails.
†No. pairs in body plus no. pairs in tail.

Figure 3. Live schistosome cercaria from a *Haminoea japonica* snail. Scale bar = 30 μm. Measurements are shown in Table 2.

bird species might serve as hosts for schistosomes from *H. japonica*.

Cercarial dermatitis is commonly acquired in fresh water (*38*). It is less common in marine or estuarine waters; most cases are reported from the northwestern Atlantic Ocean or Australia. This disease was observed on the Pacific Coast of North America during an outbreak at Robert Crown Memorial Beach in 1954–1956, when cercariae identified as *A. variglandis* were found in *I. obsoleta*, an Atlantic snail introduced before 1907. The schistosome was likely introduced with this snail and remained undetected until the 1950s (*1*). In June 2005, cercarial dermatitis was again reported at Robert Crown Memorial Beach. Initial cases were found among elementary school groups that visited the beach at the end of the academic year. Since 2005, dermatitis has occurred annually at this beach (90 cases in 2005, 3 in 2006, 14 in 2007, and 31 in 2008).

Schistosome cercariae in *H. japonica* snails, large numbers of these snails at Robert Crown Memorial Beach, and the apparent absence of schistosomes in other common snails at this site indicate that schistosomes from *H. japonica* are responsible for the recent dermatitis outbreak. *H. japonica* snails were first seen in California in 1999 (*22*) and except for the 1954 outbreak attributed to *A. variglandis*, cercarial dermatitis was not reported in San Francisco Bay until shortly after the arrival of *H. japonica*.

The most popular water-contact activities at Robert Crown Memorial Beach are kite surfing and wind surfing at high tide and wading, exploring, and playing in shallow pools at low tide. In part because of cool water temperatures at this beach, swimming is uncommon, and kite and wind surfers usually wear wetsuits. Most dermatitis cases at this beach were contracted by persons wading in tide pools. Dermatitis usually occurred on the feet or legs. Only 1 case was reported among kite surfers and wind surfers. Since 2005, three biologists working at this beach have contracted dermatitis, usually on their hands or forearms (Figure 6). In experiments with *G. huttoni* schistosomes obtained near Miami, Florida, cercariae emerged only when temperatures were >22°C, regardless of season or light intensity (*32*). In San Francisco Bay, the highest numbers of cercariae from

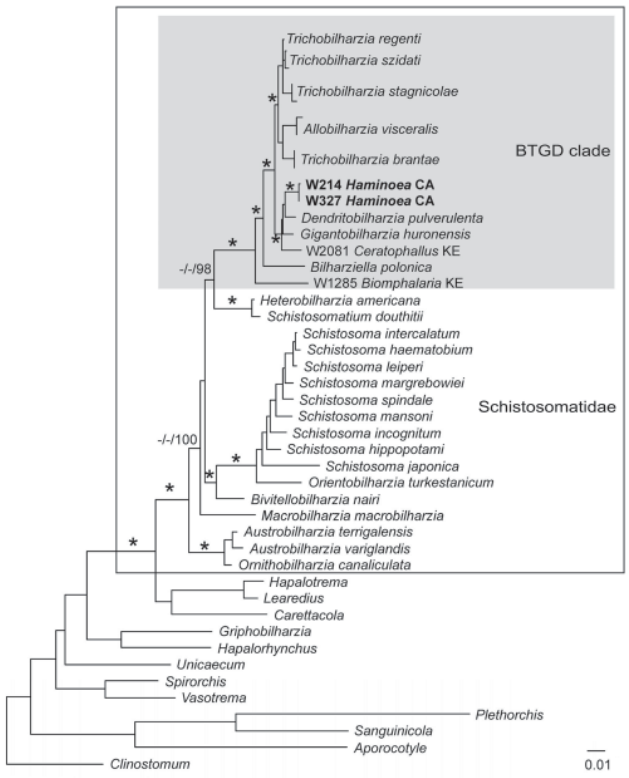

Figure 4. Maximum-likelihood phylogenetic tree based on 18S–28S rRNA sequences of schistosomes. Schistosomatids are indicated in the large box and the *Bilharziella, Trichobilharzia, Gigantobilharzia,* and *Dendritobilharzia* (BTGD) clade is indicated in the gray box. Samples in **boldface** are those obtained from *Haminoea japonica* snails. Node support is indicated by maximum parsimony (MP) and minimum evolution (ME) bootstrap values and Bayesian posterior probabilities (PPs), respectively. Asterisks indicate MP and ME bootstrap values >85 and PPs >98 and hyphens indicate no significant node support. Branch support is designated only for major clades. Scale bar indicates nucleotide substitutions per site.

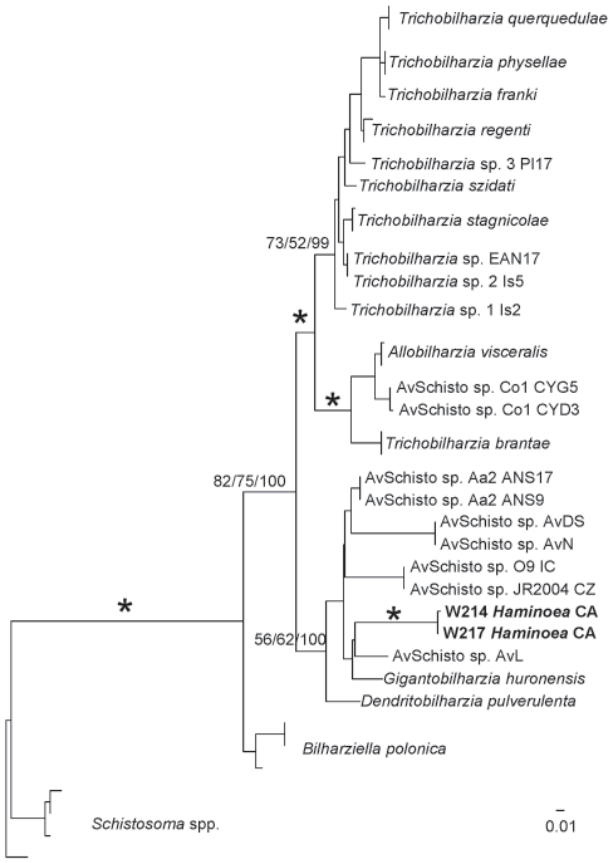

Figure 5. Maximum-likelihood phylogenetic tree based on internal transcribed spacer region 2 sequences of relationships among members of the *Bilharziella, Trichobilharzia, Gigantobilharzia,* and *Dendritobilharzia* species clade from this study and unidentified samples of avian schistosomes from GenBank (online Appendix Table, www.cdc.gov/EID/content/16/9/1357-appT.htm). Samples in **boldface** are those obtained from *Haminoea japonica* snails. Node support is indicated by maximum parsimony (MP) and minimum evolution (ME) bootstrap values and Bayesian posterior probabilities (PPs), respectively. Asterisks indicate MP and ME bootstrap values >85 and PPs >98. Branch support is designated only for major clades. Scale bar indicates nucleotide substitutions per site.

H. japonica may be released in beach flat tide pools that warm up during daytime low tides.

There are at least 3 ways in which schistosomes we found in *H. japonica* snails could have recently emerged as a disease agent in San Francisco Bay. First, an unknown native schistosome may be present in native birds and snails, which switched hosts to the introduced *H. japonica* snail. Some evidence shows that schistosomes are capable of switching hosts (*39*). Neither of 2 native *Haminoea* species found in western North America between Baja, California and Alaska (*H. vesicula* [Gould 1855] and *H. virescens*) (*22*) is known to host schistosomes or is found in the study area. However, if an undetected native schistosome is present in 1 of these species, or in a *Haminoea* species further south on the Pacific Coast, migration of its bird host over San Francisco Bay could have resulted in infection of the *H. japonica* population at Robert Crown Memorial Beach, greatly increasing the potential for human dermatitis. The range of *G. huttoni* schistosomes may extend from Florida through the Caribbean and (by bird movements) up the Pacific Coast.

Second, the schistosomes could be a species from Asia recently introduced into San Francisco Bay in infected *H. japonica* snails. This introduction would require adult snails (eggs and larvae do not contain schistosomes) that harbor male and female cercariae; both sexes would have to colonize the same bird to initiate egg-producing infections. This event occurred on 1 occasion when *A. variglandis* schistosomes and *I. obsoleta* snails were introduced into western North America. Whether *H. japonica* snails arrived in San Francisco Bay directly from Asia or indirectly through Washington State is unknown. *H. japonica* snails may have been introduced into Washington State, where they were first observed in the early 1980s (*40*), in Pacific oysters (*Crassostrea gigas* Thunberg 1793) imported from Japan for mariculture (*17*). Pacific oysters from hatcheries or oyster farms in Japan, Washington, Oregon, and California were placed in San Francisco Bay for commercial mariculture in the 1930s, for occasional experimental use until 1981, for bioaccumulation studies during 1991–2002, and were introduced illegally at 1 site in 1999. A population recently discovered in South San Francisco Bay appears to have been introduced during the late 1990s (A.N. Cohen, D. Goodwin, unpub. data). These occurrences may be related to the initial appearance of *H. japonica* snails in 1999 in South San Francisco Bay.

Third, the schistosomes could be a species from Asia found in migrating birds that infected *H. japonica* snails after these snails became established. Because some birds excrete schistosome eggs for ≤10–28 months postinfection (*19*,*23*), some schistosomes may survive in a bird host long enough to complete a long-distance migration. However, because no bird species are known to routinely migrate

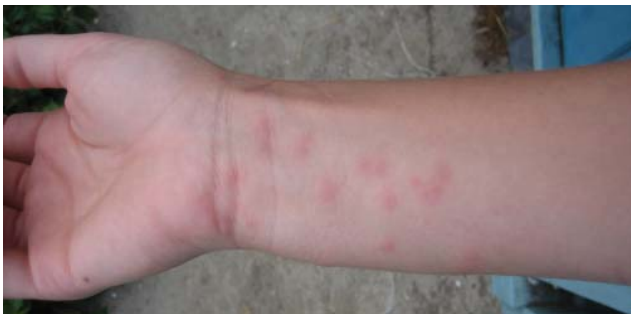

Figure 6. Cercarial dermatitis contracted in San Francisco Bay, California, USA, by one of the authors (S.V.B.).

across the Pacific Ocean between the native region of *H. japonica* snails in Asia and regions in the western United States, introduction by this mechanism seems unlikely.

Much remains to be learned about factors favoring outbreaks of cercarial dermatitis in new areas. Native *Haminoea* spp. should be surveyed for parasites to assess whether host switching may be involved, and *H. japonica* snails should be surveyed in their native range and in Washington State to determine whether trans-Pacific schistosome colonization events have occurred and by what mechanisms. Molecular analysis of *G. huttoni* schistosomes would increase the taxonomic status of the species we isolated from San Francisco Bay. The definitive avian host in this region could be determined by examination of feces for eggs and carcasses for adult schistosomes.

The molecular signatures we have provided may be present in schistosomes isolated from birds or snails in other areas, which would help establish how this zoonotic infection reached California. Potential effects on native biota, especially endangered birds that might serve as hosts (such as the California least tern or California brown pelican), should be assessed. Whether this schistosome will become established in other locations along the Pacific Coast and affect beach users is unknown. Improved understanding of the biology and mechanism of establishment of this schistosome may enable better management of human exposure and infection, control of its spread, and prevention of other schistosome introductions or outbreaks.

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Dr Brant is a research assistant professor at the University of New Mexico. Her main research interests are epidemiology of cercarial dermatitis and evolutionary history of avian schistosomes.

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Influenza in Refugees on the Thailand–Myanmar Border, May–October 2009

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We describe the epidemiology of influenza virus infections in refugees in a camp in rural Southeast Asia during May–October 2009, the first 6 months after identification of pandemic (H1N1) 2009 in Thailand. Influenza A viruses were detected in 20% of patients who had influenza-like illness and in 23% of those who had clinical pneumonia. Seasonal influenza A (H1N1) was the predominant virus circulating during weeks 26–33 (June 25–August 29) and was subsequently replaced by the pandemic strain. A review of passive surveillance for acute respiratory infection did not show an increase in acute respiratory tract infection incidence associated with the arrival of pandemic (H1N1) 2009 in the camp.

Pandemic (H1N1) 2009 emerged in April 2009 and subsequently spread around the globe. The World Health Organization issued a pandemic declaration on June 11, 2009 (*1*,*2*). By October 25, 2009, >440,000 laboratory-confirmed cases, including >5,700 deaths, had been reported to WHO (*3*). The first case of pandemic (H1N1) 2009 infection was diagnosed in Thailand on April 28, 2009, and subsequently the virus was detected in all provinces. The Thailand Ministry of Public Health reported 27,639 confirmed cases and 170 deaths as of October 10, 2009 (*4*). Myanmar (Burma) reported its first confirmed case of pandemic (H1N1) 2009 infection during the week beginning July 5, 2009, and by the end of October 2009 had reported <100 confirmed cases with no deaths (*5*). Although most infections caused by this new virus have been mild, severe disease has been reported, particularly in young adults (*6*).

Data regarding the effect of influenza in rural areas of the developing world are scarce, as are etiologic data from refugee populations (*7*–*9*). A recent review of published reports from Southeast Asia concluded that influenza infection may be identified in up to 26% of outpatients with febrile illness and in 14% of hospitalized patients with pneumonia (*10*). In Thailand, seasonal influenza virus activity peaks during the rainy season (June–September), with smaller peaks occurring during the cold months (January and February) (*11*). Incidence of influenza infections in Thailand was 64–91 cases/100,000 persons per year during 1999–2002; the influenza-related hospitalization rate was 21/100,000 persons during 1999 (*11*). Influenza infections in Myanmar are also seasonal; cases are documented predominantly in the rainy season (May–October) (*12*–*14*). Incidence data for influenza virus infections in Myanmar are not readily available.

Of 15.2 million refugees worldwide, approximately one third live in camps (*15*). These refugees often live in crowded conditions and have contact with populations from the host country and the country of origin, where public health infrastructure and surveillance may be poor (*16*,*17*).

Approximately 150,000 refugees from Myanmar are housed in several camps on the Thailand–Myanmar border. Maela Temporary Shelter (Maela, Thailand) is the largest of these camps, with a population of >40,000, predominantly of the Karen ethnic group, housed in a 4-km^2 area (*18*). This camp is located in the hills adjoining the Myanmar border, ≈500 km northwest of Bangkok, and has been in operation since 1984. Primary health and sanitation services are provided by nongovernmental or-

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ganizations. A field hospital with an inpatient area and 2 outpatient clinics provide free healthcare to the camp's population, who do not have access to healthcare facilities outside of the camp. Acute respiratory infection (ARI) is a common cause of illness in Maela, but the proportion of infections caused by influenza viruses is unknown. Seasonal influenza vaccinations and antiviral medicines are not readily available in the camp or the surrounding community.

In 2007, the US Centers for Disease Control and Prevention (CDC) and Shoklo Malaria Research Unit (www.shoklo-unit.com) established a laboratory-enhanced ARI surveillance system in Maela. Pilot data were obtained during late 2007, and formal surveillance began in 2008 with a 2-day-per-week patient review in the outpatient department of Aide Medicale Internationale Hospital. In 2009, daily patient reviews were carried out in outpatient (from January 2009) and inpatient (from April 2009) departments. We report the results of this surveillance during May–October 2009 and describe the impact of the current influenza pandemic in this rural refugee population. Data from our surveillance activities in 2008, as well as passively collected ARI incidence data, are included for comparison.

Methods

From May 1 through October 31, 2009, trained local field workers visited the hospital in Maela daily (Monday–Saturday). Patients whose illnesses met clinical case definitions for influenza-like illness (ILI) or pneumonia (Table 1) were identified by clinic staff at the time of examination, and these patients were asked to complete an additional clinical interview. Inpatient and outpatient department cases were included in the surveillance. From July 27 through October 31, 2009, original clinical case definitions were modified to capture each patient who had a history of fever during the current illness but who was not febrile at the clinic visit (either because of the intermittent nature of fever or self-administration of antipyretics).

A nasopharyngeal aspirate (NPA) was collected from each patient; a sterile 8-French infant feeding tube was inserted into the nasopharynx and then withdrawn while suction was applied with a 20-mL syringe attached to the feeding tube. The nasopharyngeal secretions and the tip of the feeding tube were transferred to a 1-mL tube of viral transport medium and stored in a cool box until transfer, within 24 h, to a –80°C freezer before analysis.

All NPA specimens were subjected to a panel of real-time reverse transcription–PCR (rRT-PCR) assays for the following viruses: influenza A (separate primer/probe sets for influenza A [universal], pandemic [H1N1] 2009, seasonal subtype H1N1, and seasonal subtype H3N1 detection) (*20*); influenza B (CDC in-house assay [details available on request]); respiratory syncytial virus (RSV; CDC in-house assay [details available on request]); and human metapneumovirus (HMPV) (*21*). An internal control PCR specific for the human RNAseP gene was used to monitor sample adequacy and to detect the presence of PCR inhibitors (*22*). Positive and negative controls were included in each PCR run. A Rotorgene 6000 real-time PCR thermocycler (Corbett Life Science, Mortlake, New South Wales, Australia) and SuperScript III One-Step RT-PCR Kits (Invitrogen, Carlsbad, CA, USA) were used throughout. All laboratory work was conducted at the Shoklo Malaria Research Unit microbiology laboratory in Mae Sot, Tak Province, Thailand.

To compare virologic results from 2009 with our surveillance data from 2008, we subsequently restricted the 2009 dataset to match data collected in 2008 (i.e., we included only patients whose illnesses met the strict case definitions and who were sampled on either Monday or Tuesday in the outpatient department). Clinical and laboratory data collected in 2008 were identical to data collected in 2009.

To estimate the incidence of influenza-associated illness, we reviewed passive disease surveillance data collected by the hospital in Maela and collated by the Com-

Table 1. Clinical case definitions for influenza virus infections in Maela Temporary Shelter, Thailand, May–October 2009*

Condition (age, y)	Strict case definition (up to 2009 Jul 27)	Expanded case definition (from 2009 Jul 28)
Influenza-like illness	Fever ≥38°C AND cough or sore throat AND does not meet criteria for pneumonia	Fever ≥38°C (or history of fever) AND cough or sore throat AND does not meet criteria for pneumonia
Pneumonia (<5)	Pneumonia: cough or difficulty breathing AND increased respiratory rate (as defined by the WHO IMCI [*19*]) Severe pneumonia: cough or difficulty breathing AND ≥1 of: lower chest wall in-drawing, nasal flaring, grunting	No change
Pneumonia (≥5)	Fever ≥38°C AND cough or difficulty breathing AND abnormal chest examination	Fever ≥38°C (or history of fever) AND cough or difficulty breathing AND abnormal chest examination

*WHO, World Health Organization; IMCI, Integrated Management of Childhood Illness.

mittee for Coordination of Services for Displaced Persons in Thailand. This surveillance system captured data only on patients visiting the hospital for treatment. The number and incidence rate (calculated by using monthly camp population census data) of clinically diagnosed upper respiratory tract infections (URTIs) and lower respiratory tract infections (LRTIs) were reported by month. No information was available to determine the number of ILI cases; therefore, we could not estimate the proportion of URTIs caused by influenza viruses in Maela. However, because most LRTIs reported are likely to be clinical pneumonia, we estimated the incidence of influenza-associated pneumonia as the incidence of LRTI multiplied by the percentage of pneumonia patients with specimens positive for influenza A. To determine the effect of pandemic (H1N1) 2009 on overall case numbers, we compared 2008 data with 2009 data.

Ethics

The Human Studies Oversight and Review Team of CDC reviewed the surveillance project and declared it to be a nonresearch activity, as defined by US 45 CFR 46.102(d). Therefore our study was exempt from the need for full review by an institutional review board.

Statistical Analysis

All statistical analyses were performed by using STATA version 10.1 software (StataCorp, College Station, TX, USA). Categorical variables were analyzed by using the Fisher exact test; continuous variables were analyzed by using the Wilcoxon rank-sum test (because none were normally distributed). Two-tailed p values <0.05 were considered significant. Epidemiologic week numbers were calculated by using standard criteria (*23*).

Results

During May 1–October 31, 2009, a total of 324 patients were included in the surveillance. Of these, 19 were excluded from further analysis; 18 patients did not meet the clinical case definitions, and no NPA specimen was received for 1 patient (Figure 1).

Pneumonia was diagnosed for 234 (77%) of the 305 eligible patients, and ILI was diagnosed for 71 (24%). For patients with pneumonia, median age was 2.0 years (range 0.1–68 years) and 55% were male; for those with ILI, median age was 1.4 years (range 0.2–10 years) and 54% were male.

Fifty seasonal influenza A infections and 17 pandemic (H1N1) 2009 infections were detected by rRT-PCR. Forty-nine of the 50 seasonal influenza A infections were subtyped as H1N1; one was subtype H3N1 (Figure 2; Table 2). No influenza B infections were detected. Median age of patients with seasonal influenza A and pandemic (H1N1) 2009 was 3 years for both groups, but pandemic (H1N1) 2009 was more restricted in age range (upper limit 27 years, compared with 68 years for seasonal influenza A). Influenza A virus was detected in 23% of patients who had pneumonia (seasonal influenza A, 17%; pandemic [H1N1] 2009, 6%) and in 20% of ILI cases (seasonal influenza A, 14%; pandemic [H1N1] 2009, 6%).

Seasonal influenza A activity spanned weeks 26–34 (June 28–August 29) and peaked in week 31 (August 2–8; the virus was detected in 80% of all NPA samples obtained from patients that week) (Figure 3). Pandemic (H1N1) 2009 was detected later, beginning on week 31 (August 3). Activity subsequently remained steady, with a maximum of 8 cases detected in a single week (15% of all patients sampled in week 36).

Seven dual virus infections were detected, all in children <14 years of age: 2 seasonal influenza A plus HMPV, 2 pandemic (H1N1) 2009 plus HMPV, and 3 pandemic (H1N1) 2009 plus RSV. Dual infections were observed significantly more frequently with pandemic (H1N1) 2009 than with seasonal influenza A (5/17 vs. 2/50; p = 0.003). Among patients admitted to the inpatient department, those

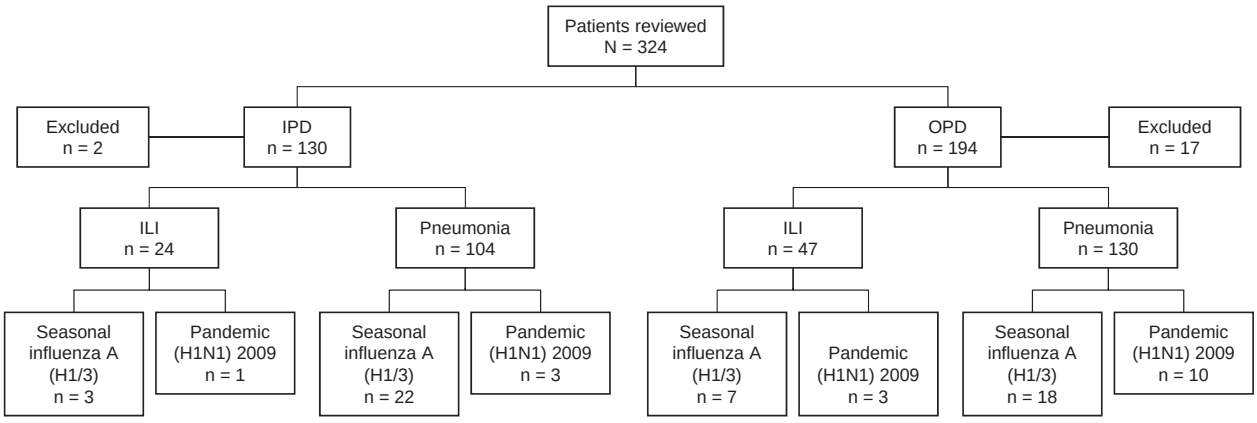

Figure 1. Influenza surveillance summary for Maela Temporary Shelter, Thailand, May–October 2009. IPD, inpatient department; OPD, outpatient department; ILI, influenza-like illness.

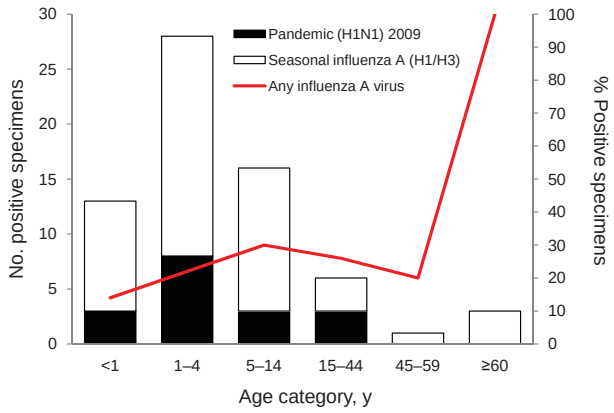

Figure 2. Age distribution of patients from whom specimens were positive for seasonal influenza (n = 50) or pandemic (H1N1) 2009 (n = 17) in Maela Temporary Shelter, Thailand, May–October 2009.

with dual virus infection were not significantly more ill than those with influenza A infection alone (3/7 vs. 26/60; p = 1.0).

Illnesses for 205 (67%) patients met the strict case definition for ILI or pneumonia; 100 (33%) met only the expanded case definitions. Age distribution and proportion of influenza A viruses did not differ significantly between the strict and expanded case definition groups. However, a significantly higher proportion of patients with ILI (18/25 vs. 6/46; p<0.001) or pneumonia (99/180 vs. 5/54; p<0.001) whose illnesses met the strict case definition were hospitalized, which suggests that the expanded case definitions captured patients with milder illnesses.

Overall, at least 1 virus was detected in 175 (57%) patients (37/71 ILI, 138/234 pneumonia). HMPV and RSV accounted for 120/187 (54%) viruses detected. These viruses were detected in ILI cases (HMPV 23%,; RSV 17%) and pneumonia (HMPV, 21%; RSV 18%). RSV was detected significantly more often in children <5 years of age (48/221 vs. 6/84; p = 0.003) and was more age restricted than all other viruses.

In 2008, NPA samples were obtained from 74 patients meeting the case definitions on Mondays or Tuesdays in outpatient departments during May 1–October 31 (2 ILI, 72 pneumonia). An influenza virus was identified in 6 patients (3 influenza A, 3 influenza B); pneumonia was diagnosed for all. In 2009, samples were obtained from 35 patients with illnesses that met the strict case definitions in operation in 2008; patients were examined in outpatient departments on the same days of the week (3 ILI, 32 pneumonia). An influenza virus was detected in 9 patients (4 seasonal influenza A, 5 pandemic [H1N1] 2009); pneumonia was diagnosed for all.

Committee for Coordination of Services for Displaced Persons in Thailand passive surveillance data for Maela showed that the median monthly incidence of URTI was 51.8/1,000 persons (range 30.8–66.1 persons) during May–October 2009; incidence peaked in August. For LRTI, median monthly incidence was 32.4/1,000 persons (range 20.0–37.3), and incidence peaked in September. For the same months of 2008, median monthly incidence was 36.1/1,000 persons (range 18.4–50.8 persons) for URTI and 22.4/1,000 persons (range 11.2–49.9 persons) for LRTI (Figure 4) (R. Sedhain, pers. comm).

Discussion

Our study demonstrates that influenza virus infections are common etiologic agents of respiratory infection in a Southeast Asian refugee population living in crowded conditions. During the 6 months of surveillance in 2009, influenza A viruses were detected by rRT-PCR in 23% of clinical pneumonia and 20% of ILI cases sampled, representing a considerable impact that this vaccine-preventable disease has among patients with ARI.

Maela is an overcrowded and relatively closed refugee camp and therefore might be considered an ideal location for a novel influenza virus to cause an explosive outbreak. However, the number of confirmed cases indicated that no major outbreak occurred in 2009. After the first case of pandemic (H1N1) 2009 was identified in August, these cases increased modestly in September, then substantially declined during October. Overall, only 25% of all influenza A viruses were determined to be the pandemic strain. However, supportive data show a change of the predominant influenza virus. In late August 2009, seasonal influenza A (H1N1) was the predominant circulating virus; during the subsequent 2 months, only cases of pandemic (H1N1)

Table 2. Characteristics of patients with influenza A infection in Maela Temporary Shelter, Thailand, May–October 2009*

Characteristic	Seasonal influenza A (H1N1/H3N1)	Pandemic (H1N1) 2009
No. cases	50	17
Median age, y (range)	3.1 (0.3–68.0)	3.7 (0.4–27.0)
Sex ratio, M:F, no. (%)	31:19 (62:38)	10:7 (59:41)
Admitted to IPD, no. (%)	25 (50)	4 (24)
Diagnosis		
Influenza-like illness, no. (%)	10 (20)	4 (24)
Pneumonia, no. (%)	40 (80)	12 (76)

*IPD, inpatient department, Aide Medicale Internationale Hospital.

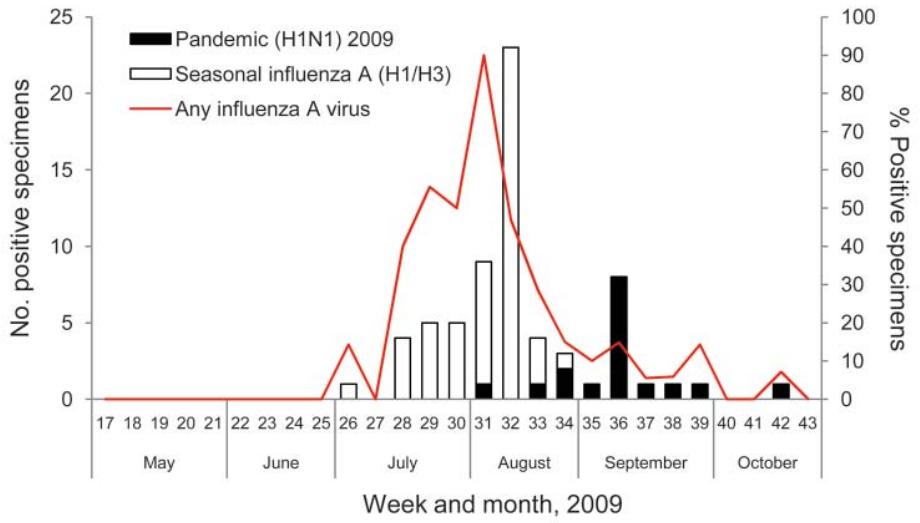

Figure 3. Influenza virus PCR results by week for Maela Temporary Shelter, Thailand, May–October 2009.

2009 were detected. During May–August, the incidence of LRTI and URTI in cases captured by the passive surveillance system was higher each month in 2009 than in 2008. The rates of URTI were similar in September and October of both years, whereas the LRTI rate was higher in October 2008 than in October 2009. Pandemic (H1N1) 2009 did not clearly increase in case-patients with ARI after its first detection in the camp in August 2009. However, surveillance did not capture mild infections that did not result in visits to the outpatient department.

The occurrence of most influenza A infections in patients who had pneumonia most likely reflects a sampling bias, although influenza is a generally underrecognized cause of pneumonia in the tropics (*24*). ILI is not a routinely used diagnosis for the clinic staff at Maela, so most of the ILI case-patients likely were not interviewed and sampled. However, when influenza A was identified, pandemic (H1N1) 2009 case-patients were less likely than seasonal influenza case-patients to have been hospitalized. This information suggests that, in this population, illness caused by pandemic (H1N1) 2009 was no more severe than illness associated with seasonal influenza A. Several confounding factors, unrelated to the innate pathogenicity of the viruses, may account for this finding: 1) the timing of the modification of case definitions in relation to the appearance of pandemic (H1N1) 2009; 2) differences in age distribution; and 3) presence of underlying illnesses in the patient groups. Data regarding underlying medical conditions were not collected as part of this surveillance so the effect of other conditions cannot be assessed. To prevent spread of infection, public health systems may request persons with ILI to self-quarantine, which might result in underestimation of the number of cases identified in clinic- or hospital-based surveillance systems. During the 2009 influenza season, announcements regarding influenza and the need for good hygiene were made on the Maela public address system; healthcare workers reinforced these messages by home visits. Whether this intervention had any effect on health-seeking behavior remains unclear. An influenza triage system was in operation at the hospital, but our surveillance staff had access to patients seen and treated in this area.

Our study has several limitations. Most importantly, not every patient eligible for sampling was included, frequently because the patient refused or clinic staff failed to identify patients with illnesses that met the case criteria. These data were not recorded, so the effect of this bias cannot be estimated. As previously discussed, ILI is not a frequently used diagnosis outside this surveillance program, and most cases with this clinical syndrome were diagnosed as common cold. Many of the ILI cases documented were miscategorized in the clinic as pneumonia but were subsequently found not to meet the case definition, explaining the presence of persons hospitalized with ILI. Overall, these factors may bias toward sampling of case-patients who had more severe symptoms. Also, screening took place in only 1 of the 2 hospital outpatient clinics. However, because both are general clinics, the impact of this screening is likely to be reflected in the absolute number of cases detected rather than in the proportion of ILI and pneumonia cases caused by influenza viruses. Regarding laboratory data, the likelihood of confirmation of influenza infection is associated with the clinical case definitions in use: the strict ILI case definition used in our surveillance has a sensitivity of 98.4%–100% but a specificity of only 7.1%–12.9% (*25*). In another study, the probability of having a positive influenza virus PCR was directly related to magnitude of fever (*26*). Therefore, given the bias toward severe cases, we may have considerably underestimated the impact of influenza in Maela.

As a result of the limitations noted above, we could

not directly calculate the incidence of influenza infections in the Maela population. Also, given the mobile nature of refugee populations, calculating accurate incidence rates is difficult, although the monthly census in Maela enabled generation of relatively accurate figures for this population. Therefore, because we detected an influenza virus in 23% of case-patients who had pneumonia during May–October 2009, we believe the virus may have been responsible for 7 pneumonia episodes per 1,000 population per month (32.4 cases × 23%), which equates to ≈900 influenza-associated pneumonia cases during the 3-month influenza season, largely because of seasonal influenza. For comparison, in 2 rural Thai provinces during 2008, influenza virus infection was associated with 18.4% of hospitalized case-patients who had clinical pneumonia (minimum incidence of 134.4/100,000 population) (*27*). Given the likely health inequalities between our refugee population and rural provinces in Thailand, direct comparison of these datasets is difficult. However, the incidence of influenza-associated pneumonia in Maela was ≈5× higher than in the Thai provinces (*27*).

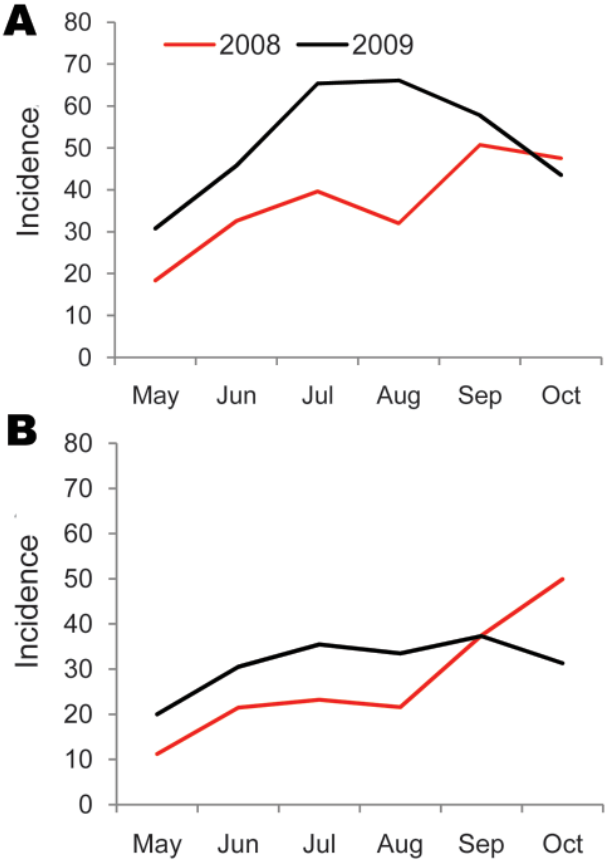

Figure 4. Incidence (per 1,000 population) of (A) upper respiratory tract infections (URTI) and (B) lower respiratory infections (LRTI) for Maela Temporary Shelter, Thailand, May–October 2008 and 2009. Passive surveillance data from Committee for the Coordination of Services for Displaced Persons in Thailand.

Population structure, such as the number of young children and elderly persons, may account for some of this difference, because the incidence of influenza infection is highest in these age groups. As with ILI, the case definitions used may have affected the data or the use of different laboratory confirmation tests for influenza infection may have resulted in considerable variation in disease rates between studies; the study in Thailand used RT-PCR for laboratory confirmation. Although the rates of influenza-associated pneumonia were different in the refugee camp, the proportions of pneumonia cases associated with influenza were similar (23% vs. 18%).

Methods of preventing or mitigating influenza outbreaks in a community include vaccination; use of antiviral drugs; and basic infection control measures, particularly good respiratory etiquette, hand washing, and social distancing (*28*). The World Health Organization has devised a specific influenza pandemic preparedness and mitigation plan for refugee and displaced populations, but implementation requires the coordinated efforts of healthcare providers (frequently nongovernmental organizations) and governments to ensure that control measures are available and used effectively (*29*). Because resources are likely to be strained during an influenza pandemic, refugee and displaced populations might not be adequately represented in a country's pandemic preparedness plan. Availability of items required to control influenza transmission (personal protective equipment, vaccines, and antiviral medication) may be limited for this population without robust planning at the local and national levels. In addition to pandemic preparedness, camp administrators and donor agencies should consider routine vaccination for seasonal influenza in these populations.

Continuation and refinement of this surveillance as the pandemic continues may provide further insight into the epidemiology of influenza in resource-poor rural Asian populations. Work such as this solidifies the need of inclusion of refugee populations in influenza vaccine strategies and pandemic planning.

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Cotton Rats and House Sparrows as Hosts for North and South American Strains of Eastern Equine Encephalitis Virus

Nicole C. Arrigo, A. Paige Adams, Douglas M. Watts, Patrick C. Newman, and Scott C. Weaver

Eastern equine encephalitis virus (EEEV; family *Togaviridae*, genus *Alphavirus*) is an arbovirus that causes severe disease in humans in North America and in equids throughout the Americas. The enzootic transmission cycle of EEEV in North America involves passerine birds and the ornithophilic mosquito vector, *Culiseta melanura*, in freshwater swamp habitats. However, the ecology of EEEV in South America is not well understood. *Culex (Melanoconion)* spp. mosquitoes are considered the principal vectors in Central and South America; however, a primary vertebrate host for EEEV in South America has not yet been identified. Therefore, to further assess the reservoir host potential of wild rodents and wild birds, we compared the infection dynamics of North American and South American EEEV in cotton rats (*Sigmodon hispidus*) and house sparrows (*Passer domesticus*). Our findings suggested that each species has the potential to serve as amplification hosts for North and South America EEEVs.

Eastern equine encephalitis virus (EEEV; family *Togaviridae*, genus *Alphavirus*) is an arbovirus that causes severe neurologic disease in humans in North America and in equids throughout the Americas (*1*). EEEV strains that circulate in North America and the Caribbean (NA EEEV, lineage I) are distinguishable from those that circulate in Central and South America (SA EEEV, lineages II–IV) by the following: antigenicity (4 distinct subtypes), genetics (20%–25% nt sequence divergence), phylogenetic and evolutionary patterns, epidemiology, human pathogenicity, and geographic distribution (*2*). One theory for their markedly different characteristics is that EEEV adapted to a unique North American ecologic niche after its introduction and evolutionary divergence from EEEV in Central and South America (*3*). Although the ecology of vectors and vertebrate hosts for NA EEEV has been well defined, the ecology for SA EEEV remains poorly characterized, which limits our understanding of the divergence of these viruses.

Enzootic circulation of EEEV in eastern North America is primarily supported by a variety of avian reservoirs in the order Passeriformes and by the highly ornithophilic mosquito vector, *Culiseta melanura*, in freshwater swamp habitats. However, under favorable amplification conditions, sporadic epizootic and epidemic transmission occurs by bridge vectors (e.g., *Aedes* spp. mosquitoes) that have more catholic feeding behaviors. These vectors have the ability to broaden the virus' amplification host range to other avian or mammalian species in habitats that pose greater risk for incidental hosts, such as humans and equids. For example, recent studies in some southeastern foci of North America suggest that enzootic and/or epizootic EEEV transmission may involve ectothermic hosts (e.g., reptiles and amphibians) and herpetophilic mosquito vectors (*4*). Rodents have not been implicated in transmission of enzootic NA EEEV; however, seroprevalence data (*5*) support their susceptibility to infection and warrant consideration of their potential to serve as vertebrate hosts during epizootic transmission.

Isolation of SA EEEV from *Culex (Melanoconion)* spp. mosquitoes in the Spissipes section (e.g., *Cx. pedroi*, *Cx. taeniopus*) suggests that they are the principal enzootic, and potentially epizootic, mosquito vectors (*6*–*8*) in Central and South America. These mosquito species have

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broad host preferences—mammalian, avian, and reptilian (*9*)—but the primary vertebrate host for SA EEEV has not yet been identified. Virus isolations and seroprevalence data demonstrate that wild birds, rodents, marsupials, and reptiles are susceptible to infection (*6*,*10*–*12*). However, the involvement of these vertebrates in the enzootic transmission of SA EEEV remains unclear.

Venezuelan equine encephalitis virus (VEEV) is the closest genetic relative to EEEV and circulates sympatrically with SA EEEV. Like SA EEEV, *Culex (Melanoconion)* spp. mosquitoes serve as the primary enzootic vectors of VEEV (*13*–*15*). Small mammals are the principal reservoir hosts of VEEV (*15*), although a wide variety of vertebrate species have antibodies against VEEV (*16*,*17*). Phylogenetic comparisons of SA EEEV and enzootic VEEV subtypes ID and IE have shown similar patterns of evolution that are consistent with the use of mammalian vertebrate hosts rather than the avian hosts involved in NA EEEV transmission (*2*). Therefore, the similarities in geographic range, vector ecology, and phylogenetic profiles of SA EEEV and VEEV support the hypothesis of similar mammalian vertebrate host usage, unlike the avian host usage for NA EEEV.

To further test this hypothesis of differential vertebrate hosts for NA versus SA EEEV strains, we compared their infection dynamics in a wild rodent (cotton rat, *Sigmodon hispidus*) known to support VEEV transmission and in a passerine bird (house sparrow, *Passer domesticus*) known to be a competent host of NA EEEV. Our goals were to better understand the ecology of SA EEEV, which will help clarify the extent to which these viruses have ecologically diverged and the parameters contributing to or limiting the potential emergence or adaptation of EEEV in naive environments.

Materials and Methods

Animals

During August and September 2007, cotton rats (*S. hispidus berlandieri*) (*18*) were collected from Galveston Island State Park, Texas, USA (29.27°N, 94.83°W) by using live-capture traps (H.B. Sherman Traps, Tallahassee, FL, USA). The weights of the feral rats ranged from 52 to 138 g, suggesting a wide range of ages (*19*). Laboratory-born progeny of captured rats were also used in experiments for a total of 3 cohorts: feral, 7–8-wk progeny, and juvenile (2–3 wk progeny). House sparrows were collected by using mist nets throughout Houston, Texas. Birds were morphologically identified, sexed, and aged (hatch-year vs. after hatch-year). To determine viremia and antibody responses, we experimentally infected 2 cohorts, collected in June and July 2008. To determine survival rates without manipulation, we infected a third cohort, collected in July and August 2009. All experimental groups of rats and sparrows were matched for sex and approximate age or life stage.

Animals were transported directly to the BioSafety Level 3 facility at the University of Texas Medical Branch, housed individually, and given food and water ad libitum. During acclimation, feral rats were determined to be seronegative for EEEV, VEEV, and western equine encephalitis virus by 80% plaque-reduction neutralization tests ($PRNT_{80}$), and they were screened by immunofluorescent assay for persistent infection with Bayou (*Hantavirus*) and Whitewater Arroyo viruses (*Arenavirus)*, known to be enzootic in the region. Hemagglutination inhibition tests also determined that the sparrows were seronegative for EEEV and western equine encephalitis virus, as well as for the flaviviruses St. Louis encephalitis virus and West Nile virus. All studies were approved by the Institutional Animal Care and Use Committee at the University of Texas Medical Branch.

Virus Isolation and Animal Infection

NA EEEV strain FL93-939 (NA FL93, lineage I) was isolated from a *Culex* spp. mosquito pool in Florida in 1993, cloned into cDNA form (*20*), and rescued from baby hamster kidney cells. SA EEEV strains 77U1104 (SA PE70, lineage II) and C49 (SA CO92, lineage III) were isolated from sentinel hamsters in Peru, 1970, or Columbia, 1992, respectively, and passaged once in Vero cells.

We inoculated each animal subcutaneously in the thigh with virus or with uninfected medium for negative controls (Table 1). The target dose was ≈3 $1og_{10}$ PFU, which is consistent with the approximate maximum amount introduced by the bite of an alphavirus-infected mosquito (*21*). Animals were monitored daily for signs of illness and killed when moribund or ≈4 wk postinfection. For viremia and antibody assays, 100-μL blood samples were collected from the retroorbital sinus of rats or from the jugular vein of sparrows for the first 5–7 d postinfection. To determine seroconversion status, we also collected samples on days 29–30 for rats and days 14, 22, 24, and/or 39 for sparrows. To reduce handling, we randomly divided the sparrow cohorts into 2 groups from which blood was collected on alternate days. Blood from rats was collected daily.

Virus Titer and Antibody Assays

Blood samples were immediately diluted 1:10 with phosphate-buffered saline supplemented with 10% heat-inactivated fetal bovine serum and penicillin (10,000 U/mL), streptomycin (10,000 μg/mL), and gentamicin (50 mg/mL). Diluted whole blood was tested to determine virus titers by plaque assay and antibody titers (maximum dilution 1:1,280) by $PRNT_{80}$ on Vero cells, as described (*22*). Diluted serum samples from ≥14 d postinfection were also tested for antibodies by $PRNT_{80}$ (*22*).

Table 1. Total cohort sizes and eastern equine encephalitis virus inoculum*

Characteristic	Eastern equine encephalitis virus strain						No. controls
	FL93-939 (FL93)		77U1104 (PE70)		C49 (CO92)		
	No.	Dose, $\log_{10}$ PFU	No.	Dose, $\log_{10}$ PFU	No.	Dose, $\log_{10}$ PFU	
Rat cohort							
Juvenile	6	3.1	6	3.5	NT	NT	1
Mature	8	2.2–3.1	13	3.8–4.2	12	2.8–3.3	4
House sparrow cohort*							
Infection	13	2.9–3.6	13	2.8–3.8	13	3.9–4.9	4
Nonmanipulation	23	2.9	23	3.2	22	3.4	13

*Total no. animals in nonmanipulation cohort also includes animals from infection cohort. NT, not tested.

Data and Statistical Analyses

Only those animals with evidence of infection (detection of virus or antibodies) were included in the statistical analyses. Viremia and antibody response profiles were determined by calculating daily geometric mean titer values. Viremia and antibody values below the limit of detection were considered halfway between 0 and limit of detection: 1.0 $\log_{10}$ PFU/mL for viremia and 1:20 neutralizing antibody. A 2-way analysis of variance with Bonferonni posttest was used to analyze viremia and antibody data. Although all cohorts were considered individually for these analyses, the feral and 7-8–week rat cohorts and the 2 sparrow cohorts were each combined for graphical clarity and because their daily mean viremia titers and survival rates did not differ statistically. House sparrow survival analysis also included a third cohort for which we assessed survival rates in those not manipulated. These combined groups are denoted mature cotton rats and house sparrows. We used the log-rank test to analyze survival data. $p<0.05$ was considered significant.

Results

Viremia Pro les

Within Species

The viremia profiles of mature cotton rats showed higher initial replication of SA PE70 than NA FL93 and SA CO92, a trend particularly evident 24 h postinfection (Figure 1, panel B). All titers peaked by 48 h; SA PE70 generated the highest titers among mature rats and sharply declined thereafter. Although not statistically significant (Table 2), peak titers of NA FL93 and SA CO92 were lower than titers of SA PE70 and declined less rapidly through 72 h postinfection. The trend for juvenile rats was also higher titers of SA PE70 than of NA FL93 (Figure 1, panel A);

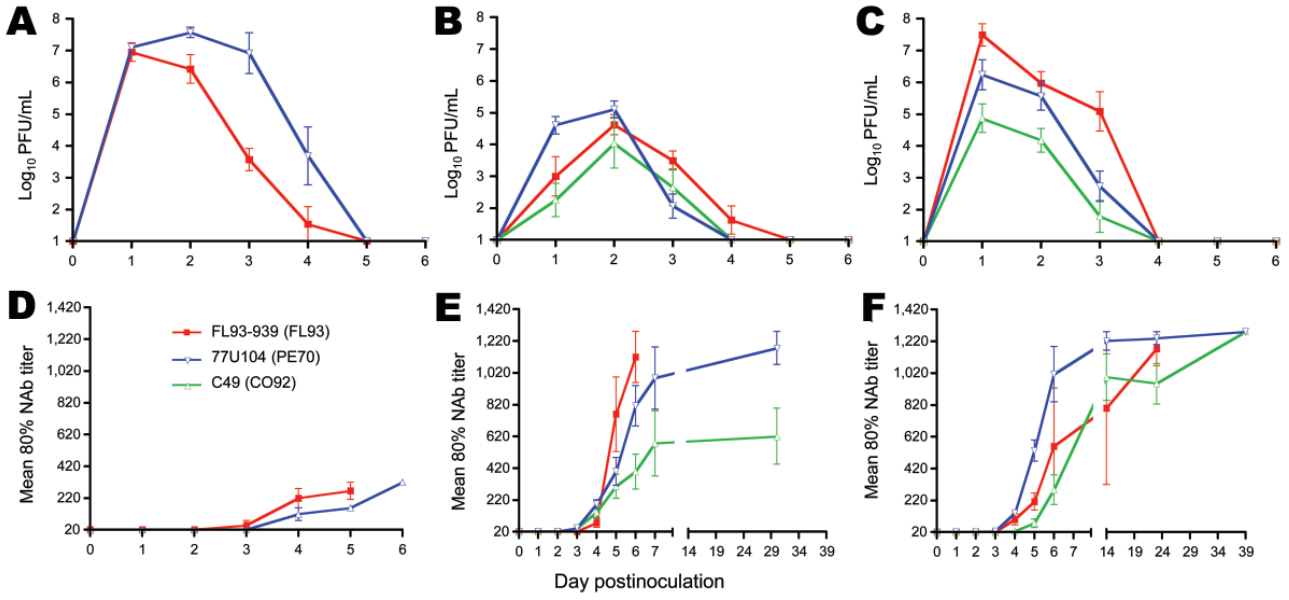

Figure 1. Mean viremia (A–C) and neutralizing antibody response (D–F) profiles in juvenile cotton rats (A, D), mature cotton rats (B, E), and house sparrows (C, F) after subcutaneous inoculation with 3–4 $\log_{10}$ PFU of North American eastern equine encephalitis virus (EEEV) strain FL93 (red lines), South American (SA) EEEV strain PE70 (blue lines), or SA EEEV strain CO92 (green lines). Note the difference in scale of the x-axis for the antibody response of juvenile rats. NAb, neutralizing antibody. Error bars represent SEM.

Table 2. Comparisons of mean peak titers of eastern equine encephalitis virus within experimental cohorts*

	Mean peak viremia titer, log_{10} PFU/mL (± SEM)			Within-cohort comparison, p value		
Cohort	FL93 (FL93-939)	PE70 (77U104)	CO92 (C49)	FL93 vs. PE70	FL93 vs. CO92	PE70 vs. CO92
Juvenile cotton rats	7.0 (0.3)	7.6 (0.2)	Not tested	0.089	NT	NT
Mature cotton rats	4.5 (0.3)	5.1 (0.3)	3.8 (0.8)	0.140	0.374	0.078
House sparrows	7.5 (0.4)	6.2 (0.5)	4.9 (0.4)	0.051	**<0.001**	0.060

*Two-tailed p-values determined by Student *t* test; p values <0.001 are not specified. **Boldface** indicates significant difference. NT, not tested.

titers for each virus strain remained significantly higher for juvenile than for mature rats (p<0.001, Tables 2, 3). NA FL93 peaked by 24 h postinfection; titers of SA PE70 were similar at 24 h, surpassed NA FL93 by 48 h, and continued to be significantly higher (p<0.001) through 96 h postinfection. SA PE70 viremia in the juvenile rats was the highest among all virus strains and rat cohorts.

House sparrows supported higher NA FL93 replication than SA PE70 throughout the experiment; SA CO92 replication was the lowest (Figure 1, panel C). NA FL93 and SA CO92 viremia profiles were similar between the 2 sparrow cohorts; however, SA PE70 titers were slightly higher in the second sparrow cohort (data not shown, differences not significant). The titer of all virus groups peaked by 24 h; the highest peak titers were in the NA FL93 infection groups (Table 2). NA FL93 and SA PE70 titers were similar at 48 h; however, NA FL93 titers were 1–3 logs higher than SA PE70 and SA CO92 at 24 and 72 h postinfection.

Between Species

Rats and sparrows were susceptible to infection with all EEEV strains; however, trends in NA and SA EEEV viremia profiles were opposite between species (Figure 1). In rats, SA PE70 titers were highest, but in sparrows, NA FL93 titers were highest. SA CO92 replication was lowest overall, and peak viremia titers were comparable between species. Viremia in mature rats peaked at 48 h postinfection and in sparrows peaked at 24 h postinfection. This rapid initial replication in sparrows also corresponded to significantly higher peak titers of NA FL93 (p<0.05–0.001) compared with those of mature rats (Table 3). SA PE70 titers were also generally higher in sparrows than in mature rats. SA CO92 titers were marginally higher in the sparrows than in mature rats; however, differences in their peak titers were not significant. In contrast, the viremia titers in juvenile rats were similar to or higher than those in sparrows. Juvenile rats sustained significantly higher SA PE70 viremia titers than the sparrows at 48, 72, and 96 h postinfection (p<0.01–001), but NA FL93 titers were comparable on all days.

Survival Rates

Of 25 mature cotton rats infected with either SA PE70 or SA CO92, 100% survived and had no signs of disease (Figure 2, panel B). In contrast, all mature rats infected with NA FL93 died. Signs of illness began on day 4 postinfection; by day 6, most animals exhibited lethargy, anorexia, dehydration, and neurologic manifestations of instability and erratic movement. Most mature rats died during days 3–6, and 1 rat died on day 17 after a prolonged illness with anorexia. One uninfected control animal died on day 7 without any detectable signs of illness. None of the juvenile rats infected with either SA PE70 or NA FL93 survived; their illness was similar to that observed in mature rats infected with NA FL93 (Figure 2, panel A). All juveniles died during days 3–6, and the mean time to death did not differ significantly between groups. Juvenile rats were not inoculated with SA CO92.

For sparrows, NA FL93-infection resulted in a 26% survival rate, which was significantly lower than the 82%–83% survival rates for SA PE70- and SA CO92–infected sparrows (p<0.001). Mortality rates for sparrows did not differ significantly from those for mature rats for all viruses (p>0.3). The NA FL93–induced mortality rate for juvenile rats was comparable to those for NAE FL93–infected sparrows and mature rats (p>0.3); however, the mortality rate for juvenile rats infected with SA PE70 was significantly greater than that for sparrows and mature rats infected with SA PE70 (p<0.001).

Antibody Responses

For rats and sparrows, antibodies were detected by day 4 postinfection (Figure 1, panels D–F). Antibodies were detected in all animals that had detectable viremia and that survived beyond day 3; however, some mature rats infected with SA CO92 had low antibody titers

Table 3. Comparisons of mean peak titers of eastern equine encephalitis virus between experimental cohorts*

	Mean peak virus titer, log_{10} PFU/mL (± SEM)			Between-cohort comparison, p value		
Virus	Juvenile cotton rats	Mature cotton rats	House sparrows	Juvenile vs. mature rats	Juvenile rats vs. house sparrows	Mature rats vs. house sparrows
FL93 (FL93-939)	7.0 (0.3)	4.5 (0.3)	7.5 (0.4)	**<0.001**	0.271	**<0.001**
PE70 (77U104)	7.6 (0.2)	5.1 (0.3)	6.2 (0.5)	**<0.001**	**0.026**	**0.036**
CO92 (C49)	NT	3.8 (0.8)	4.9 (0.4)	NT	NT	NT

*Two-tailed p values determined by Student *t* test; p values <0.001 are not specified. **Boldface** indicates significant difference. NT, not tested.

in the absence of detectable viremia. In the mature rats, the antibody response to NA FL93 was initially more robust than that to SA EEEV, but SA EEEV antibodies were detected 1–2 days earlier (Figure 1, panel E). Similar to the pattern in mature rats, juvenile rat titers in response to NA FL93 were initially higher than titers in response to SA PE70, although juvenile rat antibody responses were much lower overall (Figure 1, panel D). The antibody responses of sparrows showed the opposite pattern to those of rats (Figure 1, panel F). Although titers were similar to those of mature rats, SA PE70–infected sparrows generated a more robust initial response than those infected with NA FL93 or SA CO92. Unlike the mature rats, some NA FL93–infected sparrows survived, and the antibody response to all 3 viruses ultimately reached the highest measured titers. The early antibody responses to NA FL93 and SA PE70 in mature rats and in sparrows were inversely related to their respective viremia profiles; however, a consistent correlation at the individual animal level was not found.

Discussion

Reservoir host competence depends primarily on an animal's susceptibility to infection, the intensity of viremia, and the duration of viremia sufficient to infect appropriate mosquito vectors. Rats and sparrows were equally susceptible to infection with the NA and SA EEEV strains and doses used in this study, and their viremia lasted 4–5 days. However, the patterns of infection differed; the general trend was higher SA PE70 replication in rats and higher NA FL93 replication in sparrows, consistent with the hypothesis that SA EEEV strains use mammalian hosts as their principal reservoirs. Infections of both adult species with SA CO92 resulted in the lowest overall viremia and antibody titers, suggesting an overall attenuation of this strain.

The minimum infectious oral dose for *Cs. melanura* mosquitoes, the primary NA enzootic vector, corresponds to a viremia of ≈3 $\log_{10}$ PFU/mL, and almost all mosquito species infected experimentally become infected after blood meals of at least 6 $\log_{10}$ PFU/mL (*23–25*). Regardless of slight variations in inoculum doses, all viruses resulted in viremia titers in rats and sparrows high enough to infect enzootic and epizootic vectors in North America. The highest and longest titers of NA EEEV were found in sparrows and of SA PE70 were found in juvenile cotton rats. Although the preferred habitats of both animal species differ from the hardwood swamps inhabited by *Cs. melanura* mosquitoes, our results suggest that both species have the potential to play a role as amplification hosts during epizootic and epidemic transmission. Although mosquito vectors in North America have not been evaluated for their competence to transmit the SA EEEV strains we tested, the productive infection of both animal species we tested highlights the potential for SA EEEV emergence in North American habitats.

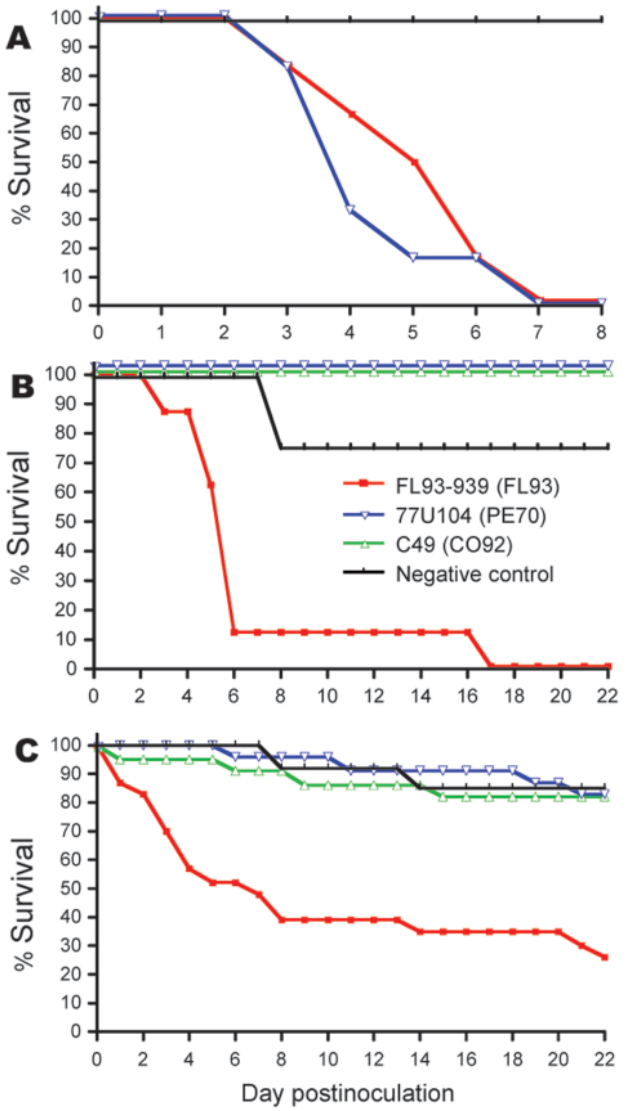

Figure 2. Survival rates for juvenile cotton rats (A), mature cotton rats (B), and house sparrows (C) after subcutaneous inoculation with ≈3–4 $\log_{10}$ PFU of North American eastern equine encephalitis virus (EEEV) strain FL93 (red lines), South American (SA) EEEV strain PE70 (blue lines), or SA EEEV strain CO92 (green lines). Survival rates beyond day 22 postinfection did not differ. Experimental infection of juvenile cotton rats with SA EEEV strain CO92 was not conducted.

Only 1 study has assessed the vector competence of mosquitoes for EEEV in South America (*8*). Turell et al. (*8*) observed that at least 50% of mosquito species in Peru, including the presumed local enzootic mosquito vector, *Cx. pedroi*, became infected after feeding on chickens or hamsters that had moderate levels of viremia (4.6–5.8 $\log_{10}$ PFU/

mL), and even more species became infected after ingesting higher doses from blood meals (7.7–8.5 $\log_{10}$ PFU/mL). Given these limited data, our study indicates that viremia sufficient in intensity and duration to serve as a source of infection for mosquito vectors in South America develops in sparrows and cotton rats. Additional vector-competence experiments with species from other foci of enzootic SA EEEV transmission (e.g., *Cx. taeniopus* mosquitoes) and experimental infections of sympatric animal species would help confirm these results and provide a more complete understanding of the ecology of EEEV in South America.

Although survival is not an essential requirement for host competence, the infection profile and pathogenicity of a virus in a host can be indicative of the host's evolutionary history. The higher virus titers induced by SA PE70 and the survival of all mature cotton rats after infection by both SA EEEV strains may indicate selection for resistance to disease or selection for attenuation of these SA viruses in this species. Selection for resistance to disease has been proposed to explain the benign outcome of experimental infections of various rodents with sympatric VEEV (*18*,*26*,*27*) as opposed to the severe disease outcome for closely related rodents from regions where the virus is not endemic. Although the subspecies of cotton rats (*S. hispidus berlandieri*) collected in Galveston does not reside sympatrically with SA EEEV, it is genetically and geographically close to members of the *S. hispidus* rat complex in areas of enzootic SA EEEV transmission (e.g., *S. hispidus hirsutus* rats) (*28*). The results of our study could reflect a long-term association between SA EEEV and ancestral *S. hispidus* rats and support their potential role in enzootic transmission of EEEV in South America.

Unlike mature rats, juvenile cotton rats experienced severe neurologic disease and 100% mortality rates after infection with either NA FL93 or SA PE70. These age-dependent disease and mortality rates have been previously observed with Sindbis virus (another alphavirus) and EEEV infection of laboratory mice (*29*,*30*). Explanations include increased virus replication in immature neurons (*31*) and metabolically active osteoblasts (*32*) and potential involvement of differential interferon induction and response (*33*). Gardner et al. (*29*) observed age-dependent survival of mice after subcutaneous inoculation with an adult mouse–attenuated strain of SA EEEV (BeAr 436087); however, NA FL93-939 resulted in severe disease and death for mice of all ages (*29*). These observations are consistent with the results of our experimental infections of mature and juvenile rats.

The survival profiles between sparrows and mature rats after experimental infection with NA or SA EEEV were similar. Although both SA strains caused slightly higher mortality rates for sparrows than for mature rats, these differences were not significant. Sparrow deaths resulting from NA FL93 correlated with the development of extremely high peak viremia titers at 1 day postinfection, suggesting the inability to control early virus replication. Although the SA EEEV virus titers at 1 day postinfection were higher in sparrows than in mature cotton rats, peak titers remained comparable between species, and no significant differences in survival rates were noted. In addition, all rats infected with NA FL93 died, despite relatively low peak viremia in mature rats. These observations suggest underlying differences in the pathogenesis of NA and SA EEEV within each species that go beyond their relative susceptibility to virus infection.

The NA EEEV-induced deaths of sparrows may also reflect the relatively recent introduction of these birds into the United States and their shorter history of exposure to EEEV. Komar et al. (*23*) reported similar mortality rates and correlation with peak viremia in NA EEEV experimental infections of European starlings (*Sturnus vulgaris*), also a nonnative species introduced into the United States in the late 1800s (*23*). Many domesticated captive birds, such as whooping cranes (*34*), emus (*35*), and ring-neck pheasants (*36*), as well as native free-ranging wild birds such as American crows (*Corvus brachyrhynchos*) (*37*) and blue jays (*Cyanocitta cristata*) (*38*), have also reportedly experienced severe disease and high mortality rates in response to EEEV infection. However, seroprevalence of EEEV antibodies in surviving wild birds in North (*39*,*40*) and South America (*11*,*12*) indicates that some avian species have the ability to survive natural infection.

Although additional ecologic studies are needed to confirm a primary vertebrate host for EEEV in Central and South America, our results demonstrate the competence of rats and of sparrows to serve as amplification hosts for NA and SA EEEV. However, the lack of detectable disease in mature rats after SA EEEV infection supports the possibility of long-term exposure of rodents to EEEV in South America. This dichotomy in rat survival rates should also be explored as a potential model for studying differences in NA and SA EEEV viral tropism and pathogenesis, which may explain differences in virulence for humans. Although enzootic transmission of NA EEEV primarily involves passerine birds, the relative competence of cotton rats and sparrows as NA EEEV hosts highlights the probable influence of *Cs. melanura* mosquito habitats and avian host preferences in shaping the ecology of EEEV in North America. NA and SA EEEV experimental infections of vertebrate and mosquito species from regions of enzootic SA EEEV transmission would complement these studies and broaden our understanding of the evolution of these viruses and their potential to emerge and adapt to new environments.

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Legionellosis Outbreak Associated with Asphalt Paving Machine, Spain, 2009

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From 1999 through 2005 in Alcoi, Spain, incidence of legionellosis was continually high. Over the next 4 years, incidence was lower, but an increase in July 2009 led health authorities to declare an epidemic outbreak. A molecular epidemiology investigation showed that the allelic profiles for all *Legionella pneumophila* samples from the 2009 outbreak patients were the same, thus pointing to a common genetic origin for their infections, and that they were identical to that of the organism that had caused the previous outbreaks. Spatial-temporal and sequence-based typing analyses indicated a milling machine used in street asphalt repaving and its water tank as the most likely sources. As opposed to other machines used for street cleaning, the responsible milling machine used water from a natural spring. When the operation of this machine was prohibited and cleaning measures were adopted, infections ceased.

Legionella pneumophila (*1*) is a gram-negative bacterium identified as the causative agent of an outbreak of pneumonia that occurred in a Philadelphia hotel during a Legionnaires' convention in 1977 (*2*). This outbreak affected 221 persons, of whom 34 died (*2*). Although other *Legionella* spp. can cause the disease, *L. pneumophila* is responsible for 90% of the cases of legionellosis globally. This species is 1 of the most common causes of community-acquired bacterial pneumonia (*3*–*7*) and the second most common cause of severe pneumonia (*8*).

L. pneumophila is a waterborne bacterium that can cause respiratory illness when a susceptible person inhales contaminated, aerosolized water. Infection sources are usually human-made aquatic habitats, such as potable water supplies (*9*), whirlpool spas (*10*), cooling towers (*11*,*12*), showers (*13*), decorative fountains (*14*,*15*), and hoses (*16*).

Molecular epidemiologic analyses of *L. pneumophila* usually compare sequence-based typing patterns from bacterial cultures derived from putative environmental sources with *L. pneumophila* cultures from patients' sputum samples. This approach consists of amplifying and sequencing internal fragments of 7 loci and assigns a number to the different alleles derived from each locus (*17*,*18*). The combination of allele variants for each locus determines the allelic profile that characterizes each sample. Isolation of *L. pneumophila* from respiratory secretions on selective media is fastidious and time-consuming. Moreover, some *L. pneumophila* strains may be viable but cannot be cultured (*19*); despite high specificity of this method, culturing *L. pneumophila* is not efficient (*20*,*21*). Therefore, the efficiency of sequence-based typing of *L. pneumophila* can be improved by direct amplification and sequencing of DNA extracted from uncultured respiratory samples (*22*).

Since 2000, a specific epidemiologic surveillance system for legionellosis has been in place in the Hospital Virgen de los Llirios in Alcoi, Spain. Every patient with signs of pneumonia is scanned by chest radiography and urine analysis for *L. pneumophila* serogroup 1 antigen. This surveillance system enables early detection and better prognosis for *L. pneumophila*–infected patients. It also helps distinguish between sporadic cases and outbreaks, thus enabling early start of epidemiologic investigations.

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At the end of July 2009, the epidemiology surveillance system detected 2 cases of legionellosis in persons who had stayed in Alcoi, Spain, during their incubation periods. New cases appeared during the first week of August, at which time an epidemic outbreak was declared and an epidemiologic investigation was started. Patients in the outbreak were questioned about clinical and personal aspects. Spatial–temporal analysis was used to identify the most likely areas of exposure for infection. *L. pneumophila* was isolated from environmental samples obtained in those areas. Because the usual facilities and municipal water systems associated with risk were not contaminated, other facilities not previously linked to legionellosis outbreaks were considered. To verify the common genetic origin of the outbreak and its environmental source, we performed an epidemiologic molecular analysis using sequence-based typing for clinical and environmental samples.

Materials and Methods

Legionellosis Cases

Cases of pneumonia reported in Alcoi during the incubation period were considered suspected cases of legionellosis unless this diagnosis could be ruled out. A confirmed case of legionellosis was defined as a case of pneumonia with laboratory evidence of acute infection with *L. pneumophila* including 1) isolation of *L. pneumophila* serogroup 1 from respiratory secretions or lung tissue, 2) a ≥4-fold rise in antibody titers (from 128) against *L. pneumophila* serogroup1 by immunofluorescence in paired acute- and convalescent-phase serum specimens, or 3) detection of *L. pneumophila* serogroup 1 antigen in urine.

Cases that conformed to the case definition and/or had positive results in at least 1 of the following tests were considered suspected cases: 1) high (>256) antibody titer against *L. pneumophila* serogroup 1 in convalescent-phase serum, 2) seroconversion to *L. pneumophila* serogroup 1 by indirect inmunofluorescence in acute- and convalescent-phase serum, or 3) direct staining of bacteria in respiratory secretions or lung tissue by direct fluorescence using monoclonal or polyclonal antibodies against any *Legionella* spp. or serogroup, including serogroup 1.

Epidemiologic Investigation

A questionnaire was used to obtain personal information (age, gender, job, and free-time activities), clinical features (signs and symptoms, date of illness onset, date of hospitalization, previous hospitalizations, and medical history), predisposing and risk factors, place of residence, and recent urban mobility within Alcoi. Information about the patients' addresses and usual itineraries (roads and places visited) in Alcoi was used to delimit an area of influence, or a buffer, for the location of the likely source of infection. A 500-m radius was considered around home and city itineraries for each patient by using vectorial cartography (scale 1:10,000), ortophotography (scale 1:5,000), and ArcView software (www.esri.com). The buffer for each patient was thus represented by a polygonal area; intersecting areas yielded a common area representing a likely location for the source of the outbreak.

Environmental Investigation

Systematic environmental investigations are regularly performed in the municipal water distribution system, but when this outbreak was detected, an active search for *L. pneumophila* was made in patients' homes (bulk water and biofilms from showerheads and taps) and the water distribution system (bulk water); results were negative. None of the other usual sources (e.g., public fountains, cooling towers, humidifiers) were found to pose a risk. The absence of usual risk sources led us to consider other possible sources of aerosols, including moving devices used in street cleaning and asphalt repaving; the latter had been observed in the risk area during the epidemiologic inspection.

Laboratory Methods

Clinical Sampling

Respiratory samples and, when available, corresponding cultures were obtained from 11 patients with a diagnosis of legionellosis made by positive urine test result at the Hospital Virgen de los Lirios. DNA was extracted from respiratory isolates by using an UltraClean BloodSpin Kit (Mobio Laboratories, Inc., Carlsbad, CA, USA). DNA from positive *L. pneumophila* cultures was extracted as described (*23*). Briefly, bacterial colonies from pure cultures were resuspended in 200 μL of 20% Chelex 100 resin (Bio-Rad Laboratories, Richmond, CA, USA). DNA was then extracted during 3 freeze–thaw cycles (–75°C for 10 min and 94°C for 10 min), and cellular debris was removed by centrifuging at 10,000 × *g* for 1 min. The amount of genomic DNA was measured by spectrophotometry at 260 nm in triplicate, and DNA purity was checked by using the A260/A280 ratio. Purified DNA was stored at –20°C until used. DNA extraction from respiratory samples (sputum, bronchoalveolar aspirated secretions, and lung biopsy tissue) was performed with a QIAamp DNA Mini Kit (QIAGEN, Valencia, CA, USA) and stored at –20°C.

Environmental Sampling

Water samples were filtered and treated with acid. Water and swab specimens were plated onto buffered charcoal yeast extract medium with and without supplemental antimicrobial drugs; standard plating techniques were used. Inoculated plates were incubated at 35°C and examined reg-

ularly for colonies resembling *Legionella* spp. Suspected colonies were inoculated onto biplates containing buffered charcoal yeast extract medium with and without L-cysteine. Nine cultures that required L-cysteine for growth were subcultured and tested with specific antiserum to determine the *Legionella* spp. and serogroup.

PCR, Sequencing, and Allelic Pro le Assignment

For cultured isolates, the 7 loci in the European Working Group for *Legionella* Infections (EWGLI, www.ewgli.org) typing scheme were amplified as detailed by Gaia et al. (*17*) and Ratzow et al. (*18*). DNA from respiratory samples was amplified by using a seminested approach. For the first PCR we used the same primers and conditions as for cultured isolates. For the second PCR we used the internal primers described by Coscollá et al. (*22*) and used 2 μL of the first PCR products as template. Amplification conditions and profiles were the same as for the first PCR except for the annealing temperature (*22*). PCR products were purified by using a High Pure PCR Product Purification Kit (Roche Diagnostics, Mannheim, Germany). PCRs were conducted in a reaction volume of 50 μL containing 20 ng of genomic DNA, 1 U of Taq DNA polymerase, 200 μmol/L of each dNTP, 2.5 mmol/L of magnesium-free buffer, 2.5 mmol/L $MgCl_2$, and 0.2 μmol/L of each pair of primers. We adopted the gene notation used in the first *L. pneumophila* genome published (*24*); consequently, locus *fliC* corresponds to locus *flaA* used in the EWGLI typing scheme (*17*).

Purified DNA was directly sequenced by the dideoxy method by using a BigDye Terminator v3.0 Ready Reaction Cycle Sequencing Kit and was analyzed in an ABI PRISM 3700 sequencer (each from Applied Biosystems, Foster City, CA, USA). Sequencing of PCR products from respiratory samples differed only in the use of the same internal primers used in the second PCR of the seminested approach. Sequence chromatogram files were analyzed by using the Staden sequence analysis package (*25*).

Allelic profiles for the 7 sequenced genes were obtained from EWGLI and were aligned and compared with sequences derived in this study. Multiple sequence alignments were obtained by using ClustalX (26) and further refined by visual inspection.

Molecular Phylogenetic Analysis

A phylogenetic reconstruction was obtained with the concatenated alignment of sequences from the 7 loci analyzed. Models of nucleotide substitution were assessed by using the maximum-likelihood approach implemented in jModeltest (*27*). Maximum-likelihood phylogenetic trees were obtained with PHYML 3.0 (*28*) by using the previously derived models of nucleotide substitution for each locus. Support for the nodes was evaluated by bootstrapping with 1,000 pseudoreplicates.

Results

Epidemiologic Findings

Among patients with positive urine antigen test results, 11 cases of legionellosis were confirmed and *L. pneumophila* was isolated from 4. All patients required hospitalization, and all except 1 recovered. (The patient who did not recover had severe signs and symptoms and subsequently died.) The main signs and symptoms were fever (100% incidence), pneumonia (100%), headache (27.3%), myalgia (27.3%), diarrhea and/or vomiting (18.2%), and confusion (45.5%). The average age was 70 years, range 49–88 years. More men than women were affected (male:female ratio = 4.5).

Confirmed cases occurred from July 21 through September 17. According to the date of disease onset, the outbreak showed 3 epidemic waves: 2 cases in the second half of July, 8 cases in the first half of August, and 1 case in the second half of August (Figure 1).

No common indoor source of exposure was found, and the initial hypothesis was that the outbreak originated from environmental contamination of an unknown source capable of producing and dispersing large quantities of aerosols contaminated with *L. pneumophila*. The first 2 patients lived in the northern part of the city, which suggested that the source could be located in that area. The spatial distribution of patients' buffers changed in August, thus indicating that the likely source of the outbreak had moved to the

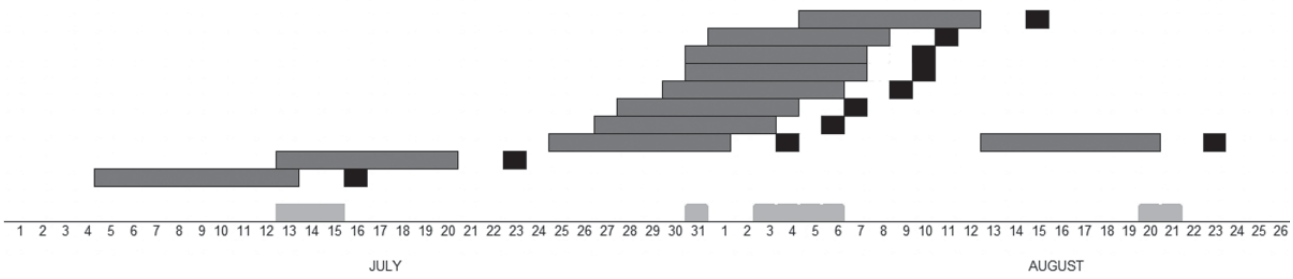

Figure 1. Timeline for epidemic of legionellosis, Alcoi, Spain, 2009. Light gray squares indicate days the paving machine was working; dark gray squares indicate incubation period; black squares indicate day of illness onset.

Santa Rosa quarter (Figure 2). The area of epidemic risk was modified accordingly, and the search for putative environmental sources focused on that neighborhood.

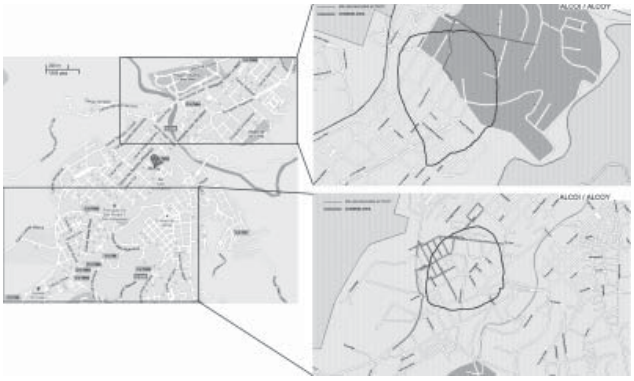

Figure 2. The 2 neighborhoods in Alcoi, Spain, with identified risk areas for the outbreak of legionellosis, 2009 (left). For each area, an enlarged map at right shows the common risk area (black lines) and the streets where the milling machine and water tank had operated during the incubation periods (gray lines).

Environmental Findings

L. pneumophila was not isolated from samples derived from the municipal water supply, traditional risk facilities, and patients' houses. The environmental investigation was extended to other potential sources, especially portable cleaning devices such as sweepers, hydrocleaners, and water tanks used to clean the streets, all of which used water from the municipal water supply. At that time, the Santa Rosa quarter was being repaved, and 1 of the machines used in the repaving process was a tank truck that carried water used by a large milling machine. The water in the tank was obtained from a natural spring untreated with chlorine or anything else. Because the average daytime temperature in Alcoi during July and August is 27°C, the water in the machine might have been warm enough for *L. pneumophila* growth. The milling machine had been working north of Alcoi around July 15 and in the Santa Rosa neighborhood from July 31 through August 20 (Figure 2). This activity fits spatially and temporally with the incubation period of confirmed cases. This machine was identified and removed from service on August 21, thus was able to cause the last infection detected on August 23 (Figure 2).

Microbiological Findings

L. pneumophila serogroup 1 was isolated from the water in the tank and from the atomizers in the milling machine (Figure 3). *L. pneumophila* was also isolated from other machines used for street cleaning in the city, but these organisms were from serogroups other than serogroup1.

The milling machine and the water tank were immediately confined outside the city, sealed, cleaned, and disinfected. When the machine was put back into use, a new cleaning and maintenance protocol was implemented to prevent future contamination. The cleaning protocol consisted of treatment with chlorine (20 ppm) and removal of the atomizers and their replacement by gravity-based water distributors. Additionally, for operation in the city, use of a separate thermo-insulated water tank in which chlorine was continuously applied at 20 ppm was required, thus preventing any further growth of *L. pneumophila*. Additionally, water had to be obtained from the municipal system.

Molecular Characteristics

L. pneumophila sequence information was obtained from 7 uncultured sputum samples and from 4 cultures derived from the 11 patients studied. Complete allelic profiles, according to the EWGLI typing scheme, were obtained for 9 clinical samples in the study. All had the same profile, which corresponded to sequence type (ST) 578 in the EWGLI database (Table 1). Partial allelic profiles for the other 2 samples were consistent with ST578. The 9 *L. pneumophila* isolates derived from putative environmental sources, including the milling machine and the water tank (Table 2), were also characterized and corresponded to 4 STs: ST578 in 4 samples, ST1 in 3 samples, ST777 in 1 sample, and a new ST in sample 4159.

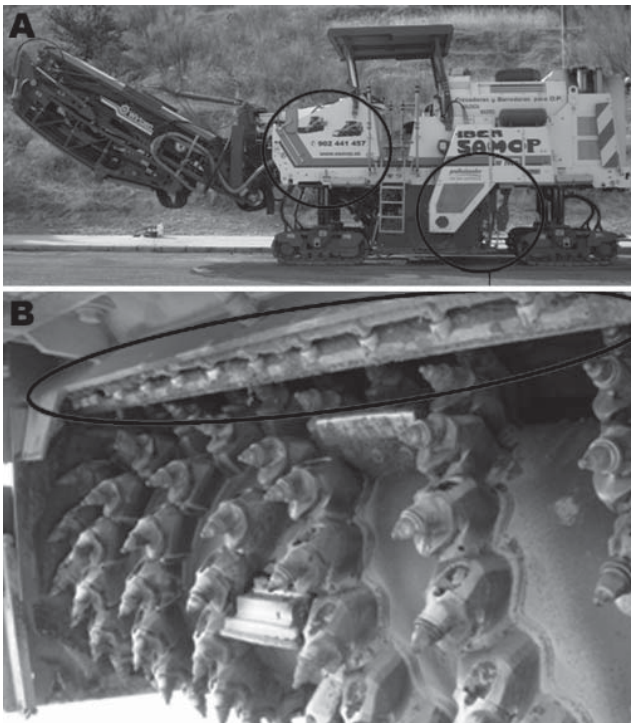

Figure 3. Milling machine and water tank used for asphalt paving in Alcoi, Spain, 2009, during outbreak of legionellosis. The 2,000-L water tank (A, left circle) supplied the 18 atomizers (A, right circle; B, oval), which sprayed 8,000 L water/d.

Table 1. Sequence information for *Legionella pneumophila* isolates from patients with legionellosis, Alcoi, Spain, August 2009*

Patient no.	Sex	Sample collection date	Sample type	EWGLI sequence-based typing pattern	ST
0203	M	13	Isolate and sputum	6, 10, 15, 13, 9, 14, 6	578
0205	F	13	Sputum	6, 10, 15, 13, 9, 14, 6	578
0260	F	17	Sputum	6, 10, 15, 13, 9, 14, 6	578
0263	F	17	Isolate and sputum	6, 10, 15, 13, 9, 14, 6	578
0293	M	18	Sputum	6, 10, 15, 13, 9, 14, 6	578
0295	M	18	Isolate and sputum	6, 10, 15, 13, 9, 14, 6	578
0300	M	18	Sputum	0, 0, 15, 0, 9, 0, 0	NA
0351	F	20	Sputum	6, 0, 15, 0, 9, 0, 0	NA
0353	M	20	Sputum	6, 10, 15, 13, 9, 14, 6	578
0372	M	20	Sputum	6, 10, 15, 13, 9, 14, 6	578
1107	F	30	Isolate and sputum	6, 10, 15, 13, 9, 14, 6	578

*EWGLI, European Working Group for *Legionella* Infections; ST, sequence type; 0, sequence not available; NA, sequence type not available.

A phylogenetic tree was obtained from the concatenated alignment of the 2,984 bp (Figure 4). The phylogenetic analysis showed that 4 environmental samples were identical to the clinical samples (represented by ST578 C/E in Figure 4). Their comparison with the remaining environmental samples showed 14–50 nt differences to strains 4159 and 4143/4160/7970 (represented by ST1 in Figure 4), respectively. The comparison of these to reference strains showed that outbreak samples were more closely related to the Corby strain (*29*), a virulent human isolate from which it differed by only 6 nt.

Discussion

During the epidemic outbreak of legionellosis in the summer of 2009 in Alcoi, Spain, 11 affected persons were identified, and their *L. pneumophila* isolates shared the same sequence-based typing profile (ST578). Spatial-temporal analysis of outbreak cases pointed to a milling machine used in street asphalt repaving and its water tank as the most likely source of infection. Molecular typing confirmed that *L. pneumophila* isolated from the machine showed the same allelic profile as the samples from the patients. When this machine was removed from service and cleaned, infections in this locality ceased.

The absence of apparent risk facilities in the area during the outbreak period and the changing spatial distribution of cases led us to consider alternative sources of contamination and spread. The heterogeneous spatial grouping led us to hypothesize that the transmission sources could be mobile. The analysis of the water tank and milling machine used in both neighborhoods where risk areas were identified during the incubation period of confirmed cases resulted in isolation of *L. pneumophila*. Molecular results confirmed that 4 of the 9 environmental isolates obtained showed a sequence-based typing profile identical to that of all clinical samples. This result highlights the fact that a device not previously considered to represent a risk for *L. pneumophila* infection, such as a street paving machine, can be associated with a legionellosis outbreak.

These kinds of machines are good candidates for spreading *L. pneumophila* infections, given their ability to generate aerosols. These machines are used in urban areas where contaminated aerosols can be inhaled by many citizens. They are continually moving, making their identification as a source of infection more difficult because by the time an outbreak is detected and the causative *L. pneumophila* strains are characterized, these machines have usually moved to another location. Lack of suitable cleaning routines for these machines makes them excellent candidates for colonization with *L. pneumophila*. In this particular outbreak, use of untreated water from a natural spring contributed to the contamination of the tank and milling machine. These devices are frequently stored and operated in and from industrial areas where

Table 2. *Legionnella pneumophila* isolated during environmental investigation, Alcoi, Spain, 2009*

Isolate no.	Sampling site	EWGLI sequence-based typing pattern	ST
4143	Cold-water tap (sports club)	1,4,3,1,1,1,1	1
4145	Water accumulator (sports club)	6,10,15, 13, 9,14,6	578
4159	Street sweeper (sprayer)	1,10,14,10,18, X†, 0‡	New
4160	Street sweeper (drain tap)	1,4,3,1,1,1,1	1
4161	Street sweeper (container)	5,2,22,10,6,25,1	777
7968	Water tank (hose pipe)	6,10,15, 13, 9,14,6	578
7969	Milling machine (tap 1)	6,10,15, 13, 9,14,6	578
7970	Milling machine (tap 2)	1,4,3,1,1,1,1	1
7973	Water tank	6,10,15, 13, 9,14,6	578

*EWGLI, European Working Group for *Legionella* Infections; ST, sequence type.
†Not obtained.
‡Unassigned new allele.

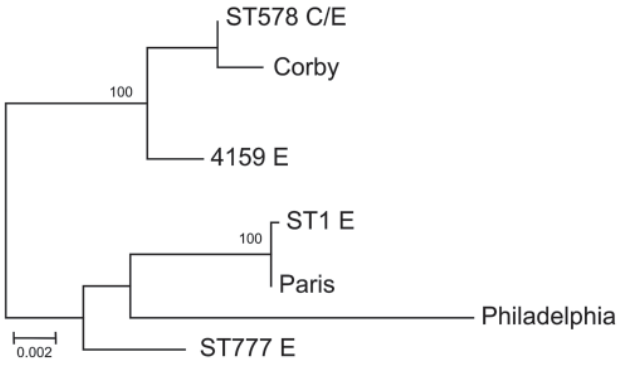

Figure 4. Maximum-likelihood phylogenetic tree obtained from the concatenated alignment of 7 *Legionella pneumophila* genome loci obtained from clinical (C) and environmental (E) samples during outbreak of legionellosis in Alcoi, Spain, 2009. Isolates with identical sequence types (Tables 1, 2) are represented as 1 isolate. Parentheses enclose the number of samples showing each sequence type. Reference sequences for Philadelphia, Paris, and Corby strains are included. Nodes supported by bootstrap values >70% are indicated. Scale bar indicates 0.002 nucleotide substitutions/position in the sequence.

nontreated water supplies are common, which increases risk for colonization by *L. pneumophila*. Whether they spread *L. pneumophila* more easily than other devices usually linked to such outbreaks, like cooling towers or spas, is unknown, but they should be considered risk devices for the reasons detailed above.

During 1999–2005, ST578 has been found in clinical samples from patients with legionellosis in Alcoi (*22*) and has caused recurrent outbreaks and sporadic cases of community-acquired pneumonia. However, to our knowledge, no identical isolate has been found in the environment in this area (M. Coscollá et al., unpub. data). Finding ST578 in a sports club (Table 2), not related to the outbreak, indicates that it can occasionally be found in other risk facilities, which would help to explain its association with past clinical cases in Alcoi. Additionally, this profile was reportedly found in an environmental strain detected in Mexico during an epidemiologic investigation of travel-associated legionellosis (EWGLI sequence-based typing database, www.ewgli.org).

Although culture isolates were available for only 4 of the 11 outbreak patients identified, our use of a sequence-based typing approach to analyze clinical samples on the basis of direct extraction and sequencing of *L. pneumophila* from sputum samples increased the number of patients we were able to study (i.e., all those identified in the outbreak). The efficiency of this approach has been demonstrated to be higher than that of sequencing after isolating *L. pneumophila* from cultures (*22*). Moreover, the 100% match between the sequences directly obtained from respiratory samples and cultured isolates from the same patient corroborates the suitability of the direct sequencing approach for the identification and molecular epidemiology studies of *L. pneumophila* (*22*). We think that this approach extends the usefulness of molecular epidemiologic tools in the study of *L. pneumophila* outbreaks, thus enabling more precise identification of their sources.

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Comparison of Pandemic (H1N1) 2009 and Seasonal Influenza, Western Australia, 2009

Dale Carcione, Carolien Giele, Gary K. Dowse, Donna B. Mak, Leigh Goggin, Kelly Kwan, Simon Williams, David Smith, and Paul Ef er

We compared confirmed pandemic (H1N1) 2009 influenza and seasonal influenza diagnosed in Western Australia during the 2009 influenza season. From 3,178 eligible reports, 984 pandemic and 356 seasonal influenza patients were selected; 871 (88.5%) and 288 (80.9%) were interviewed, respectively. Patients in both groups reported a median of 6 of 11 symptoms; the difference between groups in the proportion reporting any given symptom was ≤10%. Fewer than half the patients in both groups had ≥1 underlying condition, and only diabetes was associated with pandemic (H1N1) 2009 influenza (odds ratio [OR] 1.9, 95% confidence interval [CI] 1.1–3.5). A total of 129 (14.8%) persons with pandemic (H1N1) 2009 and 36 (12.5%) persons with seasonal influenza were hospitalized (p = 0.22). After controlling for age, we found that patient hospitalization was associated with pandemic (H1N1) 2009 influenza (OR 1.5; 95% CI 1.1–2.1). Contemporaneous pandemic and seasonal influenza infections were substantially similar in terms of patients' symptoms, risk factors, and proportion hospitalized.

Pandemic (H1N1) 2009 influenza A emerged in Mexico in March 2009 and was first reported in the United States the following month, toward the close of the 2008–09 influenza season in the Northern Hemisphere (*1*,*2*). The virus rapidly spread worldwide, with the first pandemic (H1N1) 2009 infection reported in Australia on May 9, 2009, just before the start of the traditional winter influenza season in the Southern Hemisphere (*3*).

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There are little data directly comparing confirmed pandemic (H1N1) 2009 with contemporaneous seasonal influenza over the same influenza season (*4*–*6*). Many of the reports on the epidemiology of influenza in 2009 to date have focused exclusively on pandemic (H1N1) 2009 or have used limited laboratory-based surveillance data on isolation rates for seasonal and pandemic (H1N1) 2009 influenza viruses (*7*–*13*). Other reports have compared pandemic (H1N1) 2009 and seasonal influenza infections that occurred outside the usual influenza season (*14*). Still other investigators have compared various indicators of influenza severity during the current pandemic with historical data from previous annual influenza epidemics (*15*,*16*). Interpretation of such comparisons is challenging because of variation in influenza activity from season to season. Furthermore, heightened awareness surrounding the current pandemic may have affected patient care-seeking behavior or physician diagnostic practices, thus potentially creating bias in year-to-year comparisons. Examining confirmed pandemic (H1N1) 2009 and seasonal influenza infections occurring in the same population during the 2009 influenza season enables a more straightforward comparison.

We interviewed persons with laboratory-confirmed pandemic (H1N1) 2009 or seasonal influenza infection over a 10-week period encompassing the peak of the winter influenza season. This effort enabled us to directly compare the clinical illness and predisposing medical risk factors associated with pandemic (H1N1) 2009 and seasonal influenza infections diagnosed contemporaneously from the general population of Western Australia, which has a population of 2.2 million persons.

Methods

All clinical laboratories report positive influenza test results to the Communicable Disease Control Directorate

(CDCD), Department of Health, Western Australia. For diagnosis, respiratory samples, usually combined nose and throat swab specimens, were tested by PCR. More than 90% of the specimens were tested at PathWest Laboratory Medicine Western Australia, Queen Elizabeth II Medical Centre, by using an assay that identified and distinguished between pandemic (H1N1) 2009 and seasonal influenza A/H1, A/H3, and B (*17*); positive results for pandemic (H1N1) 2009 influenza virus reported from other clinical laboratories were considered as single infections with pandemic (H1N1) 2009.

Reports of all PCR-confirmed influenza infections were reviewed. Patients were excluded if the results could not differentiate between pandemic and seasonal viruses or if the patient was identified as infected with pandemic (H1N1) 2009 and seasonal influenza. Pandemic influenza was defined as PCR-confirmed pandemic (H1N1) 2009 influenza infection, and seasonal influenza was defined as any PCR-confirmed influenza infection for which infection with pandemic (H1N1) 2009 virus had been excluded.

The study began May 29, 2009 (1 week after the illness onset in the first person with confirmed pandemic [H1N1] 2009 influenza infection in Western Australia) and concluded August 7, 2009 (Figure 1) (*18*). From the study inception through July 13, 2009, attempts were made to interview every patient with confirmed influenza illness reported to CDCD. On July 14, 2009, we instituted a sampling framework because of increasing numbers of reported infections. The sampling scheme entailed identifying the last digit of a sequentially-assigned identification number from the first patient reported each day with seasonal or pandemic (H1N1) 2009 influenza, then selecting all patients reported that day with the same last digit. If <20 patients were identified for interview, we added 1 to the digit (n + 1) and selected additional patients by using the same procedure. This process was repeated until up to 20 persons with seasonal influenza and 20 with pandemic influenza were chosen each day. If <20 influenza infections were reported on a given day for either seasonal or pandemic (H1N1) 2009 influenza, we attempted to interview all patients reported on that day.

Study participants were interviewed by a trained nurse who used a standard questionnaire. If a patient was unable to answer questions or was <18 years of age, the nurse interviewed a parent or other family member familiar with the patient's situation. We made 6 attempts to contact the patient or a proxy, after which the patient was considered not contactable. Diagnostic specimens of participating patients were collected a median of 2 d after illness onset (interquartile range [IQR] 1–3 d), and patients were interviewed a median of 6 d after onset (IQR 5–8 d).

The patient's self-reported symptoms, treatment with antiviral medications, presence of underlying medical conditions, and disease disposition were recorded. Information on hospitalization was obtained at the time of the interview and by retrospectively querying a hospital discharge database that covers all public hospitals in the state and 1 major private metropolitan facility. A cross-check with the hospital discharge database was performed for every influenza notification received at CDCD.

For our analysis, we first characterized all patients with pandemic (H1N1) 2009 or seasonal influenza infection reported to CDCD during the study period (the target population) in terms of age, sex, and hospitalization status by using univariate Mantel-Haenszel χ^2 tests for proportions and *t* tests for population means. We then compared patients who were interviewed (the study population) with the remaining patients not interviewed in the target population in terms of age, sex, and hospitalization status; if a signifi-

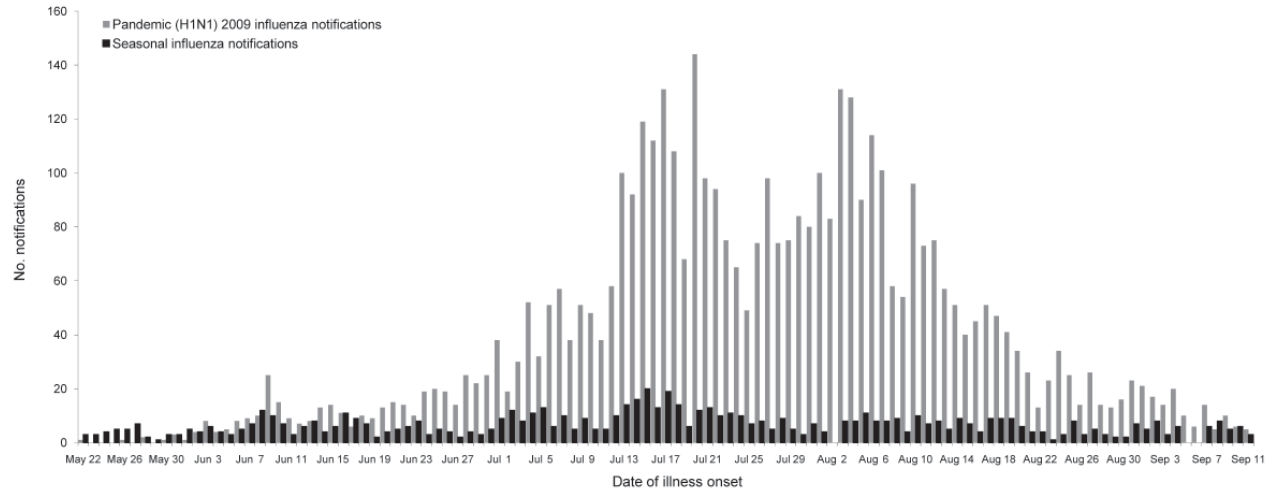

Figure 1. Number of notifications for pandemic and seasonal influenza, by date of onset and type, Western Australia, May 22–September 11, 2009. Influenza subtypes reported during the study period (n = 3,178): pandemic (H1N1) 2009, 2,794 (87.9%); influenza A (H3N2), 253 (8.0%); seasonal influenza A (H1N1), 89 (2.8%); influenza B, 36 (1.1%); and seasonal influenza A (not subtyped), 6 (0.2%).

cant difference was identified, we weighted the interview responses from the study participants to reflect the target population (*19*). Finally, by using the data obtained during interviews, we performed univariate analyses to compare patients with pandemic influenza with those with seasonal influenza with respect to reported symptoms, underlying medical conditions, and treatment. Because the age structure of the population with pandemic influenza differed from that with seasonal influenza, we also computed odds ratios (ORs) for individual symptoms or underlying medical conditions by using logistic regression to control for age. In each of the regression analyses, the dependent variable was defined as influenza type (pandemic/seasonal), and the independent variables were limited to age in years and 1 dichotomous variable representing the presence or absence of a single patient characteristic (e.g., a symptom or underlying medical condition).

To assess whether antiviral medications might have influenced the symptoms reported, we performed a subanalysis restricted to patients who were treated with antiviral agents within the first 2 days of illness onset (early use of antiviral drugs) and compared those patients with patients who were never treated, controlling for age, influenza type, and the presence of underlying medical conditions.

Influenza-like illness was defined as documented fever >38°C or a history of fever when the temperature was not known, and cough or sore throat, or both. Risk difference was defined as the absolute difference in the proportion of pandemic and seasonal influenza patients reporting a given parameter. A p value <0.05 was considered significant. Statistical analyses were performed by using Epi Info 2000 (Centers for Disease Control and Prevention, Atlanta, GA, USA).

Results

Characteristics of the Target Population

A total of 3,313 notifications of laboratory-confirmed influenza were received at CDCD during the study period. Of these notifications, 117 (3.5%) were excluded because information on the viral strain and/or subtype was incomplete, and 18 (0.5%) were excluded because pandemic and seasonal influenza viruses were detected (Figure 2). Of the remaining 3,178 influenza infections reported, 2,794 (87.9%) were pandemic (H1N1) 2009 influenza and 384 (12.1%) were seasonal influenza. The proportion of each influenza subtype identified is shown in the inset in Figure 1. The mean age of patients with pandemic influenza was significantly lower than that for patients with seasonal influenza, 27 and 35 years, respectively (p<0.005).

Most of the pandemic and seasonal influenza patients were female, but the proportion of female patients among the seasonal influenza patients was significantly greater

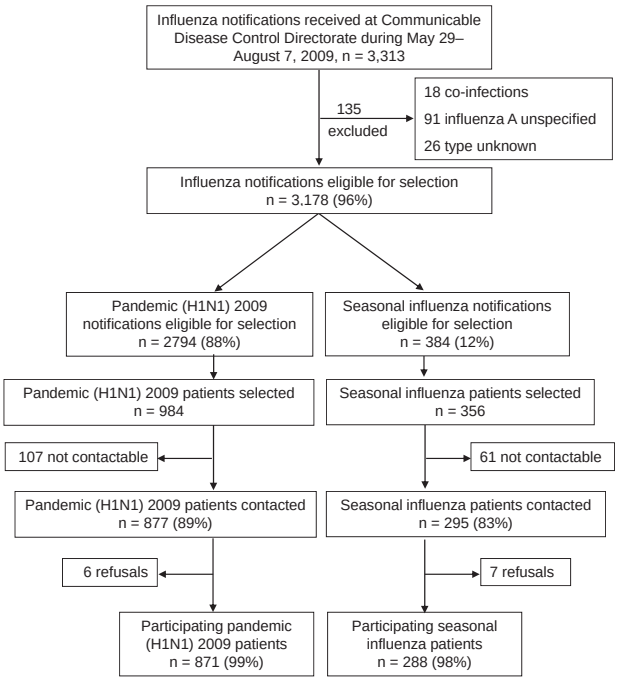

Figure 2. Recruitment of pandemic and seasonal influenza study participants, Western Australia, 2009.

than that among patients with pandemic influenza, 57.3% (220/384) and 51.2% (1,431/2,794), respectively (p = 0.03 by χ^2 test). However, the association between female sex and seasonal influenza was not significant when we controlled for age (p = 0.09).

A total of 415 (14.9%) of the 2,794 patients with pandemic influenza and 48 (12.5%) of the 384 patients with seasonal influenza were hospitalized. The difference between proportion of patients hospitalized with pandemic and seasonal influenza was not significant on univariate analysis (p = 0.22, by χ^2 test). However, when we controlled for age, the odds of hospitalization were significantly greater for persons with pandemic influenza (OR 1.53, 95% confidence interval [CI] 1.10–2.13; p = 0.011).

Selection and Representativeness of Study Participants

A total of 984 patients with pandemic (H1N1) 2009 influenza and 356 patients with seasonal influenza were selected for interview, and 871 (88.5%) and 288 (80.9%) of selected patients completed the interview, respectively (Figure 2). Of the 181 patients selected but not interviewed, 168 were not able to be contacted because they did not have a working telephone number or did not answer after 6 attempts, and 13 declined to participate.

Patients who completed interviews were very similar to the remaining notified influenza patients who were

not interviewed with respect to age and sex. The median age was 25 years (IQR 13–42 years) for study participants and 25 years (IQR 14–39 years) for the remaining notified influenza patients who were not interviewed. Women and girls accounted for 51.0% (591/1,159) of the study participants and 52.5% (1,060/2,019) of the remaining patients with notified influenza cases who were not interviewed ($p = 0.41$, by χ^2 test).

Hospitalized persons were underrepresented among the study participants compared with the remaining influenza case-patients who were not interviewed, i.e., 11.9% (138/1,159) of the interviewed patients had been hospitalized compared with 16.1% (325/2,019) of the patients not interviewed ($p<0.05$). The interview data were therefore weighted to reflect the hospitalization rate in the target population for both pandemic and seasonal influenza.

Comparison of Pandemic and Seasonal In uenza in the Study Population

The age distribution for study participants, by influenza type, is shown Figure 3. As in the target population, the mean age of study participants with pandemic (H1N1) 2009 influenza was significantly younger than the mean age of study participants with seasonal influenza, 26 and 36 years, respectively ($p<0.005$). Only 6% (49/871) of the study participants with pandemic influenza were >55 years of age compared with 23% (65/288) of those with seasonal influenza ($p<0.005$).

The distribution of the total number of symptoms reported by each patient with pandemic or seasonal influenza is shown in Figure 4. Patients with pandemic influenza and seasonal influenza reported a median of 6 symptoms (IQR 5–8 symptoms and 4–8 symptoms, respectively). When we controlled for age, no significant association was found between influenza type and the total number of symptoms that patients reported ($p = 0.19$).

The number and proportion of patients reporting specific symptoms are presented in the Table. The difference in the proportion reporting a given symptom between patients with pandemic and seasonal influenza was ≤10% for all symptoms. Univariate analyses showed that fever and diarrhea were significantly more common for patients with pandemic influenza. Controlling for age added cough and myalgia/arthralgia to the symptoms significantly associated with pandemic (H1N1) 2009 influenza compared to those with seasonal influenza. Rhinorrhea was significantly associated with seasonal influenza on univariate analysis, and this association persisted when controlling for age.

The distribution of the total number of underlying medical conditions reported by individual patients with pandemic (H1N1) 2009 or seasonal influenza is shown in Figure 5. Just over half of the patients with seasonal or pandemic influenza had no underlying medical condition(s).

The proportion of patients who reported a specific underlying medical condition is presented in the Table. The absolute difference in the proportion of patients that reported a given medical condition between those with pandemic (H1N1) 2009 and seasonal influenza was greatest for pregnant patients but still <5% for all underlying conditions queried. None of the 11 underlying medical conditions we queried were significantly associated with pandemic or seasonal influenza in univariate analysis. When controlling for age, we found that only the odds of reporting a history of diabetes were significantly greater among patients with pandemic influenza (OR 1.93, 95% CI 1.07–3.51; $p = 0.03$).

Having ≥1 underlying medical condition was not significantly associated with pandemic influenza in the univariate analyses. However, after we controlled for age, we observed that the odds of reporting ≥1 underlying medical conditions were significantly greater among patients with pandemic influenza (Table).

By design, the analysis that used weighted data shown in the Table mirrors the hospitalization rates observed for pandemic and seasonal influenza in the target population (14.9% vs. 12.5%, $p = 0.22$). The distribution of the length of stay for hospitalized study participants is shown in Figure 6. The mean duration of hospitalization was 5.1 d (median 3 d, IQR 2–6 d) for patients with pandemic influenza and 3.4 d (median 2 d, IQR 1–4 d) for those with seasonal influenza ($p = 0.13$). Although the findings were not significant, a trend toward longer hospital stays did appear for those with pandemic (H1N1) 2009 versus seasonal illness, based on the proportion of patients hospitalized an additional ≥7 d (21% vs. 8%, $p = 0.07$).

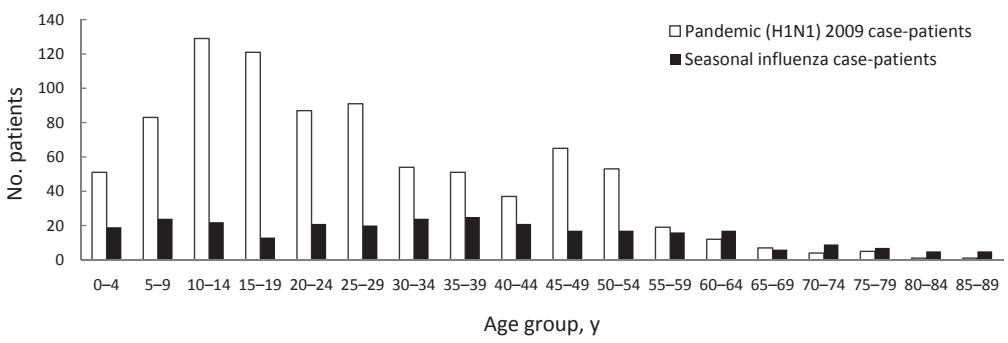

Figure 3. Age distribution for study participants, by influenza type, Western Australia, 2009.

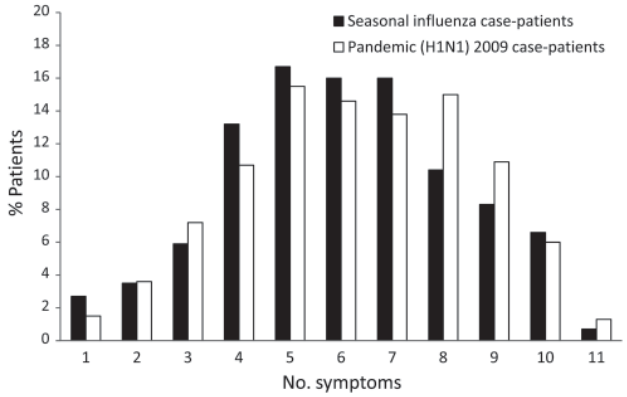

Figure 4. Number of symptoms reported by study participants with influenza, by influenza type, Western Australia, 2009.

Of the 10 patients hospitalized for ≥14 d, all had ≥1 underlying medical condition, and 6 had ≥2 conditions. Two patients in the study population died; both had pandemic (H1N1) 2009.

The proportion of patients reporting treatment with antiviral medication was significantly greater among those with pandemic influenza (Table). Information on the type of antiviral drug received was recorded for 427 (94.9%) of 450 patients treated with antiviral drugs; 426 reported taking oseltamivir and 1 reported taking zanamivir. The mean lag time between illness onset and starting antiviral treatment was 2.7 d (median 2 d, IQR 1–3 d) for patients with pandemic influenza and 2.3 d (median 2 d, IQR 1–3 d) for patients with seasonal influenza (p = 0.39).

Comparing patients given antiviral medications with those who were not, we found a significant inverse relationship (i.e., a protective effect) between early antiviral drug use and reported rhinorrhea for patients with pandemic influenza (OR 0.5, 95% CI 0.4–0.8; p = 0.005). We also observed a positive association between early antiviral drug use and nausea and vomiting; this association was robust and persisted when the analysis was simultaneously controlled for age, influenza type, underlying conditions, as well as other symptoms commonly associated with influenza and/or gastrointestinal illness (i.e., fever, cough, sore throat, diarrhea) (OR 1.6, 95% CI 1.2–2.1; p = 0.02). No other symptoms were associated, positively or negatively, with antiviral drug use in the first 2 days of illness onset.

Discussion

This comparison of >1,000 total confirmed seasonal and pandemic (H1N1) 2009 influenza infections occurring contemporaneously over the peak of the traditional influenza season yielded several findings. First, the spectrum of clinical illness due to pandemic influenza was similar to that caused by seasonal influenza. Although several symptoms were more common in patients with pandemic influenza, the differences were modest and of limited clinical importance.

Our findings generally parallel those from a recent comparative analysis in Singapore, with some differences. For example, in Singapore, the proportions of patients with seasonal and pandemic influenza who reported diarrhea were 0% and 4%, respectively; these figures are substantially lower than those found in our study (12% and 19%, respectively), despite the fact that in both settings most nonpandemic influenza viruses identified were influenza A (H3N2). These differences highlight the need to consider data from diverse geographic, cultural, and healthcare environments when characterizing the clinical manifestations of influenza.

Second, we observed that the hospitalization rates for pandemic (H1N1) 2009 and seasonal influenza infections were similar. Our ability to use a comprehensive statewide database to identify hospital admissions in the broader target population permitted a robust analysis that found the overall proportion of confirmed pandemic and seasonal illnesses hospitalized was not significantly different when aggregated data were used in univariate analyses (p>0.05). However, if the analysis was controlled for age, the odds of being hospitalized were significantly greater for the population with pandemic influenza. These seemingly dissonant results actually reflect the fact that for many age groups there was a higher risk for hospitalization with pandemic (H1N1) 2009, but because patients with seasonal influenza were older relative to those with pandemic influenza and elderly patients are more likely to be admitted to hospital when ill with influenza, the cumulative hospitalization rate in the 2 patient groups was similar.

In addition, in this study, the mean duration of hospitalization was not statistically different between patients with pandemic (H1N1) 2009 and seasonal influenza even though other indicators suggested pandemic patients were hospitalized for longer periods. An analysis of a larger sample of hospitalized patients is under way.

Third, the underlying medical conditions associated with pandemic (H1N1) 2009 and seasonal influenza illnesses diagnosed in the community were nearly identical in terms of the type and number of conditions reported. Most patients in both groups reported no risk factors, and only when we controlled for age did we find an association between having ≥1 underlying medical condition and pandemic influenza. Notably, the largest risk difference we observed was for was pregnancy (4%). Univariate analyses showed that the association between pregnancy and pandemic influenza approached statistical significance (p = 0.08; analysis not shown). When we restricted our analysis to women 15–45 years of age, the risk difference nearly doubled, but significance was still not attained, perhaps as a consequence of the smaller sample size. Seasonal

influenza is a well-established cause of serious illness during pregnancy, and several reports indicate that the risk for severe illness from pandemic (H1N1) 2009 may be even greater (*20–23*).

Obesity, newly recognized as a risk factor for severe influenza illness during the 2009 pandemic, was reported as often by patients with seasonal influenza as by those with pandemic influenza (11% vs. 9%; p>0.05). This finding suggests that obesity may be equally important as a risk factor for seasonal and pandemic (H1N1) 2009 (*24*).

Finally, because our study was not a randomized controlled trial, inferences about the effect of antiviral medications should be viewed with caution. For example, our observation that antiviral drug use was negatively associated with reported rhinorrhea may be due to the effect of treatment or may have resulted from a relative disinclination of providers to prescribe antiviral drugs for patients with rhinorrhea, on the basis of an assumption that nasal symptoms make influenza infection less likely (*25*). However, the robust positive association we observed in our population between antiviral agent use and nausea/vomiting suggests that there was a causal relationship, a conclusion consistent with that of a recent metaanalysis on oseltamivir use (*26*).

The limitations of our study include the following: reported underlying medical conditions were not objectively

Table. Symptoms, underlying medical conditions, and medical care reported by study participants in a comparison of pandemic (H1N1) 2009 and seasonal influenza, Western Australia, 2009*

Parameter	No. respondents	Pandemic (H1N1) 2009, no. (%)	Seasonal influenza, no. (%)	RD	Univariate χ^2 p value	OR† (95% CI)	p value
Symptoms							
Fever‡	1,159	762 (88)	225 (78)	10	0.001	1.64 (1.15–2.35)	0.01§
Cough	1,159	743 (85)	236 (82)	3	NS	1.45 (1.01–2.34)	0.01§
Myalgia/arthalgia	1,159	565 (65)	173 (60)	5	NS	1.40 (1.06–1.87)	0.02§
Diarrhea	1,159	165 (19)	35 (12)	7	0.008	1.72 (1.15–2.57)	0.01§
Rhinorrhea	1,159	494 (57)	189 (66)	–9	0.007	0.60 (0.45–0.80)	0.01§
Sore throat	1,159	488 (56)	169 (59)	–3	NS	0.82 (0.62–1.09)	0.17
Shortness of breath	1,159	289 (33)	99 (35)	–2	NS	1.14 (0.85–1.53)	0.38
Headache	1,159	537 (62)	176 (61)	1	NS	1.02 (0.77–1.35)	0.91
Vomiting or nausea	1,159	284 (33)	80 (28)	5	NS	1.14 (0.84–1.54)	0.40
Fatigue	1,159	639 (73)	205 (71)	2	NS	1.12 (0.83–1.51)	0.47
Rigors	1,159	471 (54)	148 (52)	2	NS	1.13 (0.86–1.48)	0.40
ILI criteria met¶	1,159	706 (81	209 (73)	8	0.002	1.50 (1.09–2.06)	0.01§
Underlying medical conditions							
Diabetes	1,032	49 (7)	18 (6)	1	NS	1.93 (1.07–3.51)	0.03§
Heart disease	1,027	34 (5)	20 (7)	–2	NS	1.16 (0.63–2.16)	0.63
Respiratory disease	1,031	178 (24)	62 (22)	2	NS	1.33 (0.94–1.87)	0.10
Renal disease	1,028	13 (2)	7 (2)	0	NS	1.17 (0.44–3.10)	0.76
Neurologic disease	1,028	12 (2)	7 (2)	0	NS	0.91 (0.33–2.53)	0.86
Hematologic disorder	1,028	19 (3)	5 (2)	1	NS	2.33 (0.82–6.66)	0.11
Metabolic disease (not diabetes)	1,028	12 (2)	4 (1)	1	NS	1.25 (0.38–4.06)	0.71
Immune impairment	1,028	26 (3)	16 (6)	–3	NS	0.88 (0.45–1.71)	0.70
Morbid obesity	1,031	64 (9)	32 (11)	–2	NS	1.12 (0.70–1.80)	0.64
Current smoker	1,032	98 (13)	35 (12)	1	NS	1.36 (0.89–2.08)	0.16
Pregnancy (women only)	556	36 (9)	8 (5)	4	NS	1.85 (0.84–4.10)	0.13
Any	1,051	366 (48)	135 (47)	1	NS	1.44 (1.07–1.94)	0.02§
Medical care							
Hospitalization	1,159	129 (15)	36 (12)	3	NS	1.58 (1.04–2.39)	0.03§
Antiviral treatment	1,103	388 (47)	71 (26)	21	0.001	3.12 (2.27–4.29)	0.01§

*Totals respondents may not sum to 1,159 for all parameters because questions regarding underlying medical conditions and antiviral treatment were added shortly after the study was initiated, and there are intermittent missing values to individual questions for some respondents. RD, risk difference (absolute difference in the proportion of pandemic and seasonal influenza patients reporting a given parameter); OR, odds ratio; CI, confidence interval; NS, not significant; ILI, influenza–like illness (patient had fever and cough or sore throat).
†ORs were computed by using logistic regression to control for age. Each row depicts data from a separate regression equation, where the dependent variable was defined as influenza type and age (in years) and a single patient characteristic, as listed in the first column of the row (coded as a dichotomous variable indicating the presence or absence of the respective symptom or underlying medical condition) were included as the predictor variables. In all of the logistic analyses performed, age remained significantly associated with influenza type, i.e., younger patients had a higher odds of having pandemic influenza compared with seasonal influenza.
‡Fever was defined as temperature >38°C or subjective fever if temperature was not measured.
§Significant OR obtained using logistic regression.
¶ Patient reported ≥1 of the underlying medical conditions listed.

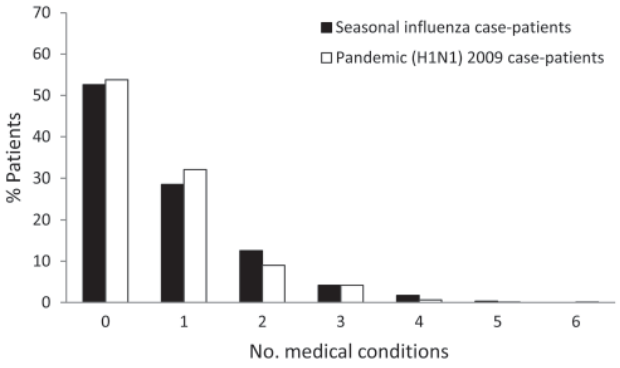

Figure 5. Number of underlying medical conditions reported by study participants, by influenza type, Western Australia, 2009.

verified, data on the duration of symptoms were not collected, and interviewers were not blinded to influenza type when administering the questionnaire. Also, because this was a public health evaluation of notified influenza infections principally detected through routine healthcare practices in the community at large, we were unable to control for potential biases stemming from who was tested and who was not. However, because the healthcare provider could not be confident of whether the patient had pandemic (H1N1) 2009 or seasonal influenza at the time of testing, any bias in who was selected for testing should be approximately equal for pandemic (H1N1) 2009 and seasonal influenza patient groups. Lastly, a limitation inherent in the case–control study design we used was that we are unable to assess the extent to which the underlying medical conditions reported increased the risk for a diagnosis of influenza of either type, when compared with persons without underlying medical conditions.

In summary, our head-to-head comparison of confirmed pandemic (H1N1) 2009 and contemporaneous seasonal influenza infections found little to differentiate the 2 in terms of symptoms, underlying medical conditions, and the proportion of patients hospitalized. These results add to the growing body of knowledge about pandemic (H1N1) 2009

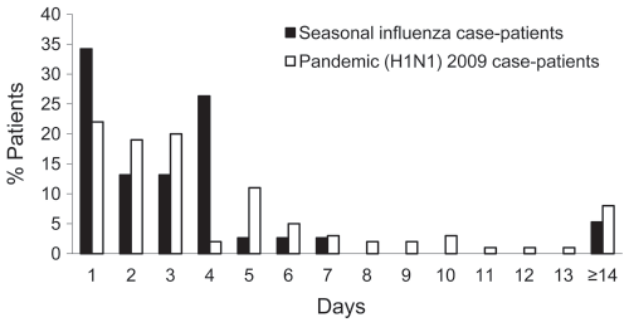

Figure 6. Duration of hospital stay for study participants, by influenza type, Western Australia, 2009.

and are in general agreement with several studies that used different methods in other settings (*27*). These data are important because early in the pandemic some reports espoused different conclusions; 1 report estimated the lethality of pandemic (H1N1) 2009 to be ≈1 death per 10,000 infections, about 100× greater than that for regular seasonal influenza (*28*,*29*). Worldwide, unprecedented levels of resources have been expended to mitigate the impact of pandemic (H1N1) 2009. In the United States alone, the federal government appropriated $7.65 billion for this effort (*30*). This commitment to controlling pandemic (H1N1) 2009 is to be lauded, but we must not lose sight of the fact that seasonal influenza remains an important, albeit relatively uncelebrated, cause of illness and death each year. As the pandemic (H1N1) 2009 response draws to a close, it may be prudent to revisit the level of effort directed toward reducing the enormous effects, in terms of costs and health outcomes, associated with annually recurring influenza epidemics (*31*).

Acknowledgments

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All-Cause Mortality during First Wave of Pandemic (H1N1) 2009, New South Wales, Australia, 2009

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In temperate countries, death rates increase in winter, but influenza epidemics often cause greater increases. The death rate time series that occurs without epidemic influenza can be called a seasonal baseline. Differentiating observed death rates from the seasonally oscillating baseline provides estimated influenza-associated death rates. During 2003–2009 in New South Wales, Australia, we used a Serfling approach with robust regression to estimate age-specific weekly baseline all-cause death rates. Total differences between weekly observed and baseline rates during May–September provided annual estimates of influenza-associated death rates. In 2009, which included our first wave of pandemic (H1N1) 2009, the all-age death rate was 6.0 (95% confidence interval 3.1–8.9) per 100,000 persons lower than baseline. In persons ≥80 years of age, it was 131.6 (95% confidence interval 126.2–137.1) per 100,000 lower. This estimate is consistent with a pandemic virus causing mild illness in most persons infected and sparing older persons.

Influenza epidemics can be associated with large increases in all-cause and cause-specific death rates (*1*–*4*). Increases have been observed in the rate of deaths attributed to influenza, pneumonia, circulatory disease, and diabetes (*4*,*5*). Many deaths in which influenza infection is a factor do not have laboratory confirmation of infection. Public health surveillance thus incorporates indirect measures of disease caused by influenza. Analyses based on the Serfling approach (*1*) frequently have been used to assess the effects of influenza epidemics on mortality time series (*2*,*3*,*6*–*10*). Because deaths increase in winter, even when influenza epidemics are not occurring, these models are required so that observed death rates during influenza epidemics can be differentiated from a seasonally changing baseline. The models typically do not incorporate virologic data but nevertheless produce estimates similar to models that do incorporate markers of specific virologic activity (*4*,*11*).

Although the first winter wave of pandemic (H1N1) 2009 in New South Wales, Australia, substantially increased demand for acute health services, impact on death from pneumonia and influenza (P&I) was limited, even in comparison with recent seasonal influenza epidemics (*12*). New South Wales is Australia's most populous state (population 7.0 million) and includes Australia's largest city and primary entry port, Sydney (4.4 million persons).

We applied a Serfling-based approach to time series of age-specific population rates of all-cause death rates for 2003–2009. Our analysis more completely assesses the impact of death temporally associated with the first wave of pandemic (H1N1) 2009 in New South Wales.

Methods

For scheduled disease surveillance, death registrations from the New South Wales Registry of Births, Deaths and Marriages are provided daily to the New South Wales Department of Health and securely stored in the Health Outcomes and Information Statistical Toolkit database (*13*). The database also includes the Australian Bureau of Statistics (ABS) Cause of Death Data Collection (*14*), which is less current than the registry data source.

We assembled a time series of weekly rates of deaths per 100,000 population for 5 age groups (0–19, 20–49, 50–64, 65–79, and ≥80 years) for weeks ending on Fridays from January 1, 2003, through September 30, 2009. ABS

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death data were used for deaths occurring before January 1, 2006, and New South Wales Registry data were used for subsequent years. We calculated weekly death rates using estimated resident populations linearly interpolated from mid-year estimates (*15*) and projections (*16*).

To forecast the seasonally varying baseline time series of mortality that would be expected when influenza was not at epidemic levels, Serfling (*1*) used an ordinary linear regression model with terms that describe a consistent and cyclic winter rise and summer decline in mortality (harmonic terms). Before fitting the model, he removed observed values during influenza epidemics to prevent the baseline being raised by the epidemics. We adopted a similar approach, but we used robust regression, which does not require removal of observations during epidemic periods. This approach is used for seasonal P&I mortality surveillance by the US Centers for Disease Control and Prevention and the New South Wales Department of Health (*13*,*17*).

Briefly, robust regression is a model-fitting procedure that limits the influence of statistical outliers (extreme observations) on the resulting model. This model is important for influenza surveillance because influenza epidemics cause outliers in the time series, and the influence of influenza epidemics needs to be limited when background seasonal pattern is estimated. During cooler months, the difference between observed and baseline mortality can then be attributed to the impact of influenza on mortality.

For this analysis, we included a sequential week number in the model to capture long-term linear trend, the square of week number to capture any long-term curved trend, and harmonic terms with annual periodicity for the seasonal pattern. Categorical age group was included in the model. To enable the trend and seasonal terms to vary by age group, we also included terms for interactions between age group and each of the time variables (online Technical Appendix, www.cdc.gov/eid/content/16/9/1396-Techapp.pdf).

To estimate annual differences between observed and expected death rates for each age group, we first calculated the difference between the observed all-cause death rate and the modeled baseline rate in each week during May–September. This 5-month period was sufficiently long to include seasonal influenza activity in most years and the time during which pandemic (H1N1) 2009 circulated during 2009. The weekly rate difference was applied to the interpolated population estimate for the month to obtain an estimated count difference for the week. These differences, whether positive or negative, were summed over all weeks during May–September to obtain total count differences for the year's influenza season. We then divided the resulting total count difference by the average population during May–September of that year to obtain the total rate difference. Negative differences indicate that the observed death rates were lower than expected compared with the seasonal baseline.

To obtain crude all-age difference in death rates, we first calculated totals for age-specific observed deaths, baseline estimated deaths, and population estimates for each week in the study period. Confidence intervals (CIs) for the total of the predicted baseline rates were obtained (online Technical Appendix). We then calculated the all-age rate and count differences and their CIs in the same way as the age-specific differences.

To permit an age-adjusted comparison between years, we also calculated standardized rates from the age-specific rates using the 2009 mid-year population as the standard. The upper 95% confidence limit of each year's standardized rate was obtained by standardizing the upper 95% confidence limit of each of the age-specific rates. The equivalent procedure was followed to obtain the lower 95% confidence limit in each year.

For visual comparison, weekly P&I death rates per 250,000 population by age were calculated from the same death databases used for all-cause death rates. For the pre-2006 ABS vital statistics dataset, we included any death with an underlying or contributing cause of death in the International Classification of Diseases, 10th Revision, codes J09–J18. For the 2006–2009 registry dataset, we included any death registration with pneumonia or influenza mentioned anywhere in the causes of death using the same algorithm as our weekly P&I mortality surveillance (*13*). The rate per 250,000 rather than 100,000 population was presented to enable visual inspection of detail in the time series.

To compare death rates with influenza activity, the weekly proportion of respiratory specimens that tested positive for influenza was obtained from the New South Wales Department of Health's routine influenza surveillance information reported by up to 8 major public pathology laboratories in New South Wales during the years studied. Reporting occurs from early May through the end of September each year. Testing was by direct immunofluorescence or PCR (*18*).

To assess consistency between observed mortality patterns and circulating influenza strains, subtyping information for New South Wales specimens was obtained from the Australian World Health Organization Collaborating Centre for Reference and Research on Influenza (Melbourne, Victoria, Australia) (A. Hurt, pers. comm.). In some years, 2 strains predominated, so we defined predominant strains as those identified in at least 33% of specimens in the year.

The death registration data files used for the study had identifying details, including names, addresses, and dates of birth, removed. Therefore, ethics approval was not required.

To assess the plausibility of our results, we repeated the analysis using 2 alternative methods. First, the analysis was rerun fitting a separate robust regression model to each

age group without any age-specific parameters. Second, we applied a more traditional Serfling approach to our original age-specific analysis, which involved removing weekly rates during May–September and fitting an ordinary linear regression model without robust estimation. The methods are described in more detail in the online Technical Appendix.

Results

In New South Wales during January 1, 2003–September 30, 2009, median weekly all-cause death rates for persons 0–19 years of age were <1/100,000 in all years. For persons 20–49 years of age, they were ≈2/100,000; for persons 50–64 years of age, ≈10/100,000; for persons 65–79 years of age, ≈40/100,000; and for persons ≥80 years of age, ≈180/100,000 (Table 1).

The seasonal fluctuations in mortality were more evident in the older age groups, and death rates in persons 65–79 years of age also declined steadily over the study period (online Appendix Figure, www.cdc.gov/eid/content/16/9/1396-appF.htm). Among persons ≥80 years of age, periods of increased death rates in the cooler months relative to baseline occurred in 2003, 2004, 2007, and 2008. These correspond to increases in P&I deaths and influenza circulation. Among persons 65–79 years of age, sustained peaks above baseline that correspond to influenza activity and peaks in P&I deaths are evident in 2003, 2007, and 2008. Among persons aged 50–64 years, a clear and consistent peak is evident in 2007, and possibly in 2009. Among persons 20–49 years of age, a peak is evident only in 2007, although the time series in that age group varied substantially. Among persons 0–19 years of age, no peaks clearly coincide with influenza activity. An increase coinciding with influenza circulation can be discerned in 2003, and a short-lived peak is evident in 2009, but some similar and larger peaks occurred at various times during the 7 years, not always when influenza was circulating (online Appendix Figure).

Estimates of age-specific differences between observed and predicted baseline death rates for each year are shown in Table 2, and the all-age and age-standardized differences in Table 3. Predominant strains are also shown in Table 3, but no consistent association between antigenic characteristics and positive or negative rate differences is evident. In years with significant positive increases above baseline (those in which the 95% CI excludes zero), the magnitude of the difference increased dramatically with age (Table 2). Significant all-age increases in death rates above baseline occurred in 2003 with 10.0 (95% CI 7.3–12.6) deaths per 100,000 population more than expected; 2004 with 7.2 (95% CI 5.3–9.1) per 100,000 more; 2007 with 7.3 (95% CI 5.3–9.2) per 100,000 more; and 2008 with 8.6 (95% CI 6.5–10.6) per 100,000 more. Age-standardizing increased the all-age rate difference in 2003 and 2004 by ≈10% (Table 3).

In years with all-age increases above baseline, the greatest increases occurred in persons ≥80 years of age, from 53.1 (95% CI 49.4–56.7) per 100,000 in 2007 to 186.2 (95% CI 181.4–191.1) per 100,000 in 2003. Death rates in persons 65–79 years of age also significantly increased during the same years, from 15.6 (95% CI 12.1–19.2) per 100,000 in 2004 to 25.3 (95% CI 21.7–28.9) per 100,000 in 2007. Death rates were significantly increased in persons 50–64 years of age only in 2004 and 2007. Death rates for all other age groups did not differ significantly from baseline (Table 2), but CIs were wide.

In years with significantly increased all-age death rates, estimated excess deaths ranged from 482 (95% CI 352–612) in 2004 to 666 (95% CI 488–843) in 2003. Except during 2007 in which the count difference was greater both for persons 50–64 and 65–79 years of age, most deaths above baseline were in persons ≥80 years of age (Table 3).

During May–September 2009, when pandemic (H1N1) 2009 virus first circulated in New South Wales, death rates for persons <65 years of age did not differ significantly from baseline, although the CIs included wide ranges of possible counts. For persons ≥65 years of age, estimates were significantly lower than expected, particularly in persons ≥80 years of age, in which the estimated count difference was 371 (95% CI 356–387) below baseline (Table 2). Only in 2005 were the overall estimates further below baseline, but the difference between the 2 years was not significant (Table 3).

Repeating the modeling separately for each age group led to a median difference of 0.5 deaths/100,000 popula-

Table 1. Weekly all-cause death rates per 100,000 population used in the regression model, New South Wales, Australia, January 2003–September 2009

	Median (interquartile range), by age group, y				
Year	0–19	20–49	50–64	65–79	≥80
2003	0.8 (0.4)	1.9 (0.4)	9.3 (0.9)	43.3 (6.9)	187.0 (46.5)
2004	0.8 (0.3)	1.8 (0.3)	9.1 (1.6)	42.0 (4.9)	183.3 (50.6)
2005	0.8 (0.3)	1.8 (0.4)	8.7 (1.3)	39.6 (4.3)	176.5 (40.3)
2006	0.8 (0.3)	1.8 (0.4)	8.5 (1.4)	38.2 (4.7)	180.4 (29.1)
2007	0.7 (0.3)	1.8 (0.3)	8.9 (1.1)	37.9 (6.3)	179.0 (36.7)
2008	0.7 (0.4)	1.7 (0.3)	8.4 (1.3)	36.5 (4.5)	181.4 (40.1)
2009	0.7 (0.3)	1.8 (0.3)	8.6 (1.2)	35.2 (3.3)	175.1 (31.2)

tion between the age-specific rate differences using this method and our original method. The mean difference was 3.9/100,000 (range 0.0–29.4/100,000); larger differences occurred in the older age groups where estimates were larger.

We also repeated the original analysis after removing observations from the May–September period in each year and using ordinary linear regression rather than robust regression. Annual all-age estimates of the difference between observed and baseline all-cause death rates were an average 19.8/100,000 greater than those estimated by using robust regression. The year 2003 showed the highest increase above the baseline of 2,096 deaths (31.4/100,000). The year 2009 showed the smallest difference with 594 additional deaths (8.4/100,000) above baseline.

Table 2. Difference between observed and baseline all-cause death rates and counts, New South Wales, Australia, January 2003–September 2009*

Age group, y, and year	Rate (95% CI)	No. (95% CI)
0–19		
2003	1.7 (–3.1 to 6.6)	31 (–56 to 117)
2004	–1.1 (–4.7 to 2.4)	–20 (–83 to 43)
2005	–1.0 (–4.7 to 2.8)	–17 (–84 to 50)
2006	–0.3 (–4.1 to 3.6)	–5 (–74 to 64)
2007	–0.8 (–4.5 to 2.8)	–15 (–81 to 51)
2008	0.8 (–3.0 to 4.6)	15 (–54 to 83)
2009	–0.1 (–5.5 to 5.4)	–2 (–100 to 97)
20–49		
2003	0.8 (–4.1 to 5.7)	23 (–118 to 164)
2004	–1.6 (–5.1 to 2.0)	–45 (–148 to 58)
2005	0.3 (–3.5 to 4.1)	9 (–101 to 119)
2006	1.7 (–2.2 to 5.5)	49 (–64 to 161)
2007	1.0 (–2.7 to 4.6)	29 (–79 to 136)
2008	0.2 (–3.6 to 3.9)	5 (–107 to 117)
2009	–0.1 (–5.6 to 5.3)	–4 (–166 to 159)
50–64		
2003	3.0 (–1.9 to 7.8)	33 (–21 to 87)
2004	9.8 (6.2 to 13.3)	111 (71 to 151)
2005	–1.8 (–5.6 to 2.0)	–21 (–64 to 23)
2006	–4.7 (–8.6 to –0.9)	–56 (–102 to –10)
2007	14.5 (10.9 to 18.2)	176 (132 to 221)
2008	–2.1 (–5.9 to 1.7)	–26 (–73 to 21)
2009	0.5 (–5.0 to 5.9)	6 (–63 to 75)
65–79		
2003	23.5 (18.7 to 28.4)	154 (122 to 186)
2004	15.6 (12.1 to 19.2)	103 (80 to 126)
2005	–22.1 (–25.9 to –18.4)	–147 (–172 to –122)
2006	–29.3 (–33.2 to –25.5)	–196 (–222 to –171)
2007	25.3 (21.7 to 28.9)	172 (147 to 197)
2008	16.5 (12.8 to 20.3)	114 (88 to 140)
2009	–7.4 (–12.9 to –2.0)	–53 (–91 to –14)
≥80		
2003	186.2 (181.4 to 191.1)	425 (414 to 436)
2004	141.3 (137.7 to 144.8)	333 (325 to 342)
2005	–104.7 (–108.5 to –100.9)	–257 (–266 to –247)
2006	–32.6 (–36.4 to –28.7)	–82 (–92 to –73)
2007	53.1 (49.4 to 56.7)	140 (130 to 149)
2008	180.1 (176.4 to 183.9)	492 (482 to 502)
2009	–131.6 (–137.1 to –126.2)	–371 (–387 to –356)

*Rate per 100,000 persons. CI, confidence interval.

Discussion

In New South Wales, during May–September 2009, the epidemic of pandemic (H1N1) 2009 during the first Southern Hemisphere winter after its emergence was associated with a decline in rates of all-cause deaths relative to seasonal expectation. The all-age reduction was similar to that of 2005. Despite a small apparent peak in weekly death rates for persons 50–64 and possibly 0–19 years of age that coincided with the peak in influenza activity in 2009, overall May–September differences in 2009 did not differ significantly from zero in persons <65 years of age and were significantly lower than expected in persons ≥65 years of age. This is consistent with the epidemiologic evidence from reports from Australia (*12*,*19*,*20*) and internationally (*21*) that suggest that persons ≥60 years of age were relatively protected from infection. Reduced susceptibility in older age may be due to past exposure either through natural infection or vaccination to a similar H1 strain or a strain that provided cross-protective immunity (*22*). Previous pandemics also have spared the older population (*23*). The differences in 2009 for younger persons that were close to zero reflect the limited statistical precision of the indirect method we used rather than reflecting an actual death rate from influenza close to zero. This results from the combination of our relatively small population size and low overall death rates for younger persons.

The apparently low influenza-related mortality in 2009 contrasts with the substantially higher-than-usual demand for emergency department, general inpatient, and intensive care services, particularly among persons <60 years of age (*12*). This could reflect high attack rates for younger persons but fatal outcomes in a small proportion because of greater resilience in younger persons or intensive treatment in younger persons who have serious complications (*24*). The small peak in death rates for persons 50–64 years of age in 2009 (online Appendix Figure) is consistent with the increased relative risk for admission to intensive care units and death in that age group among persons with confirmed pandemic strain infection (*12*). This may reflect increasing prevalence with age of risk factors for death tempered in persons <65 years of age by reduced susceptibility to infection.

In New South Wales, during May–September 2009, with intensive case ascertainment, 51 deaths with confirmed pandemic (H1N1) 2009 virus infection were reported. Most occurred in persons <60 years of age, and one fourth of infected persons were >70 years of age (*25*). Even though these figures cannot be directly compared with our indirect estimates, the count for younger persons would be

Table 3. All-age differences between observed and baseline all-cause death rates* and counts, and predominant influenza virus strains, by year, New South Wales, Australia, January 2003–September 2009

Year	Crude rate (95% CI)	Standardized† rate (95% CI)	No. (95% CI)	Predominant strain(s)
2003	10.0 (7.3 to 12.6)	11.1 (6.3 to 16.0)	666 (488 to 843)	A/Fujian/411/2002 (H3N2)-like
2004	7.2 (5.3 to 9.1)	8.0 (4.5 to 11.6)	482 (352 to 612)	A/Fujian/411/2002 (H3N2)-like
2005	–6.4 (–8.4 to –4.3)	–6.9 (–10.6 to –3.1)	–432 (–571 to –294)	A/California/7/2004 (H3N2)-like
2006	–4.3 (–6.4 to –2.2)	–4.5 (–8.3 to –0.6)	–291 (–433 to –149)	B/Malaysia/2506/2004-like (Victoria lineage)
2007	7.3 (5.3 to 9.2)	7.5 (3.8 to 11.1)	502 (366 to 638)	A/Brisbane/10/2007 (H3N2)-like, A/Solomon Islands/3/2006 (H1N1)-like
2008	8.6 (6.5 to 10.6)	8.8 (5.0 to 12.5)	600 (457 to 742)	B/Florida/4/2006-like (Yamagata lineage)
2009	–6.0 (–8.9 to –3.1)	–6.0 (–11.5 to –0.6)	–423 (–630 to –217)	A/California/7/2009 (H1N1)-like, A/Brisbane/10/2007 (H3N2)-like

*Rates are per 100,000 population. CI, confidence interval.
†Age standardized by using the 2009 mid-year age-specific population estimates as the standard population.

within the upper range of CIs we estimated in younger age groups. For older age groups, we estimated fewer deaths than baseline, and CIs did not include positive counts. This finding suggests that background noninfluenza death rates may have been lower than usual or strain replacement by the pandemic virus led to lower death rates from nonpandemic influenza later in the season. The online Appendix Figure does show unusually low death rates for elderly persons late in the 2009 season.

In Australia, seasonal influenza vaccination is offered free to all persons ≥65 years of age and to younger persons in high-risk groups. In persons ≥65 years of age, vaccination levels in our state were >70% during 2003–2007 (*26*). We therefore would expect influenza-related mortality to reflect the innate virulence of the circulating virus strains, preexisting population immunity from past exposure to related influenza strains, and the degree of mismatch between the circulating strain and the available influenza vaccine.

The emergence of the Fujian strain in the Northern Hemisphere winter of 2002–03 led to widespread outbreaks. These outbreaks would explain the greatest relative increase in mortality that we observed in 2003. In that year, antigenic match to the available vaccine was poor, and a matching strain was unable to be included in the Southern Hemisphere vaccine until 2004 (*27–29*). In 2004, further antigenic drift occurred away from this vaccine strain (*30*) which may explain the continuing increased mortality in 2004, despite the apparently low level of influenza circulation generally.

In 2005, a variant of the Fujian strain, the California 2004 H3N2 strain, predominated in New South Wales. Mortality did not increase, possibly because of cross-protection from the A/Wellington/1/2004 (H3N2)–like strain included in the 2005 Southern Hemisphere vaccine (*31*). In 2006, mortality also did not increase. This was a relatively mild epidemic year in Australia, with the B Malaysia strain, which was included in the local 2006 vaccine (*32*), predominating.

The 2007 epidemic in Australia, in which we next observed substantially increased mortality, including a distinct peak in persons 50–64 years of age, and the 2007–08 epidemic in the United States were relatively severe. These epidemics caused unusually high illness and death in young children (*33*,*34*), consistent with the antigenic drift away from both the 2007 Southern Hemisphere subtype H3N2 and H1N1 vaccine strains (*33*).

In 2008, we observed the second highest estimate of increased mortality of the years we studied. This finding is somewhat surprising because, compared with influenza A (H3N2), influenza B is uncommonly associated with increased mortality (*35*). Yet, the double peak in influenza isolates also was evident in the all-cause and P&I mortality curves for persons ≥80 years of age in 2008 (online Appendix Figure). Morbidity appeared relatively low in 2008 in our state, but influenza B strains dominated locally and nationally (*19*,*36*). The New South Wales strain data received from the national influenza collaborating center indicated that most influenza B strains of both Yamagata and Victoria lineages from New South Wales in 2008 showed low reactivity to reference strains, suggesting antigenic drift. This could have led to reduced effectiveness of the 2008 and 2007 vaccines, which alternately included a B strain from each lineage.

The alternative analyses we conducted to check the plausibility of our results did not alter our conclusions. Fitting the model separately to each age group made little difference. However, our modeling approach with age group included in a single model ensured consistency of the age-based estimates with all-age estimates. Using a more traditional Serfling approach with exclusion of cooler month data did not change our original conclusion that, in the 2009 pandemic year, influenza-related mortality was relatively low compared with recent influenza seasons. However, this approach led to possibly excessive estimates of influenza-associated mortality. This more conventional method may be overly sensitive to choices made on which periods to exclude. On the other hand, the method we used

may have been overly conservative. Nevertheless, the age-specific rate differences we observed using the robust regression method in years with excess influenza activity were broadly similar to those in recent studies in Norway (*37*), the Netherlands (*38*), Italy (*8*), and Canada (*39*) that used various methods.

A limitation of this study was the relatively short time series used. Nevertheless, the number of years included was sufficient to provide a reasonable comparison with the year of primary interest, 2009. The modeling approach does not account for other time-varying factors that might influence death rates during the year, such as other circulating pathogens and meteorologic factors. An implicit assumption in the Serfling method is that the magnitude and timing of the background seasonal mortality pattern is rigidly consistent from year to year, but in reality noninfluenza factors vary from year to year and within years, and the rigidity of the Serfling model would therefore vary in its successful distinction between influenza and noninfluenza mortality. On the other hand, using more flexible models has the risk of following rather than excluding changing mortality associated with influenza activity.

The arrival of pandemic (H1N1) 2009 virus in New South Wales during winter 2009 was associated with a decline in all-cause mortality compared with the usual seasonal pattern. This lower mortality may reflect the relatively low virulence of the virus in most persons infected; the reduced susceptibility of older age groups, who usually are most at risk for complications and death from influenza infection; the success of public health measures, including intensive deployment of the antiviral medication stockpile, the high quality of healthcare available in Australia, or a combination of these factors.

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Determinants of Multidrug-Resistant Tuberculosis Clusters, California, USA, 2004–2007

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Laboratory and epidemiologic evidence suggests that pathogen-specific factors may affect multidrug-resistant (MDR) tuberculosis (TB) transmission and pathogenesis. To identify demographic and clinical characteristics of MDR TB case clustering and to estimate the effect of specific isoniazid resistance–conferring mutations and strain lineage on genotypic clustering, we conducted a population-based cohort study of all MDR TB cases reported in California from January 1, 2004, through December 31, 2007. Of 8,899 incident culture-positive cases for which drug susceptibility information was available, 141 (2%) were MDR. Of 123 (87%) strains with genotype data, 25 (20%) were aggregated in 8 clusters; 113 (92%) of all MDR TB cases and 21 (84%) of clustered MDR TB cases occurred among foreign-born patients. In multivariate analysis, the *katG* S315T mutation (odds ratio 11.2, 95% confidence interval 2.2–∞; p = 0.004), but not strain lineage, was independently associated with case clustering.

In 2007, >500,000 cases of multidrug-resistant (MDR) tuberculosis (TB), defined as resistance to at least isoniazid and rifampin, occurred worldwide (*1*). Although demographic and clinical risk factors for transmission and pathogenesis of both drug-susceptible and drug-resistant *Mycobacterium tuberculosis* have been well described (*2*,*3*), little is known about the microbial factors that influence the generation of secondary MDR TB cases (*4*,*5*).

Community- and population-based molecular epidemiologic studies of isoniazid-monoresistant *M. tuberculosis* (*6*–*8*) have shown that specific resistance-conferring mutations are associated with variable degrees of genotypic clustering, a measure of strain pathogenicity that incorporates host factors, transmissibility of the organism, and capacity of the organism to cause active disease. For example, isoniazid-monoresistant strains with a serine-to-threonine substitution at position 315 (S315T) are more often associated with secondary cases than are strains without the S315T mutation (*6*,*7*), likely because of reduced or absent catalase–peroxidase production (*9*). However, the effects of specific isoniazid resistance–conferring mutations on genotypic clustering in multidrug resistance are less well characterized. The studies reported to date have been limited by inadequate genotypic discrimination (*10*,*11*) and/or nonrepresentative sampling of cases (*10*,*12*–*14*).

California reports the highest annual number of TB cases (*15*), more than one fourth of all MDR TB cases (*16*), and the highest immigration rates in the United States (*17*). We conducted a population-based cohort study of all incident MDR TB cases in California during a 4-year period (January 2004–December 2007) to 1) describe demographic and clinical characteristics of clustering and 2) estimate the effect of specific isoniazid resistance–conferring mutations and strain lineage on genotypic clustering of MDR *M. tuberculosis*.

Methods

We analyzed culture-positive cases of MDR TB reported to the California TB registry from January 1, 2004, through December 31, 2007. California state law (Health and Safety Code Title 17 §2505) requires reporting of all verified cases of TB, submission of all *M. tuberculosis* isolates to local public health laboratories, and submission of all MDR *M. tuberculosis* isolates to the California Department of Public Health Microbial Diseases Laboratory. Testing for first- and second-line drug susceptibilities was

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performed at local laboratories or at the Microbial Diseases Laboratory by using BACTEC 460 (Becton Dickinson Diagnostic Instruments, Sparks, MD, USA), MGIT 960 (Becton Dickinson), or the agar proportion method. Some isolates were forwarded to the Centers for Disease Control and Prevention (CDC; Atlanta, GA, USA) for additional second-line drug testing or for confirmation of drug resistance. Patients were included in the study if MDR *M. tuberculosis* was identified on ≥1 isolate. Demographic and clinical information for all patients with MDR TB was abstracted from state TB surveillance forms (Report of Verified Case of Tuberculosis), which have high validity (*18*). All protocols were approved by the California Committee for the Protection of Human Subjects and University of California, San Francisco, Committee for the Protection of Human Subjects.

Characterization of Mutations Associated with Isoniazid and Rifampin Resistance

For each isolate, genomic DNA was extracted from solid media (Lowenstein-Jensen slants, Middlebrook 7H10 or 7H11 agar), liquid media (BACTEC 12 B or MGIT [Becton Dickinson]), or smear-positive sputum sediments. A real-time PCR assay with 6 molecular beacon probes was performed by using an iQ5 iCycler instrument (Bio-Rad, Hercules, CA, USA) to screen for mutations associated with isoniazid and rifampin resistance (*19*). Two molecular beacons that targeted *katG* (codon 311–317) and the *inhA* promoter were used to detect isoniazid resistance–conferring mutations, and 4 molecular beacons that targeted the core region of *rpoB* were used to detect rifampin resistance–conferring mutations. Isolates with mutations in *katG* detected by the wild-type probe were further tested with another molecular beacon that specifically targeted *katG* S315T (AGC–ACC). When molecular beacon analysis did not show *katG* S315T or -c15t *inh*A promoter mutations, the entire *fur*A-*katG* locus (H37Rv: 2153626–2156657, 3,031 bp) was sequenced as described (*6*). Sequence data were generated by using ABI BigDye v3.1 dye terminator sequencing chemistry and the ABI PRISM 3730xl capillary DNA analyzer (Applied Biosystems, Foster City, CA, USA) at the Genomic Core Facility, University of California, San Francisco (www.genomics.ucsf.edu/Sequencing/index.aspx), and were analyzed with ClustalW (www.ebi.ac.uk/Tools/clustalw/index.html).

Genotyping and Lineage Determination

Spacer oligonucleotide typing (spoligotyping) and mycobacterial interspersed repetitive unit (MIRU) typing were performed in accordance with the Centers for Disease Control and Prevention Universal Genotyping Program procedures (*20*). Spoligotyping was performed by using Luminex-based methods to detect 43 known spacer sequences in the direct repeat locus (*21*). MIRU typing was performed by using the protocol described by Cowan et al. (*22*). A capillary sequencer, CEQ 8000 (Beckman, Fullerton, CA, USA), was used to analyze the number of repeated sequences at each of the 12 loci. IS*6110*-based restriction fragment length polymorphism (RFLP) genotyping was performed following standardized methods (*23*). RFLP patterns were compared by using Bioimage Whole Band Analyzer software version 4.2.1 (Bioimage Corp., Ann Arbor, MI, USA) (*24*). RFLP patterns with ≤20 identical bands, or >20 bands and differing by no more than a single band, were considered matched. IS*6110* RFLP band assignment was edited by 2 independent readers, and the cluster assignment was confirmed visually.

The phylogeographic lineage of strains was determined from spoligotyping results. Spoligotype families H, LAM, and T, X, S were considered to be of Euro-American lineage; Beijing of East-Asian lineage; EAI of Indo-Oceanic lineage; and CAS of East African-Indian lineage (*5*).

De nitions

Cases were defined as clustered if ≥2 isolates from cases reported during the study period shared the same MIRU and spoligotype, matched IS*6110* RFLP, and had specific drug resistance–conferring mutations for isoniazid and rifampin. Clustering was assumed to represent both transmission of *M. tuberculosis* and progression to active disease, leading to secondary case generation within the period of the study. Cases not in a cluster were considered to be the result of reactivation of latent infection. Patients with the earliest case report date within a cluster were regarded as index cases.

Statistical Analysis

The proportion of clustered MDR TB cases was analyzed as the number of clustered cases divided by the total number of culture-positive cases that occurred during the study period. Because of limited sample size, the independent effects of *katG* S315T and phylogeographic lineage on clustering were estimated by using exact logistic regression methods. Refugee status and sputum smear positivity were included in the model as relevant host risk factors. Refugee resettlement during the study period could bias our results in that overrepresentation of ethnic groups or geographic locations with a high prevalence of particular strain-specific factors (phylogeographic lineage or drug-resistance mutations) could confound the association under study. To examine this influence and potential clustering of TB cases within households or communities related to refugee resettlement, sensitivity analysis was conducted by reestimation of study results after excluding 1) patients immigrating from refugee settings within the past 3 years and 2) the single largest patient cluster, which accounted for

40% of all clustered cases. In a separate analysis, standard logistic regression was used to estimate the effect of *katG* S315T and phylogeographic lineage on sputum smear positivity, again controlling for refugee status.

Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to measure associations of interest. Categorical data (e.g., sex, foreign vs. US birth, homelessness) were compared by using Fisher exact tests. The Wilcoxon rank-sum test was used to determine differences in the distribution of continuous variables (e.g., age, time from entry into the United States to TB diagnosis). Interaction between the independent variables was assessed separately for each factor by stratification and statistical testing (Breslow-Day with the Tarone correction [*25*] and Zelen test [*26*]), with $p<0.2$ assumed to indicate the presence of interaction. All p values were 2-sided with $\alpha = 0.05$ as the significance level. Analyses were performed by using Stata 10 (StataCorp., College Station, TX, USA) and StatExact 8 (CYTEL Software Corp., Cambridge, MA, USA).

Results

During the study period, 11,395 cases of TB were reported in California, of which 9,037 (79%) had positive cultures. Of these, 8,899 (98%) had isoniazid and rifampin susceptibility results available. Of the 141 (2%) incident MDR TB cases, 123 (87%) had isolates available for MIRU, spoligotyping, and IS*6110* RFLP analysis. Isolates unavailable for genotyping (n = 18) were more often from Los Angeles County; other demographic and clinical characteristics were similar to those of analyzed cases.

Twenty-five MDR *M. tuberculosis* isolates were aggregated in 8 clusters (1 cluster of 10 cases, 1 cluster of 3 cases, and 6 clusters of 2 cases) for an overall cluster proportion of 20% (25/123). Excluding the 8 index cases, 14% (17/123) of all cases were considered to have resulted from recent transmission and rapid progression to disease (secondary cases). Within clusters, a median of 3 months elapsed between successive secondary cases (range 0–20 months). Of the 123 total cases, 113 (92%) occurred among foreign-born patients, with more than half (56%) occurring among immigrants from Mexico, Philippines, the People's Republic of China, or Vietnam (Table 1). Seven of 8 index cases occurred either in Mexican immigrants (3/8) or recent refugees from Thailand, Lao People's Democratic Republic, or India (4/8). Median time from patient arrival in the United States to TB diagnosis was approximately twice as long for clustered as for nonclustered cases (4.3 years vs. 2.4 years) and 3 times as long for cases in Mexican-born persons as for cases in persons from other countries (7.3 years vs. 2.3 years).

Younger persons were more likely than older persons to harbor strains involved in MDR TB clusters (Table 1). HIV infection was unusual in this patient population; only 3 (4%) of 75 patients with known HIV status were HIV-infected. Twenty-eight percent (35/123) of patients reported a history of active TB; this proportion did not vary between clustered and nonclustered cases (p = 0.75). Eight patients had documented previous treatment in California. Time to culture negativity (2.4 vs. 2.8 months, p = 0.95), treatment failure, or death did not differ between clustered and nonclustered cases (p = 0.57) among 105 (85%) of 123 cases for which data were available.

Seventy-five percent of MDR *M. tuberculosis* strains harbored the isoniazid resistance–conferring mutation *katG* S315T, including all (100%) clustered strains (Table 2). When we controlled for strain lineage and refugee status, *katG* S315T was inversely associated (OR 0.28, 95% CI 0.09–0.89, p = 0.03) with sputum smear positivity (Table 3). Most rifampin resistance–conferring mutations were found between positions 529 and 534 of the *rpoB* gene, likely indicative of the serine to leucine substitution at position 531 (S531L) of the *rpoB* gene (*27*). The association between this mutation and clustering did not reach statistical significance (OR 2.2, 95% CI 0.8–7.4; p = 0.16).

MDR *M. tuberculosis* isolates were distributed among East-Asian (47%), Euro-American (30%), Indo-Oceanic (20%), and East African–Indian (3%) phylogeographic lineages. Lineage could not be established for 11 cases. On univariate analysis, East-Asian strain lineage was associated with clustering (Table 4), but not with adverse outcome (death or treatment failure). Indo-Oceanic strains produced no secondary cases.

Clustering was independently associated with *katG* S315T (OR 11.2, 95% CI 2.2-∞; p = 0.004) and refugee status (OR 6.0, 95% CI 1.2–36.2; p = 0.03) in exact multivariate analyses in which strain lineage and sputum smear positivity were controlled for (Table 5). The estimated association between *katG* S315T and case clustering did not appreciably change in sensitivity analyses that excluded either all recently arrived refugees or the single largest patient cluster.

Discussion

In this 4-year population-based molecular epidemiologic study, transmission followed by secondary case generation contributed to ≈14%, or 1 of every 7, MDR TB cases in California. Clustered cases occurred more often among younger persons and persons who had emigrated from Mexico and refugee settings in Southeast Asia. In addition, pathogen-specific factors were associated with clustering of MDR TB cases, independent of traditional clinical and demographic risk factors.

The proportion of MDR TB cases attributed to transmission in California was higher than that reported by other investigators in most low incidence settings (*28–32*). The largest clusters in our study resulted from MDR TB out-

Table 1. Demographic and clinical characteristics of 123 patients with clustered and nonclustered MDR TB infections, California, USA, 2004–2007*

Characteristic	Nonclustered, n = 98†	Clustered, n = 25†	OR‡ (95% CI)	p value
Median age, y (IQR)	39 (30–50)	27 (18–53)	–	0.05
Female gender	47 (48)	11 (44)	0.9 (0.3–2.3)	0.72
Foreign birth	92 (94)	21 (84)	0.3 (0.1–1.8)	0.11
Nation or region of origin				
Philippines	20 (20)	1 (4)	–	<0.001
Mexico	16 (16)	5 (20)		
Vietnam	14 (14)	1 (4)		
China	12 (12)	0		
Central America	6 (6)	0		
India	3 (3)	3 (12)		
Laos	2 (2)	6 (24)		
Thailand	1 (1)	4 (16)		
United States	6 (6)	4 (16)		
All other nations§	18 (18)	1 (4)		
Recent immigration from refugee setting	4 (4)	7 (28)	9.1 (2–45)	<0.001
Median time from US entry to diagnosis, y (IQR)	2.4 (0.2–8.7)	4.2 (0.2–11)	–	0.77
Time from US entry to MDR diagnosis¶				
<3 mo	25 (27)	6 (29)	–	0.42
3 mo–3 y	25 (27)	4 (19)		
>3 y	40 (44)	11 (52)		
Known HIV/TB co-infection	3 (6)	0	–	–
Private healthcare provider	11 (11)	4 (16)	1.3 (0.3–5.4)	0.74
Homelessness	6 (6)	1 (4)	0.7 (0.01–5.9)	1.0
Previous active TB	28 (29)	7 (28)	1.0 (0.3–2.9)	1.0
Sputum-positive AFB smear	60 (66)	16 (70)	1.2 (0.4–3.8)	0.81
Extrapulmonary disease#	7 (7)	1 (4)	0.6 (0.1–3.9)	1.0
Cavitary disease	25 (26)	10 (42)	1.9 (0.7–5.8)	0.22
Median time to culture conversion, mo (IQR)	2.2 (1.3–4.6)	3 (1.4–4.4)		0.57
Median total treatment time, mo (IQR)	25.8 (21.4–28.9)	24.4 (22.6–27.3)		0.56
Treatment failure**	4 (7)	1 (6)	0.9 (0.02–9.7)	0.56
Treatment outcome††				0.70
Completed treatment	70 (83)	17 (81)		
Moved	7 (8)	1 (5)		
Defaulted	2 (2)	0		
Died	5 (6)	3 (14)		

*MDR TB, multidrug-resistant tuberculosis; OR, odds ratios; CI, confidence interval; IQR, interquartile range; AFB, acid-fast bacilli. – indicates OR had no meaning for those specific comparisons.
†Values are no. (%) except as indicated.
‡ORs describe the association between the characteristic of interest and MDR TB case-clustered status. The denominator for each characteristic excludes missing or unknown values.
§Afghanistan (1), Burma (1), Cambodia (5), Ethiopia (1), Indonesia (1), Mongolia (1), Nepal (1), Peru (2), South Korea (5), and Ukraine (1).
¶Date of US entry was missing for 2 persons.
#Nonclustered cases: cervical lymph node (5), bone (1), other (1); clustered cases: pleural (1).
**Culture positive after ≥8 months of treatment; limited to pulmonary TB patients who were alive at diagnosis and had an initial positive sputum culture.
††Treatment outcome available for 105 (85%) cases.

breaks in California after resettlement of Hmong refugees in 2005–2006 (*33*) and resettlement of Tibetan refugees in 2001–2006. The associations between pathogen-specific factors and case clustering could be due to regional differences in strain prevalence and preferential migration of persons with specific strains to California. We attempted to control for these factors by using highly stringent criteria to define clustered cases and by adjusting for refugee status in our multivariate model. However, without detailed contact information and contact tracing, we cannot be certain of the extent to which transmission or progression to active disease occurred within or outside California. Given that most clustered cases occurred among persons residing in the United States for >3 years and that US-born persons were involved in 3 of 8 clusters, at least some proportion of MDR TB transmission is likely to have occurred in California. This observation suggests that although most MDR TB cases in the United States are related to the migration of persons already infected with drug-resistant *M. tuberculosis*, a small but notable proportion may be due to ongoing transmission.

Heterogeneity in the reproductive success of drug-resistant *M. tuberculosis* is now well established (*34*,*35*). In this study, the *katG* S315T mutation was the only isoni-

Table 2. Isoniazid and rifampin resistance–conferring mutations among 121 clustered and nonclustered MDR TB infections, California, USA, 2004–2007*

Molecular basis for drug resistance	Nonclustered, n = 96, no. (%)	Clustered, n = 25, no. (%)
Isoniazid resistance		
katG S315T mutation	66 (69)	25 (100)
Other *katG* mutation†	8 (8)	0
inhA promoter‡	23 (26)	0
No *katG* S315T or *inhA* promoter mutation detected§	5 (5)	0
Rifampin resistance¶		
rpoB codons 511–518	8 (9)	2 (8)
rpoB codons 523–529	25 (27)	4 (16)
rpoB codons 529–534	57 (62)	19 (76)
rpoB codons 515–521	2 (2)	0

*Two isolates with otherwise complete genotyping data were unavailable for molecular beacon analysis. MDR TB, multidrug-resistant tuberculosis; S315T, serine-to-threonine substitution at position 315.
†Novel mutations detected: Y413STOP, T314T (silent), W161G, D61E (Fur A), R145P, P325L, and V633F.
‡*inhA* promoter mutation was concomitant with 4/91 (4%) isolates harboring the *katG* S315T and 2/8 (25%) isolates with *katG* mutations other than S315T.
§No mutations detected by molecular beacons; sequencing was not possible for these isolates because of degraded DNA.
¶Rifampin resistance–conferring mutations were not detected by the molecular beacon assay for 4 isolates.

Table 3. Multivariate associations with sputum smear positivity in 102 MDR TB infections, California, USA, 2004–2007*

Strain	OR (95% CI)	p value
*kat*G S315T	0.28 (0.09–0.89)	0.03
Euro-American lineage†	1.0	–
East-Asian lineage	0.31 (0.11–0.88)	0.03
Indo-Oceanic lineage	0.22 (0.06–0.86)	0.03
Refugee status	2.02 (0.43–9.45)	0.37

*MDR TB, multidrug-resistant tuberculosis; S315T, serine-to-threonine substitution at position 315; OR, odds ratio; CI, confidence interval.
†Reference.

azid resistance–conferring mutation found among clustered MDR TB cases. The high prevalence of *katG* S315T among MDR strains (*12*,*14*,*36*–*38*) and the association of this mutation with increased secondary case generation among isoniazid-monoresistant strains (*6*,*7*) have been documented. We report that the *katG* S315T isoniazid resistance–conferring mutation retains an independent effect on clustering of MDR TB cases despite the presence of mutations that confer resistance to additional drugs. In particular, secondary case generation did not significantly vary according to site of *rpoB* mutations that confer rifampin resistance, which supports the hypothesis that these are no-cost mutations or that compensatory mutations commonly exist (*39*).

The *katG* S315T mutation is thought to preserve fitness through the relative preservation of catalase-peroxidase production (*9*), although whether this mutation is associated with different clinical phenotypes is unknown. In this study, we noted an inverse association between presence of the *katG* S315T mutation and sputum smear positivity. In addition, the *katG* S315T mutation was associated with case clustering, independent of sputum smear status. These findings suggest that the *katG* S315T mutation may preserve the ability of *M. tuberculosis* to transmit and cause secondary cases through mechanisms unrelated to conventional indices of disease severity, such as the presence of abundant acid-fast bacilli in sputum.

Our findings are clinically useful for at least 2 reasons. First, MDR TB in California is occurring predominantly among patients who were not born in the United States, with some cases from recent transmission and rapid progression to disease. Our study suggests that in California, younger persons and persons who have emigrated from Mexico and from refugee settings may be at higher risk for transmitting MDR *M. tuberculosis*. Likewise, our findings reinforce the need for giving priority to screening and prevention activities in immigrant communities and US investment in international TB control (*40*). Second, if our results are verified in other settings, TB-control programs should consider pathogen-specific factors such as isoniazid resistance–conferring mutations when planning the intensity of contact investigation and secondary case-finding activities.

This study has several limitations. First, our estimates of case clustering are imprecise because of the limited num-

Table 4. Univariate associations of phylogeographic lineage with 112 clustered and nonclustered MDR TB infections, California, USA, 2004–2007*

Strain lineage	Nonclustered, n = 87, no. (%)	Clustered, n = 25, no. (%)	OR (95% CI)	p value
East-Asian	37 (43)	17 (68)	2.87 (1.03–8.48)	0.04
Euro-American	26 (30)	8 (32)	1.10 (0.36–3.12)	0.81
Indo-Oceanic	21 (24)	0	–	0.003
East African–Indian	3 (4)	0	–	1.0

*N = 112. Lineage could not be established by spoligotyping for 11 (8.9%) cases. MDR, multidrug-resistant tuberculosis; OR, odds ratio; CI, confidence interval.

Table 5. Multivariate exact logistic regression for associations with clustering among MDR TB infections, California, USA, 2004–2007*

Strain	OR (95% CI)	p value
*kat*G S315T	11.2 (2.2–∞)	0.004
East-Asian lineage	3.1 (0.9–12.2)	0.08
Sputum smear–positivity	3.1 (0.9–11.7)	0.07
Refugee status	6.0 (1.2–36.2)	0.03

*MDR TB, multidrug-resistant tuberculosis; OR, odds ratio; CI, confidence interval.

ber of MDR TB cases observed during the study period. However, these estimates are the best currently available, given that the data make up the largest population-based TB registry in the United States. Second, although our definition of genotypic clustering was highly rigorous, the lack of detailed epidemiologic information precluded confirmation of transmission within California. Third, because our study did not include pan-susceptible or isoniazid-monoresistant strains, we cannot comment directly on MDR *M. tuberculosis* pathogenicity relative to these groups. Lastly, our analyses implicitly assume independence of outcome events, and household or community-level factors potentially associated with clustering were not available. Future studies should be designed so that statistical methods can be used that are able to accommodate the possible effects of within-household clustering.

We found a substantial proportion of MDR TB cases and case clustering in California among non–US-born persons, and the *katG* S315T mutation was independently associated with clustering. Validation of these findings in larger cohorts and in different population settings may have crucial public health consequences.

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Trends in Hospitalizations for Peptic Ulcer Disease, United States, 1998–2005[1]

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Infection with *Helicobacter pylori* increases the risk for peptic ulcer disease (PUD) and its complications. To determine whether hospitalization rates for PUD have declined since antimicrobial drugs to eradicate *H. pylori* became available, we examined 1998–2005 hospitalization records (using the Nationwide Inpatient Sample) in which the primary discharge diagnosis was PUD. Hospitalizations for which the diagnosis was *H. pylori* infection were also considered. The age-adjusted hospitalization rate for PUD decreased 21% from 71.1/100,000 population (95% confidence interval [CI] 68.9–73.4) in 1998 to 56.5/100,000 in 2005 (95% CI 54.6–58.3). The hospitalization rate for PUD was highest for adults ≥65 years of age and was higher for men than for women. The age-adjusted rate was lowest for whites and declined for all racial/ethnic groups, except Hispanics. The age-adjusted *H. pylori* hospitalization rate also decreased. The decrease in PUD hospitalization rates suggests that the incidence of complications caused by *H. pylori* infection has declined.

Peptic ulcer disease (PUD) is a common illness that affects >6 million persons in the United States each year, causing considerable illness and a large economic cost to the healthcare system (*1*). Infection with *Helicobacter pylori* substantially increases the risk for PUD and its complications (*2*). Appropriate antimicrobial drug regimens to eradicate the infection and cure ulcers have been available since Marshall and Warren discovered *H. pylori* as an etiologic agent of ulcers in the early 1980s (*3*). Eradicating these infections prevents recurrence and ulcer complications such as bleeding or perforation (*4*–*6*). Therefore, a decline in hospitalizations for PUD and its complications could be expected since treatment for *H. pylori* infection became available.

Although rates of hospitalization for PUD declined in the United States during the 1980s and 1990s, rates remained high (*7*,*8*). One reason was the lack of knowledge among the general public and clinicians about the link between *H. pylori* and PUD (*9*–*11*). The Centers for Disease Control and Prevention, in collaboration with partners from other federal agencies, academic institutions, and private industry, initiated an educational campaign in 1997 to increase awareness of the relationship (*9*). The goals of the campaign were to promote the increased use of appropriate antimicrobial drug treatment to eradicate *H. pylori,* which would thus lead to a further decline in rates of hospitalization for PUD and its complications. Accordingly, reducing hospitalizations for PUD 35% from the 1998 baseline rate of 71/100,000 population to 46/100,000 population by the year 2010 was included in the Healthy People 2010 objectives that were developed in 1998 by the US Department of Health and Human Services (*12*).

The prevalence of *H. pylori* infections and their associated conditions can vary considerably among population groups within the same country. Racial and ethnic differences have been noted, with blacks more affected than whites and Mexican-Americans more affected than non-Hispanic whites and non-Hispanic blacks (*12*,*13*). A recent meta-analysis in which researchers adjusted for age and socioeconomic status, showed that *H. pylori* infection

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was significantly associated with male sex in 18 adult populations (*14*). In addition, male patients were hospitalized more often for duodenal ulcers than were female patients (*15*,*16*). The prevalence of *H. pylori* infection and PUD can also vary by geographic location, socioeconomic status, and age (*13*,*17*).

Although recent studies have suggested that rates of PUD have declined in European countries and in non-European countries outside the United States (*16*,*18*–*20*), the overall recent national trends of PUD in the United States have not been described. To determine whether rates of hospitalization due to PUD and its complications have decreased and to describe the demographic characteristics of hospitalized persons with PUD, we conducted a retrospective analysis of hospital discharge data for PUD in the United States from 1998 through 2005.

Methods

Using the Nationwide Inpatient Sample (NIS) (*21*), we analyzed hospital discharge data during 1998–2005 for the general US population. The NIS is the largest all-payer, inpatient-care database in the United States; it is produced by the Healthcare Cost and Utilization Project (HCUP), sponsored by the Agency for Healthcare Research and Quality in partnership with public and private statewide data organizations (*21*,*22*). The NIS is a stratified probability sample of hospitals in participating states designed to approximate a 20% sample of all US community hospitals. Hospitals in the sampling frame include short-term, nonfederal general and specialty hospitals from as many as 37 states (in 2005). We calculated national hospitalization estimates using discharge weights developed by HCUP.

Diagnosis codes from the International Classification of Diseases, 9th revision, Clinical Modification (ICD-9-CM), were used to define PUD in the following terms: peptic ulcer (533), gastric ulcer (531), gastrojejunal ulcer (534), and duodenal ulcer (532) (*23*). Since the first-listed diagnosis is the condition chiefly responsible for the hospitalization, hospitalizations in which 1 of these codes was listed as the first diagnosis were considered to be more specific than hospitalizations in which 1 of these codes was listed as 1 of as many as 15 diagnoses. We limited the analysis to rates of hospitalization for first-listed PUD diagnoses, unless otherwise stated. In a separate analysis, we selected records with *H. pylori* infection as 1 of as many as 15 diagnoses (ICD-9-CM code 041.86), without regard to PUD on the record.

The PUD and *H. pylori* hospitalizations were examined by age group (<20, 20–44, 45–64, and ≥65 years of age), sex, race/ethnicity (white, black, Hispanic, and Asian or Pacific Islander), geographic region (Northeast, Midwest, South, and West), designated ulcer type (peptic, gastric, gastrojejunal, and duodenal), other diagnoses listed along a PUD diagnosis, and procedures. Race/ethnicity was missing in the record for 26.0% of hospitalizations.

We selected first-listed hospitalizations for gastritis/duodenitis (ICD-9-CM code 535) as a comparison group to ensure that a change in the PUD hospitalization rate was not attributable to changes in diagnoses resulting from the increased specificity associated with endoscopy. Hospitalizations for all diagnoses were also examined as a comparison group to ensure that a change in hospitalizations for PUD was not merely a reflection of a change in the total number of hospitalizations for all diagnoses.

Annual average hospitalization rates were expressed as the number of hospitalizations per 100,000 population. The hospitalization rates were calculated by using the weighted number of hospitalizations and the census population for each year of the study period from HCUP (*24*,*25*). SEs for the hospitalization estimates were calculated by using SUDAAN software and discharge weights provided by HCUP to account for the sampling design; SEs were used to calculate 95% confidence intervals (CIs) for the rates (*25*). If the relative SE of national estimates exceeded 0.30, or if the number of unweighted outpatient visits or hospitalizations in a strata was <30, the estimates were considered unreliable and were not shown here (*26*).

A weighted least-squares technique was used to assess a linear trend in the annual hospitalization rate for ulcer types during the 8-year study period of 1998–2005; the independent variable was year and the dependent variable was the rate. A modification to the classical regression technique was necessary to account for the changing NIS survey design over the study period, as described by Gillum et al. (*27*). In this method, a regression line is fit to the data, and the resulting slope is tested for difference from zero by using a Wald test for significance; autocorrelation was not assessed. A p value <0.05 was considered significant in this study.

Age-adjusted rates were calculated by using the direct method, taking into account the survey design, and using the projected 2000 US Census population as the reference population (*28*). In the direct method, the age-adjusted rate represents what the rate would be if the study population had the same age distribution as a reference population (i.e., the projected US Census 2000 age distribution). This method is used to remove confounding by age when rates are compared over time or across populations with different age distributions. To calculate age-adjusted rates, the age group–specific rates (hospitalizations/population) were multiplied by weights representing the proportion of the reference population belonging in the corresponding age group; the resulting quantities were summed to obtain the age-adjusted rate (*28*). We report the age-adjusted PUD hospitalization rates; the overall age-adjusted rate did not

differ from the unadjusted rate, although the rates for some groups did differ.

Results

Overall PUD Hospitalization Rates

A total of 1,453,892 first-listed PUD hospitalizations were estimated for 1998–2005, with an average annual age-adjusted hospitalization rate of 63.6/100,000 population (95% CI 62.9–64.3) (Table 1). The hospitalization rate was highest for adults ≥65 years of age (299.8/100,000 population) and decreased with decreasing age group. Overall, age-adjusted hospitalization rates were significantly higher for male patients than for female patients (71.9/100,000 population [95% CI 71.0–72.7] and 56.3/100,000 population [95% CI 55.6–57.0], respectively). The rates were significantly higher for male patients of all age groups and of all race/ethnicity groups.

Overall, age-adjusted hospitalization rates were significantly lower for whites (44.2/100,000 population; 95% CI 43.3–45.0) than for each of the other racial/ethnic groups (Table 1). This rate difference for male patients was similar across groups of various races and ethnicities. Among female patients, the rate was significantly higher for blacks than those for each of the other racial/ethnic groups.

The average annual age-adjusted hospitalization rate was higher for patients with ulcers designated gastric (33.7/100,000 population; 95% CI 33.3–34.2) than for all patients with other ulcer designations (Table 1). However, a gender-specific comparison showed that for male patients, the hospitalization rate for ulcers designated gastric was comparable to that for ulcers designated duodenal (33.5/100,000 population [95% CI 33.1–34.0] and 33.1/100,000 population [95% CI 32.9–33.5]). Among female patients, the hospitalization rate for ulcers designated gastric (33.6/100,000 population; 95% CI 33.1–34.0) was almost double that for ulcers designated duodenal (17.1/100,000 population; 95% CI 16.8–17.4).

Trends over Time in PUD Hospitalization Rates

The overall age-adjusted hospitalization rate for PUD decreased 21%, from 71.1/100,000 population (95% CI

Table 1. Number of hospitalizations and age-adjusted and age-specific rates of hospitalization for first-listed discharge diagnoses of peptic ulcer disease, overall and by sex, United States, 1998–2005*

	Male patients		Female patients		Overall	
Characteristic	No. (SE)	Rate† (95% CI)	No. (SE)	Rate† (95% CI)	No. (SE)	Rate† (95% CI)
Age, y						
<20	7,851 (320)	2.4 (2.2–2.6)	4,926 (349)	1.6 (1.4–1.8)	12,803 (602)	2.0 (1.8–2.2)
20–44	113,742 (1,344)	27.0 (26.4–27.6)	72,638 (945)	17.5 (17.1–18.0)	186,557 (1,979)	22.3 (21.8–22.8)
45–64	236,381 (2,206)	92.6 (90.9–94.3)	165,013 (1,628)	61.3 (60.2–62.5)	401,581 (3,480)	76.6 (75.3–77.9)
≥65	380,016 (3,555)	322.5 (316.6–328.4)	472,590 (4,224)	283.6 (278.6–288.6)	852,720 (7,409)	299.8 (294.7–304.9)
Race/ethnicity‡						
White	392,199 (5,508)	48.7 (47.7–49.6)	408,120 (5,712)	40.4 (39.5–41.2)	800,358 (10,974)	44.2 (43.3–45.0)
Black	70,453 (1,968)	69.7 (67.1–72.3)	59,045 (1,733)	46.4 (44.5–48.2)	129,499 (3,577)	56.8 (54.7–58.8)
Hispanic	48,076 (1,661)	58.4 (55.1–61.7)	33,179 (1,324)	38.7 (36.1–41.2)	81,270 (2,887)	48.0 (45.3–50.8)
Asian/Pacific Islander	23,268 (1,158)	68.2 (63.0–73.5)	14,789 (767)	38.1 (34.7–41.6)	38,056 (1,846)	51.8 (47.8–55.8)
Ulcer type						
Gastric	343,079 (3,011)	33.5 (33.1–34.0)	427,469 (3,750)	33.6 (33.1–34.0)	770,785 (6,347)	33.7 (33.3–34.2)
Peptic	40,009 (678)	3.8 (3.7–3.9)	51,452 (818)	4.1 (4.0–4.2)	91,524 (1,313)	4.0 (3.9–4.1)
Duodenal	340,161 2,973	33.1 (32.9–33.5)	217,946 (2,055)	17.1 (16.8–17.4)	558,443 (4,676)	24.4 (24.1–24.7)
Gastrojejunal	14,772 (335)	1.4 (1.4–1.5)	18,336 (504)	1.5 (1.4–1.6)	33,142 (699)	1.4 (1.4–1.5)
Total	738,020 (5,897)	71.9 (71.0–72.7)	715,203 (5,824)	56.3 (55.6–57.0)	1,453,892 (11,201)	63.6 (62.9–64.3)

*Diagnosis codes 531–534 from the International Classification of Diseases, 9th revision, Clinical Modification. National estimates determined by using the Nationwide Inpatient Sample (*21*).
†Per 100,000 population.
‡Race/ethnicity was missing for 26.0% of hospitalized patients. Data were insufficient for the race/ethnicity category of American Indian/Alaska Native.

68.9–73.4) in 1998 to 56.5/100,000 population (95% CI 54.6–58.3) in 2005 (Table 2; Figure 1). The hospitalization rate appeared to decline for all age groups (19%–22%), except children <20 years of age, for whom no significant change occurred during the study period. Although the hospitalization rate was higher for male patients than for female patients in 1998 (83.1/100,000 and 60.8/100,000 population, respectively) and in 2005 (62.2/100,000 and 51.3/100,000 population, respectively), the difference decreased because of a greater decline for male patients (25%) than for female patients (16%). The hospitalization rate was significantly lower in 2005 than in 1998 for all racial/ethnic groups, except for Hispanics; the greatest decline was found for blacks (40%) and the least decline was observed for whites (22%). The hospitalization rate was also significantly lower in 2005 than in 1998 in all regions.

The age-adjusted rate for hospitalizations for gastritis/duodenitis (selected as a comparison group to ensure that a change in the PUD hospitalization rate was not attributable to changes in diagnosis coding practices) decreased 16%, from 55.0/100,000 population (95% CI 52.6–57.4) in 1998 to 46.0/100,000 population (95% CI 44.4–47.7) in 2005. The rate of hospitalization for all diagnoses (included as a comparison group to ensure that a change in hospitalizations for PUD was not merely a reflection of a change in the total number of hospitalizations for all diagnoses) did not change significantly during the study period.

Procedures and Other Listed Diagnoses

Esophago-gastroduodenoscopy (EGD) with closed biopsy of ≥1 sites involving the esophagus, stomach, or duodenum was the most common procedure performed in patients with PUD listed as first reason for hospitalization (Figure 2). Transfusion of packed red blood cells, endoscopic control of gastric or duodenal bleeding and flexible fiberoptic colonoscopy were also common. Many diagnoses frequently were listed with PUD hospitalizations. Among these, unspecified essential hypertension, acute posthemorrhagic anemia, iron deficiency anemia secondary to blood loss, diaphragmatic hernia, and *H. pylori* infection were the most common (Figure 3). A greater proportion of ulcers designated duodenal were listed with an *H. pylori* co-diagnosis than any other ulcer designation considered (Figure 4).

Hospitalization Rates for *H. pylori* Infections

The overall age-adjusted rate of hospitalization that included any discharge diagnosis of *H. pylori* infection decreased 47%, from 35.9/100,000 population (95% CI

Table 2. Number of hospitalizations and the age-adjusted and age-specific hospitalization rates for first-listed discharge diagnoses of peptic ulcer disease, United States, 1998–2005*

Characteristic	1998		2005		% Rate change	β-coefficient‡
	No. (SE)	Rate† (95% CI)	No. (SE)	Rate† (95% CI)		
Age, y						
<20	1,524 (133)	1.9 (1.6–2.2)	1,924 (290)	2.4 (1.7–3.0)	+26	0.016§
20–44	26,420 (817)	25.5 (23.9–27.0)	21,523 (656)	20.5 (19.3–21.7)	–20	–0.648¶
45–64	50,446 (1,164)	86.6 (82.7–90.5)	50,993 (1,326)	70.0 (66.4–73.6)	–19	–1.911¶
≥65	115,181 (2,750)	332.7 (317.1–348.3)	95,349 (2,449)	259.2 (246.1–272.2)	–22	–8.759¶
Sex						
M	100,721 (2,159)	83.1 (80.4–85.6)	84,883 (2,013)	62.2 (59.9–64.4)	–25	–2.164¶
F	92,831 (2,080)	60.8 (58.7–62.9)	84,808 (2,040)	51.3 (49.5–53.2)	–16	–0.886¶
Race/ethnicity#						
White	111,092 (4,086)	50.3 (47.7–52.9)	91,939 (3,765)	39.5 (37.3–41.8)	–22	–1.696¶
Black	18,188 (1,428)	68.6 (61.7–75.6)	12,794 (899)	41.4 (37.5–45.4)	–40	–2.093¶
Hispanic	10,850 (1,310)	63.3 (50.7–75.9)	11,079 (1,034)	43.4 (37.3–49.6)	–31	0.308§
Asian/Pacific Islander	4,526 (673)	59.7 (45.4–73.9)	4,220 (520)	38.2 (30.9–45.4)	–36	–1.620**
Region						
Northeast	33,578 (1,746)	60.1 (55.5–64.7)	29,046 (1,740)	49.3 (45.1–53.6)	–18	–1.697¶
Midwest	44,910 (1,909)	70.1 (65.5–74.7)	39,097 (1,562)	57.5 (53.8–61.1)	–18	–0.964**
South	76,773 (2,551)	79.5 (75.6–83.4)	66,969 (2,711)	61.3 (57.8–64.8)	–23	–2.046¶
West	38,315 (1,707)	68.9 (64.2–73.7)	34,746 (1,519)	53.8 (50.2–57.3)	–22	–1.129††
Total	193,576 (4,014)	71.1 (68.9–73.4)	169,858 (3,889)	56.5 (54.6–58.3)	–21	–1.501¶

*Diagnosis codes 531–534 from the International Classification of Diseases, 9th revision, Clinical Modification. National estimates were determined by using the Nationwide Inpatient Sample (*21*).
†Per 100,000 population.
‡β-coefficient from linear regression analysis, using a weighted least-squares technique, of the annual rate over the years of the study period. The p value corresponds to the test of the null hypothesis that the coefficient for year is zero.
§ $p \geq 0.05$.
¶$p < 0.001$.
#Race/ethnicity was missing for 26.0% of the hospitalizations. Data were insufficient for the race/ethnicity category American Indian/Alaska Native.
**$0.01 \leq p < 0.05$.
††$0.001 \leq p < 0.01$.

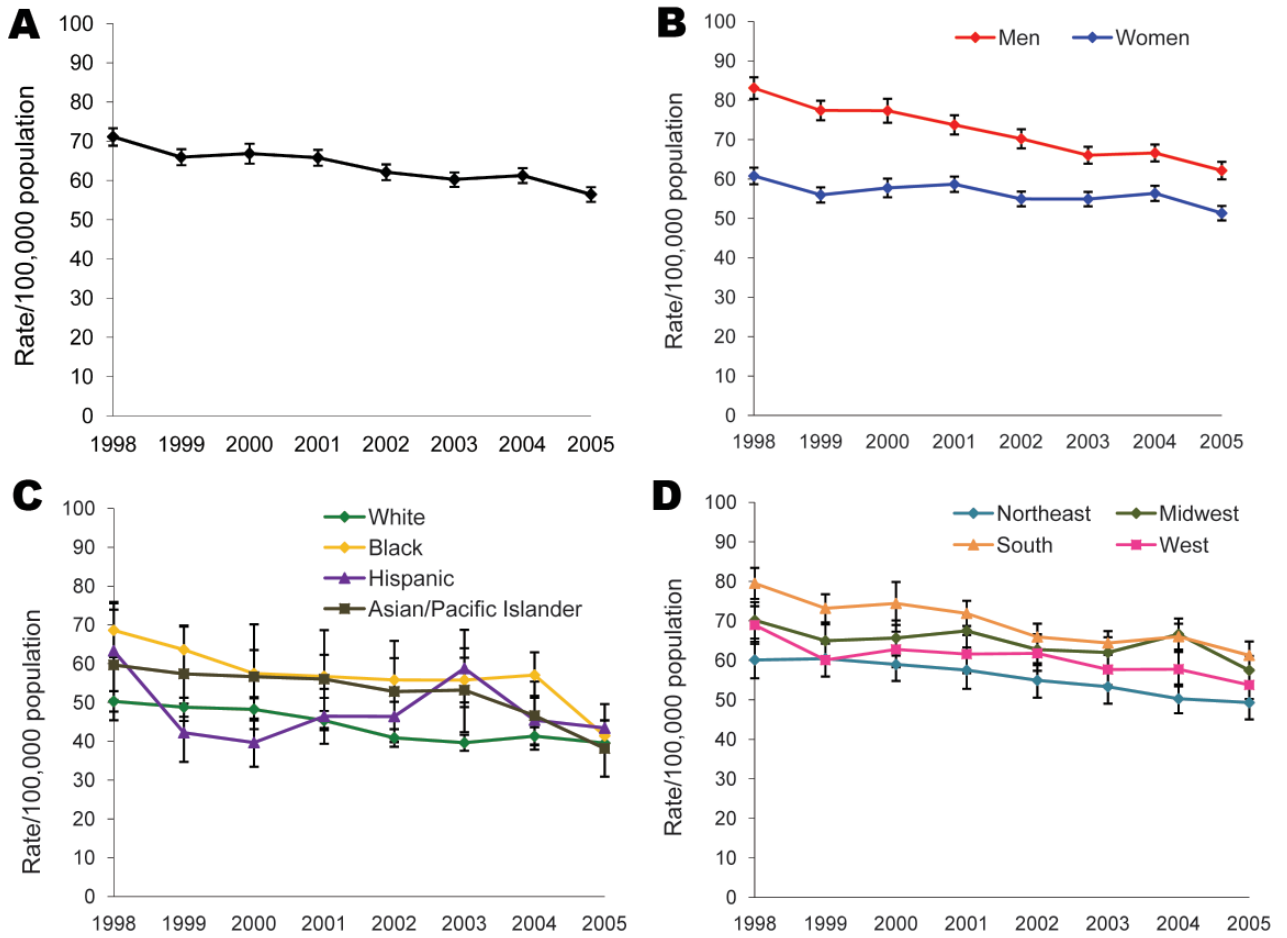

Figure 1. Age-adjusted hospitalization rates for first-listed discharge diagnoses of peptic ulcer disease (diagnosis codes 531–534 from the International Classification of Diseases, 9th revision, Clinical Modification), United States, 1998–2005. A) Overall age-adjusted hospitalization rate. B) Age-adjusted hospitalization rate by gender. C) Age-adjusted hospitalization rate by race/ethnicity. D) Age-adjusted hospitalization rate by region. Source: Nationwide Inpatient Sample (*21*). Race/ethnicity information was missing for 26.0% of hospitalizations.

34.3–37.5) in 1998 to 19.2/100,000 population (95% CI 18.3–20.1) in 2005 (Table 3). The hospitalization rate increased with age and declined during 1998–2005 for all age groups except children <20 years of age. The greatest percentage rate decrease was observed for adults ≥65 years of age, for whom the hospitalization rate decreased 54%, from 163.5/100,000 population (95% CI 152.9–174.0) in 1998 to 75.4/100,000 population (95% CI 70.0–80.8) in 2005. The hospitalization rate for male patients was only slightly higher than that for female patients, and the decline in rates was similar for both groups. In 1998, the hospitalization rate was higher for blacks (44.1/100,000 population; 95% CI 39.7–48.6) and Hispanics (41.8/100,000 population; 95% CI 34.0–49.6) than for whites (23.2/100,000 population; 95% CI. 21.7–27.9) and Asian/Pacific Islanders (34.0/100,000 population; 95% CI 26.2–41.9). In 2005, the same pattern was observed; hospitalization rate was significantly higher for blacks (23.6/100,000 population; 95% CI 21.1–26.1) and Hispanics (24.5/100,000 population; 95% CI 21.1–27.9) than for whites (10.3/100,000 population; 95% CI 9.6–11.0) and Asian/Pacific Islanders (15.8/100,000 population; 95% CI 12.0–19.6). Rates declined significantly for all racial/ethnic groups, except for Hispanics; the greatest percentage rate decrease was observed for whites (56%). The hospitalization rates were significantly different in 1998 and 2005 in all geographic regions. In 1998, the hospitalization rate was higher in the South (39.3/100,000 population; 95% CI 36.4–42.1) than in the West and Northeast regions (33.6/100,000 and 31.4/100,000 population, respectively; 95% CI 30.8–36.3 and 27.8–35.1, respectively). By 2005, the hospitalization rate was higher in the South (21.6/100,000 population; 95% CI 19.8–23.4) and in the Northeast (19.3/100,000 population; 95% CI. 17.6–21.0) than in the West (16.5/100,000 population; 95% CI 14.8–18.1) because the decline apparently occurred more slowly in the Northeast (39%) than in all other regions.

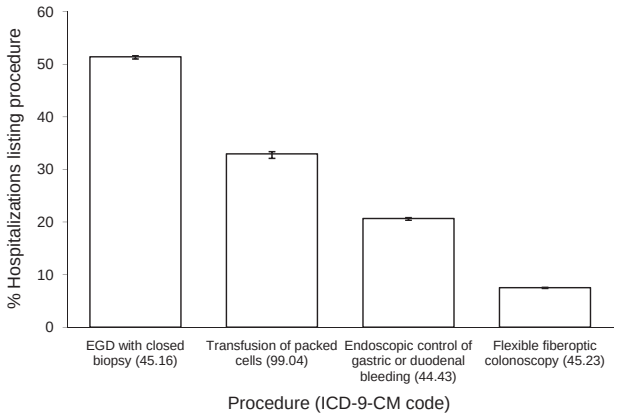

Figure 2. Hospital procedures most frequently listed with first-listed discharge diagnoses of peptic ulcer disease (diagnosis codes 531–534 from the International Classification of Diseases, 9th revision, Clinical Modification [ICD-9-CM]), United States, 1998–2005. Source: Nationwide Inpatient Sample (*21*). EGD, esophagogastroduodenoscopy.

Discussion

Our analysis of hospital discharge records from a nationally representative sample of US hospitals indicates that the overall age-adjusted rate of hospitalization for PUD declined during 1998–2005. This finding is consistent with decreases previously observed for PUD hospitalizations in the United States in several studies from the 1970s through the 1990s (*7*,*8*,*14*,*17*,*29*). One study by Manuel et al. did not observe a downward trend in PUD hospitalizations during 1996–2005; however, that study included only 5 hospitals (*30*). We analyzed data for first-listed PUD hospitalizations to limit data to hospitalizations for care specifically for ulcer-related issues. In addition, >50% of patients in our study had an EGD with closed biopsy of ≥1 sites involving the esophagus, stomach, or duodenum (Figure 2). EGD with biopsy is a reliable technique to differentiate between PUD and other causes of abdominal pain such as gastritis (*17*). Thus, it appears that the data in this study are reflective of patients who were truly hospitalized primarily for PUD or its complications.

Decreases in PUD hospitalizations are likely attributable to an underlying decline in *H. pylori* prevalence (*7*,*15*). However, declines in PUD hospitalizations have also been attributed to changes in diagnosis coding because of improved diagnostic specificity associated with endoscopy (*31*). If the decrease in PUD hospitalizations was attributable to changes in diagnosis coding, the decrease in PUD hospitalizations would be inversely related to a rise in hospitalizations for gastritis/duodenitis. We found that hospitalization rates declined for gastritis/duodenitis and

Table 3. Number of hospitalizations and the age-adjusted and age-specific hospitalization rates for any-listed discharge diagnoses that included *Helicobacter pylori* infection, United States, 1998–2005*

Characteristic	1998		2005		% Rate change	β-coefficient‡
	No. (SE)	Rate† (95% CI)	No. (SE)	Rate† (95% CI)		
Age, y						
<20	1,320 (151)	1.7 (1.3–2.0)	1,718 (276)	2.1 (1.4–2.8)	+24	0.040§
20–44	14,617 (648)	14.1 (12.9–15.3)	10,391 (457)	9.9 (9.0–10.7)	–30	–0.436¶
45–64	25,287 (955)	43.4 (40.2–46.6)	17,923 (724)	24.6 (22.7–26.6)	–43	–2.265¶
≥65	56,593 (1,866)	163.5 (152.9–174.0)	27,733 (1,009)	75.4 (70.0–80.8)	–54	–10.647¶
Sex						
M	47,162 (1,450)	38.9 (37.2–40.6)	28,535 (946)	20.6 (19.6–21.6)	–47	–1.854¶
F	50,656 (1,830)	33.4 (31.7–35.0)	29,220 (1,078)	18.0 (17.0–18.9)	–46	–1.909¶
Race/ethnicity#						
White	51,235 (2,353)	23.2 (21.7–24.7)	23,735 (1,117)	10.3 (9.6–11.0)	–56	–1.649¶
Black	11,724 (887)	44.1 (39.7–48.6)	7,480 (561)	23.6 (21.1–26.1)	–47	–1.926¶
Hispanic	7,305 (839)	41.8 (34.0–49.6)	7,075 (720)	24.5 (21.1–27.9)	–41	0.131§
Asian/Pacific Islander	2,563 (361)	34.0 (26.2–41.9)	1,771 (252)	15.8 (12.0–19.6)	–54	–1.471¶
Region						
Northeast	17,551 (1,588)	31.4 (27.8–35.1)	11,285 (681)	19.3 (17.6–21.0)	–39	–1.392¶
Midwest	23,644 (1,430)	36.9 (33.6–40.2)	12,116 (722)	17.9 (16.3–19.5)	–52	–2.229¶
South	37,929 (2,100)	39.3 (36.4–42.1)	23,603 (1,476)	21.6 (19.8–23.4)	–45	–2.193¶
West	18,699 (1,038)	33.6 (30.8–36.3)	10,766 (738)	16.5 (14.8–18.1)	–51	–1.493¶
Total	97,823 (3,156)	35.9 (34.3–37.5)	57,770 (1,925)	19.2 (18.3–20.1)	–47	–1.884¶

*Diagnosis codes 41.86 from the International Classification of Diseases, 9th revision, Clinical Modification. National estimates were determined by using the Nationwide Inpatient Sample (*21*). All *H. pylori* diagnoses are 1 of as many as 15 diagnoses.
†Per 100,000 population.
‡β-coefficient from linear regression analysis, using a weighted least-squares technique, of the annual rate over the years of the study period. The p value corresponds to the test of the null hypothesis that the coefficient for year is zero.
§p≥0.05.
¶p<0.001.
#Race/ethnicity was missing for 26.0% of the patients hospitalized. Data were insufficient for the race/ethnicity category American Indian/Alaska Native.

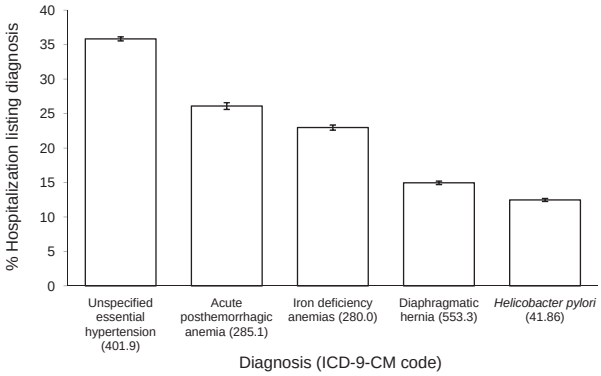

Figure 3. Other diagnoses most frequently listed with first-listed discharge diagnoses of peptic ulcer disease (PUD) (diagnosis codes 531–534 from the International Classification of Diseases, 9th revision, Clinical Modification [ICD-9-CM]), United States, 1998–2005. Source: Nationwide Inpatient Sample (*21*). Iron deficiency anemias, iron deficiency anemias secondary to blood loss.

for PUD, which indicates that the results cannot be attributable to changes in diagnosis coding practices. Furthermore, because the hospitalization rate for all diagnoses did not change significantly from 1998 to 2005, the decline in the PUD hospitalization rate observed would not merely reflect general trends in hospitalization. The overall rate for any listed *H. pylori* diagnosis declined significantly during the study period, which suggests that a decrease in rates of *H. pylori* infections may be partially responsible for the decrease in hospitalizations for PUD.

In our study, the overall rate of hospitalizations for PUD differed according to the patient's age, sex, race/ethnicity, and region. The highest rates of hospitalization for those with both PUD and *H. pylori* infection were for adults ≥65 years of age and decreased with each subsequent age group. This finding may result from an underlying birth cohort effect, in this case a decrease in *H. pylori* incidence for younger generations because of improved sanitation and fewer risk factors for transmission (*18*,*32*,*33*). A similar percentage change in rate of PUD hospitalizations was observed for all age groups ≥20 years. The comparable declines for these age groups may be partially attributable to increased use of *H. pylori* eradication therapy during 1998–2005, perhaps because of increased awareness among clinicians and patients of the association between *H. pylori* and PUD (*9*).

In this study, the overall rate of hospitalization for PUD in 1998 was higher for male patients than for female patients. However, by 2005 this difference had narrowed considerably because of a greater decrease in for male patients than for female patients. A 1985 study that examined data from the National Center for Health Statistics also recognized a trend toward comparable rates of hospitalization for both sexes (*34*). Our study also found differences in hospitalization rates between sexes by designated ulcer type; duodenal ulcer hospitalization rates were higher for male patients than for female patients. This finding is consistent with hospital admission data from the United Kingdom (*15*,*16*).

A study that used a national sample of US hospital discharge records noted differences in the hospitalization rate for PUD between racial/ethnic groups; blacks were more frequently hospitalized for PUD than whites in 1998 (*13*). Although we also found that rate of hospitalization for PUD was higher for blacks than for whites, the rate appears to be declining more rapidly for blacks than for whites. In addition, although rates were significantly lower for whites than for those in other racial/ethnic categories in 1998, by 2005 this rate difference was no longer significant because for whites, the decline apparently occurred more slowly than it did for all other racial/ethnic groups. Differences also varied by sex, as well as race/ethnicity, and suggest that hospitalizations for PUD among nonwhite men may merit further investigation. Race/ethnicity information was missing for patients in 26% of hospitalization records, possibly making comparisons between racial/ethnic groups inaccurate. A study of underreporting of race/ethnicity information in the National Hospital Discharge Survey suggests that hospitals that do not report race/ethnicity information may have a higher proportion of discharges for whites and a lower proportion of discharges for blacks than hospitals that do report race/ethnicity information (*35*). Our study did not examine PUD hospitalizations for American Indians and Alaska Natives because the survey's sample size was not large enough and did not include visits to Indian Health Service or tribal facilities. However, a previous study showed that among American Indians and Alaska Natives, the prevalence of ulcer-associated conditions was high during 1996–2005, which indicates that hospitalizations for PUD among this group may warrant further study (*36*).

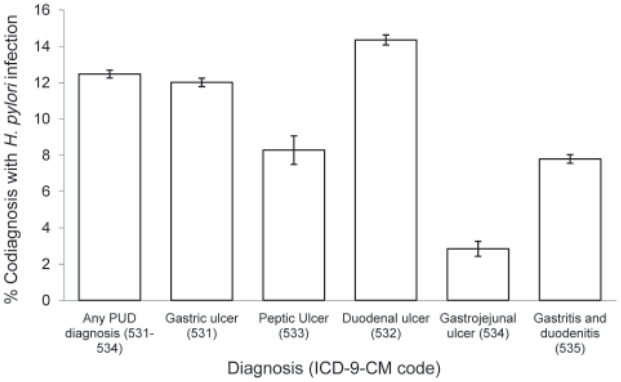

Figure 4. Proportion of first-listed ulcer diagnoses with a co-diagnosis of *Helicobacter pylori* infection (diagnosis codes 531–534 from the International Classification of Diseases, 9th revision, Clinical Modification [ICD-9-CM]), by ulcer type, United States, 1998–2005. Source: Nationwide Inpatient Sample (*21*). PUD, peptic ulcer disease.

Our study showed similar trends for hospitalizations for PUD and *H. pylori* infection, although we noted some differences. For both PUD and *H. pylori* infections, the rate of hospitalization increased with age, and the age-adjusted hospitalization rate was lower for whites than for persons in any other racial/ethnic group category. In addition, the overall age-adjusted rate of both PUD and *H. pylori* hospitalizations was higher for male patients than for female patients. However, although the age-adjusted PUD hospitalization rate appears to be declining more rapidly among male patients than among female patients, the age-adjusted *H. pylori* infection hospitalization rate appears to be declining at a similar pace for female patients and male patients. The age-adjusted PUD hospitalization rate for Hispanics did not decline significantly, and a decline in the age-adjusted *H. pylori* hospitalization rate for this group was only borderline significant, which suggests that rates among this group may deserve special attention. However, this finding may be biased because of missing information on race/ethnicity.

Our findings in this study show a continued downward trend in the rate of hospitalizations for PUD in the United States. Differences in the rate of decline for PUD hospitalization rates between sexes, racial/ethnic groups, and regions warrant further study. The overall downward trend observed in this study does not seem to be attributable to increases in gastritis/duodenitis hospitalizations or to a decline in total hospitalizations. The decline in the PUD hospitalization rate may be attributable to a birth cohort effect with subsequent declines in *H. pylori* infection prevalence and increased use of successful antibiotic treatments to eradicate *H. pylori* infections. Other factors possibly contributed to the decline in PUD hospitalizations observed in this study, including trends in use of nonsteroidal anti-inflammatory drugs and the availability of over-the-counter H2 antagonists and proton pump inhibitors. Studies on the relationship between PUD hospitalizations and nonsteroidal anti-inflammatory drug use, the possibility of undercoding for *H. pylori* on hospitalization discharge records, and subpopulation analyses would help further guide recommendations and show how to focus interventions. To facilitate further declines in hospitalizations for PUD, patients and clinicians should continue to be educated about the association between *H. pylori* and PUD.

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Ms Feinstein previously worked in the area of foodborne epidemiology at the Centers for Disease Control and Prevention. She is now a PhD candidate at the University of North Carolina, Chapel Hill, focusing on the epidemiology of infectious diseases.

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etymologia

Klebsiella

[kleb´´se-el′ə]

The genus *Klebsiella*, family Enterobacteriaceae, was named by V. Trevisan in 1885 in honor of German bacteriologist Theodor Albrecht Edwin Klebs (1834–1913). Dr Klebs is known for his pioneering work demonstrating that microorganisms are responsible for infectious diseases. He also studied the pathologic and bacteriologic features of gunshot wounds; investigated tuberculosis and successfully transmitted the disease to cattle; did research on the bacteriologic characteristics of malaria and anthrax; and, with Friedrich A.J. Löffler in 1884, discovered the etiologic agent of diphtheria, first called the Klebs-Löffler bacillus (later called *Corynebacterium diphtheriae*).

Source: Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E. The prokaryotes. *Klebsiella*. New York: Springer; 2006. p. 159–96; Carter KC. Koch's postulates in relation to the work of Jacob Henle and Edwin Klebs. Medical History. 1985;29:353–74; Dorland's illustrated medical dictionary, 31st edition. Philadelphia: Saunders Elsevier; 2007.

Illicit Drug Use and Risk for USA300 Methicillin-Resistant *Staphylococcus aureus* Infections with Bacteremia

Kristen M. Kreisel, J. Kristie Johnson, O. Colin Stine, Michelle D. Shardell, Eli N. Perencevich, Alan J. Lesse, Fred M. Gordin, Michael W. Climo, and Mary-Claire Roghmann

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Learning Objectives

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

- Examine the association of illicit drug use with USA300 MRSA infection, including risk factors for acquisition and transmission.
- Describe characteristics of illicit drug users who acquire MRSA and the changing pattern of risk from 2004 to 2008 in the United States with implications for management and prevention.

Editor

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Authors

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To assess the association of illicit drug use and USA300 methicillin-resistant *Staphylococcus aureus* (MRSA) bacteremia, a multicenter study was conducted at 4 Veterans Affairs medical centers during 2004–2008. The study showed that users of illicit drugs were more likely to have USA300 MRSA bacteremia (in contrast to bacteremia caused by other *S. aureus* strains) than were patients who did not use illicit drugs (adjusted relative risk 3.0; 95% confidence interval 1.9–4.4). The association of illicit drug use with USA300 MRSA bacteremia decreased over time (p = 0.23 for trend). Notably, the proportion of patients with USA300 MRSA bacteremia who did not use illicit drugs increased over time. This finding suggests that this strain has spread from users of illicit drugs to other populations.

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Infections caused by community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) are in-

creasing. Outbreaks have been described in a variety of populations, including sports teams, men who have sex with men, prisoners, and children (*1–10*). The USA300 MRSA clone has been recognized as the most common strain causing CA-MRSA infections (*11*).

CA-MRSA was first reported in illicit drug users in Detroit in 1980 (*12*). The drug-using population has been identified as a reservoir of CA-MRSA (*13*). Because of the repeated injection or inhalation of drugs, the opportunity for a person to cause and spread infection with one's own colonizing strain is multiplied (*13*,*14*). Skin and soft tissue infections are the most common infections in illicit drug users; the USA300 MRSA strain is the cause of up to 75% of these infections (*13–15*). Once this strain colonizes or otherwise infects a person, it can then enter the patient's bloodstream and become a potentially life-threatening bloodstream infection.

If admitted to the hospital, illicit drug–using patients with a USA300 MRSA infection complicated by bacteremia serve as a potential reservoir for transmission to other patient populations. This mechanism may be contributing to the replacement of other MRSA strains typically associated with nosocomial infections by USA300 MRSA and may aid this strain in becoming the predominant isolate causing MRSA infections in both healthcare and community settings (*16*,*17*). The objective of this study was to evaluate the association of illicit drug use with USA300 MRSA bacteremia and whether the association is static or has changed over a 5-year period as the USA300 MRSA epidemic has progressed.

Methods

Study Design and Population

A multicenter retrospective cohort study was conducted by using patients from the population of veterans enrolled from January 2004 through June 2008 at the Veterans Affairs medical centers (VAMCs) in Baltimore, Maryland; Washington, DC; Buffalo, New York; and Richmond, Virginia. Patients who used illicit drugs were compared with those who did not use illicit drugs with respect to having USA300 MRSA bacteremia vs. bacteremia caused by all other types of *S. aureus* (this group includes non-USA300 MRSA and methicillin-susceptible *S. aureus* [MSSA]). Inclusion criteria for patients included in the study were the following: 1) age ≥18 years, 2) enrollment in patient care services at 1 of the 4 VAMCs, 3) a positive blood culture for *S. aureus*, 4) having first known invasive infection caused by *S. aureus*, and 5) having a bacterial isolate from the infection available for testing. We excluded patients from the analysis for whom the infection was found to be polymicrobial, or for whom the bacteremic episode was considered to be clinically insignificant (i.e., the patient did not have clinical symptoms consistent with the presence of infection, such as fever) (*18*). The Institutional Review Boards at all participating sites approved this protocol.

Data Collection and De nitions

Data were collected from patient electronic medical records, which included administrative coding data. An infection control nurse, who was blinded to the outcome of each patient, conducted chart review by using a standardized form. Illicit drug use was defined by International Classification of Diseases, 9th Revision (ICD-9), codes indicating abuse of or dependence on cocaine (ICD-9 codes: 304.21, 304.20, 304.23, 305.61, 305.62, 305.60) or opioids (ICD-9 codes: 304.01, 304.71, 304.00, 305.51, 304.73), designated at any hospitalization up to 1 year prior to the time of the patient's presentation. The electronic medical record of any patient identified as an illicit drug user was further evaluated to determine whether the abuse was by injection.

An infection was defined as nosocomial if the patient's blood culture was positive for *S. aureus* >48 hours after hospital admission, if the patient was transferred from another healthcare facility, or if the infection was central-line associated. Infections were defined as central-line associated if a primary source was identified (i.e., no other source of infection could be found) and if the patient had a central line in place for 48 hours before the onset of bacteremia (*19*). If the patient had pneumonia, a skin and soft tissue infection, a urinary tract infection, or some other source that could explain the basis for infection, the bacteremia was defined as a secondary infection. Infective endocarditis was defined by using the modified Duke criteria (*20*).

Information on risk factors for MRSA acquisition and infection was also obtained. They included whether the patient had been hospitalized, had surgery, resided in a long-term care facility, or had undergone hemodialysis in the year before infection, as well as if any foreign medical device was present at the time of infection or if the patient had been previously colonized or infected with MRSA. The presence of HIV was assessed, as well as the presence of comorbid conditions to calculate each patient's Charlson score (*21*).

Laboratory Evaluations

All *S. aureus* isolates were sent for testing to the Baltimore VAMC. *S. aureus* was confirmed by standard microbiologic techniques. Any isolate with growth on oxacillin screen agar was defined as MRSA; any isolate without growth on this agar was defined as MSSA. All *S. aureus* isolates were screened for the presence of the Panton-Valentine leukocidin gene (PVL; *luk-F-PV, luk-S-PV*), as previously described (*22*). Further screening for the presence

of the arginine catabolic mobile element gene (ACME; *arc*A) and sequencing of the protein A (*spa*) gene hypervariable region was also performed on MRSA isolates only, as previously described (*23*,*24*). Patient sequences were compared with sequences found in the Ridom *spa* Server (www.ridom.de/spaserver).

USA300 MRSA isolates were identified by using an algorithm previously described (*5*). Any MRSA isolate that tested positive for the genes for PVL and ACME, and was *spa* type motif MBQBLO, was classified as USA300 MRSA. These isolates were confirmed as USA300 MRSA by pulsed-field gel electrophoresis (PFGE) by using a 24% random sample (13/55 suspected USA300 MRSA isolates were tested) (*11*). MRSA isolates that were negative for all 3 genetic factors were classified as non-USA300 MRSA. A 26% random sample of the non-USA300 MRSA isolates was also confirmed by PFGE (27/103 suspected non-USA300 MRSA isolates were tested) (*11*). All MRSA isolates testing positive for at least 1 of the 3 (PVL, ACME, or *spa* type motif MBQBLO) were further characterized by PFGE to determine whether any were the USA300 MRSA strain. If pulsed-field type USA300 by PFGE, the isolates were classified as USA300 MRSA. All other MRSA isolates (non-USA300 MRSA) and MSSA isolates were classified as "all other *S. aureus*." The Fingerprinting II software was used to analyze the electronic images of the gels (Bio-Rad Laboratories, Hercules, CA, USA). The banding patterns of each isolate were compared with the USA PFGE types described by McDougal et al. (*11*); the similarity between isolates was assessed by using the criteria established by Tenover et al. (*25*)

Statistical Analysis

Data were analyzed by using the SAS statistical software package, version 9.1 (SAS Institute Inc., Cary, NC, USA). The Pearson χ^2 or Fisher exact tests were used to compare categorical variables, and the Student *t* test or Wilcoxon signed-rank test was used to compare continuous variables. A p value ≤ 0.05 was considered significant. Unadjusted relative risks (RR) were calculated to estimate the association between illicit drug use and USA300 MRSA bacteremia. Stratified analyses were conducted to test for effect modification and confounding; any variable with a Breslow-Day p value ≤ 0.05 was considered significant, while a 10% difference between the unadjusted and adjusted RR was used to identify confounding. A binomial regression using a log link was fit to estimate the association between illicit drug use and USA300 MRSA bacteremia, adjusting for identified confounders and/or effect modifiers.

Results

We identified 300 patients with *S. aureus* bacteremia at the 4 participating sites during the study period. Strains having all 3 genetic factors (PVL, ACME, and *spa* type motif MBQBLO) were classified as USA300 MRSA, and a random sample of these isolates showed 100% sensitivity and specificity by PFGE. Isolates with none of these genetic factors were classified as non-USA300 MRSA, and a random sample of these isolates also showed 100% sensitivity and specificity by PFGE. Isolates with 1 or 2 of the genetic factors were also designated as non-USA300 MRSA; 18% of these isolates were found to be USA300 MRSA by PFGE, resulting in 100% sensitivity and 82% specificity of the laboratory algorithm for identifying USA300 MRSA. Sixty-seven (22%) of the infections were caused by USA300 MRSA, 117 (39%) by non-USA300 MRSA, and 116 (39%) by MSSA.

Patient and infection characteristics of the study population are presented in Tables 1 and 2. Of all patients with *S. aureus* bacteremia, 22 (7%) were illicit drug users, 13 (59%) with injection drugs. The patients had a mean age of 68 years and were almost all male (98%). Infections were classified as nosocomial in 172 (57%) patients, 83 (48%) of which were central-line associated. Sixteen (5%) patients were infected with HIV and 80 (27%) had a previous episode of colonization or infection with MRSA.

Compared with patients who did not use illicit drugs, illicit drug users were younger (mean age 51 vs. 69; $p<0.0001$; Table 3) and more likely to be African American (17% vs. 3%; $p<0.0001$). Illicit drug users were more likely to have HIV (38% vs. 6%; $p<0.0001$) or endocarditis (16% vs. 6%; $p = 0.04$) and less likely to have acquired their infection nosocomially (3% vs. 13%; $p = 0.003$) than patients who did not use illicit drugs. Illicit drug users were significantly more likely to have a bacteremic infection caused by USA300 MRSA than by all other *S. aureus* strains, compared with patients who did not use illicit drugs (RR 3.04, 95% confidence interval [CI] 1.99–4.64; $p<0.0001$; Table 4). Age (mean age 63 vs. 69 years; $p = 0.0004$) and a nosocomial acquisition of infection (RR 0.44, 95% CI 0.29–0.69; $p = 0.0002$) were both negatively associated with USA300 MRSA bacteremia.

Using binomial regression, illicit drug users were significantly more likely to have USA300 MRSA bacteremia compared with patients not using illicit drugs, controlling for year of presentation (adjusted RR [aRR] 3.00, 95% CI 1.88–4.36; $p<0.0001$; Table 5). This result was due to an increase in the proportion of *S. aureus* bacteremic infections caused by the USA300 MRSA strain in patients who did not use illicit drugs over the study period, while the proportion in patients using illicit drugs remained relatively stable after 2004. Stratified by year of presentation (categorized as early [January 1, 2004–March 31, 2006] vs. late [April 1, 2006–June 30, 2008] years of presentation), the association of illicit drug use with USA300 MRSA bacteremia decreased over the study period (RR in early years

Table 1. Isolate and patient characteristics for 300 veterans who had *Staphylococcus aureus* bacteremia at 4 Veterans Affairs medical centers, USA, 2004–2008*

Characteristic	No. (%)
S. aureus isolate characteristics	
USA300 MRSA	67 (22)
All other *S. aureus*	233 (78)
Non-USA300 MRSA	117 (39)
MSSA	116 (39)
Patient characteristics	
Age, y, mean ± SD	68 ± 13
Sex	
M	295 (98)
F	5 (2)
Race	
Black	95 (32)
Other	205 (68)
Year of presentation†	
Late	169 (56)
Early	131 (44)
Charlson score, mean ± SD	4.7 ± 3.0
HIV infection	
Yes	16 (5)
No	284 (95)
Illicit drug use	
Yes	22 (7)
No	278 (93)
History of colonization or infection with MRSA	
Yes	80 (27)
No	220 (73)
Hospitalized in year before infection	
Yes	195 (65)
No	105 (35)
Surgery in year before infection	
Yes	109 (36)
No	191 (64)
Residence in LTCF in year before infection	
Yes	34 (11)
No	266 (89)
Renal failure in year before infection	
Yes	114 (38)
No	186 (62)

*Values are no. (%) except as indicated. MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-susceptible *S. aureus*; LTCF, long-term care facility.
†Year of presentation stratified into 2 periods: early (January 1, 2004–March 31, 2006) and late (April 1, 2006–June 30, 2008).

of presentation 4.63, 95% CI 2.36–9.07; p<0.0001; RR for late years of presentation 2.45, 96% CI 1.38–4.35; p = 0.02). The association of illicit drug use and USA300 MRSA bacteremia weakened over the study period, although the trend was not statistically significant (p = 0.23 for trend over time; Figure).

Discussion

In this multicenter study, illicit drug users were more likely to have a bacteremic infection caused by USA300 MRSA than any other type of *S. aureus*, regardless of when the patient's infection occurred. The data were consistent with a decrease in the association over the study period, from a RR of 4.63 in the early years of the study to a RR of 2.45 in the late years of the study.

The finding that the association between illicit drug use and USA300 MRSA bacteremia declined over the study period is clinically important. This illustrates that, although USA300 MRSA infections emerged in drug users, this epidemic is now spreading to other populations. The illicit drug–using population has been recognized as a reservoir for USA300 MRSA (*14*). The spread to other populations observed in this study could be the result of transmission and dissemination of the strains among at-risk patients in the community, as suggested by the fact that 43% of cases of bacteremia were not classified as nosocomial infections in this population. This means that patients who have bacteremia could have acquired the bacteria during a previous healthcare exposure or in the community. In contrast, illicit drug users who are colonized or infected with USA300 MRSA could serve as a reservoir for transmission to other patient populations while hospitalized for their infections (*12–14*,*26*,*27*). Further investigation is warranted.

The increased risk of acquiring an infection caused by USA300 MRSA among illicit drug users found in this study is consistent with the findings of other studies. Gilbert et al. reported that the incidence of USA300 MRSA was higher among high-risk case-patients (defined as including a history of illicit drug use) than low-risk case-patients (*28*).

Table 2. Infection characteristics for 300 veterans who had *Staphylococcus aureus* bacteremia at 4 Veterans Affairs medical centers, USA, 2004–2008

Infection characteristic	Value
Nosocomial infection	
Yes	172 (57)
No	128 (43)
Central line at time of infection	
Yes	83 (28)
No	217 (72)
Permanent hardware at time of infection	
Yes	88 (29)
No	212 (71)
Source of infection	
Primary	126 (42)
Secondary	174 (58)
Infection complicated by endocarditis*	
Yes	38 (13)
No	262 (87)
Infection complicated by pneumonia†	
Yes	45 (15)
No	255 (85)

*Data missing for 4 patients (1%).
†Data missing for 11 patients (4%).

In a study of the New York State Prison System, inmates with drug charges were more likely to have an infection. The USA300 MRSA strain was the predominant clone that caused infections in inmates (*29*). Although our study used different sampling methods and slightly different case definitions than previous studies, the conclusions are the

Table 3. Patient and infection characteristics for 300 veterans with *Staphylococcus aureus* bacteremia and association with illicit drug use at 4 Veterans Affairs medical centers, USA, 2004–2008*

Variable	Used illicit drugs,† n = 22	No illicit drug use,† n = 278	p value‡
Patient characteristics			
Age, y, mean ± SD	51 ± 5	69 ± 12	<0.0001
Sex			
M	20 (7)	275 (93)	0.05
F	2 (40)	3 (60)	
Race			
Black	16 (17)	79 (83)	<0.0001
Other	6 (3)	199 (97)	
Year of presentation§			
Late	10 (6)	159 (94)	0.29
Early	12 (9)	119 (91)	
Charlson score, mean ± SD	5.4 ± 4.1	4.7 ± 2.9	0.28
HIV infection			
Yes	6 (38)	10 (62)	<0.0001
No	16 (6)	268 (94)	
History of colonization or infection with MRSA			
Yes	5 (6)	75 (94)	0.66
No	17 (8)	203 (92)	
Hospitalized in year before infection			
Yes	15 (8)	180 (92)	0.75
No	7 (7)	98 (93)	
Surgery in year before infection			
Yes	1 (1)	108 (99)	<0.0001
No	21 (11)	170 (89)	
Residence in LTCF in year before infection			
Yes	3 (9)	31 (91)	0.73
No	19 (7)	247 (93)	
Renal failure in year before infection			
Yes	14 (8)	172 (92)	0.87
No	8 (7)	106 (93)	
Infection characteristics			
Nosocomial infection			
Yes	6 (3)	166 (97)	0.003
No	16 (13)	112 (87)	
Central line at time of infection			
Yes	5 (6)	78 (94)	0.59
No	17 (8)	200 (92)	
Permanent hardware at time of infection			
Yes	4 (5)	84 (95)	0.33
No	18 (8)	194 (92)	
Source of infection			
Primary	10 (8)	116 (92)	0.73
Secondary	12 (7)	162 (93)	
Infection complicated by endocarditis			
Yes	6 (16)	32 (84)	0.04
No	16 (6)	242 (94)	
Infection complicated by pneumonia			
Yes	3 (7)	42 (93)	1.00
No	19 (7)	236 (93)	

*MRSA, methicillin-resistant *S. aureus*; LTCF, long-term care facility.
†Values are no. (%) except as indicated.
‡Calculated by using Pearson χ^2 or Fisher exact tests for categorical variables, Student *t* test or Wilcoxon signed-rank test for continuous variables.
§Year of presentation stratified into 2 periods: early (January 1, 2004–March 31, 2006) and late (April 1, 2006–June 30, 2008).

Table 4. Patient and infection characteristics for 300 veterans with *Staphylococcus aureus* bacteremia and association with USA300 MRSA at 4 Veterans Affairs medical centers, USA, 2004–2008*

Variable	USA300 MRSA bacteremia,† n = 67	Bacteremia due to all other *S. aureus,*† n = 233	p value‡
Patient characteristics			
Age, y, mean ± SD	63 ± 12	69 ± 13	0.0004
Sex			
M	65 (22)	230 (78)	0.31
F	2 (40)	3 (60)	
Race			
Black	34 (36)	61 (64)	0.0001
Other	33 (16)	172 (84)	
Year of presentation§			
Late	45 (27)	124 (73)	0.04
Early	22 (17)	109 (83)	
Charlson score, mean ± SD	5.2 ± 3.6	4.6 ± 2.8	0.19
HIV infection			
Yes	5 (31)	11 (69)	0.36
No	62 (22)	222 (78)	
Illicit drug use			
Yes	13 (59)	9 (41)	<0.0001
No	54 (19)	224 (81)	
History of colonization or infection with MRSA*			
Yes	21 (26)	59 (74)	0.33
No	46 (21)	174 (79)	
Hospitalized in year before infection			
Yes	40 (21)	155 (79)	0.30
No	27 (26)	78 (74)	
Surgery in year before infection			
Yes	17 (16)	92 (84)	0.03
No	50 (26)	141 (74)	
Residence in LTCF in year before infection			
Yes	10 (29)	24 (71)	0.29
No	57 (21)	209 (79)	
Renal failure in year before infection			
Yes	42 (23)	144 (77)	0.90
No	25 (22)	89 (78)	
Infection characteristics			
Nosocomial infection			
Yes	25 (15)	147 (85)	0.0002
No	42 (33)	86 (67)	
Central line at time of infection			
Yes	11 (13)	72 (87)	0.02
No	56 (26)	161 (74)	
Permanent hardware at time of infection			
Yes	20 (33)	68 (77)	0.92
No	48 (28)	126 (72)	
Source of infection			
Primary	19 (15)	107 (85)	0.01
Secondary	48 (28)	126 (72)	
Infection complicated by endocarditis			
Yes	9 (24)	29 (76)	0.83
No	57 (22)	201 (78)	
Infection complicated by pneumonia			
Yes	8 (18)	37 (82)	0.40
No	57 (23)	186 (77)	

*MRSA, methicillin-resistant *S. aureus*, LTCF, long-term care facility.
†Values are no. (%) except as indicated.
‡Calculated by using Pearson χ^2 or Fisher exact tests for categorical variables, Student *t* test or Wilcoxon signed-rank test for continuous variables.
§Year of presentation stratified into 2 periods: early (January 1, 2004–March 31, 2006) and late (April 1, 2006–June 30, 2008).

Table 5. Independent risk factors for USA300 MRSA bacteremia among 300 veterans at 4 Veterans Affairs medical centers, USA, 2004–2008*

Variable	Adjusted RR (95% CI)	p value
Illicit drug use	3.00 (1.88–4.36)	<0.0001
Late year of presentation†	1.56 (1.03–2.48)	0.04

*MRSA, methicillin-resistant *Staphylococcus aureus*; RR, relative risk; CI, confidence interval.
†Year of presentation stratified into 2 periods: early (January 1, 2004–March 31, 2006) and late (April 1, 2006–June 30, 2008).

same. Illicit drug users are at increased risk for an infection caused by USA300 MRSA compared with persons who do not use illicit drugs.

The association between illicit drug use and USA300 MRSA bacteremia could be explained by the fact that *S. aureus* infections are a common complication of drug use. Drug users are colonized with *S. aureus* more often than persons who do not use drugs (*13*,*14*). Colonization is a main risk factor for infection; the patient is usually infected with his own colonizing strain. In addition, invasive infections can occur from transfer of the colonizing strain directly into the patient's bloodstream (*13*,*14*). If USA300 MRSA is the colonizing strain, the frequent inhalation or injection of drugs could transfer this strain into the bloodstream to cause a life-threatening invasive infection.

Our study has some limitations. First, data regarding illicit drug use at the various VAMCs is based on self-report. Information regarding drug use is of a sensitive nature and, therefore, subject to recall bias. Also, to locate in the patient's electronic medical record whether the drugs were injected depended on whether the healthcare professional specifically asked and made note of this, a situation which is prone to information bias. Second, the ICD-9 codes used to define illicit drug use are imperfect measurements because they are used for insurance billing, rather than clinical purposes. Also, if any illicit drug–using patients were not hospitalized in the year before enrollment, or if drug-using patients did not report drug use during the hospitalization of interest, drug use would have been misclassified. In addition, using ICD-9 codes to define drug use up to 1 year before admission may not accurately measure current patterns of drug use in patients; the result would be a differential misclassification of exposure and could overestimate or underestimate the true association. Third, the study population was comprised only of veterans. The use of such a distinct population could reduce the generalizability of these findings. However, the relationship of illicit drug use with USA300 MRSA among veterans would likely not differ from the relationship among nonveterans, so the results should still be generalizable. Finally, USA300 MRSA isolates could have been misclassified with the use of our laboratory algorithm; however, the validation of isolates by PFGE, which showed a high sensitivity and specificity, makes this unlikely.

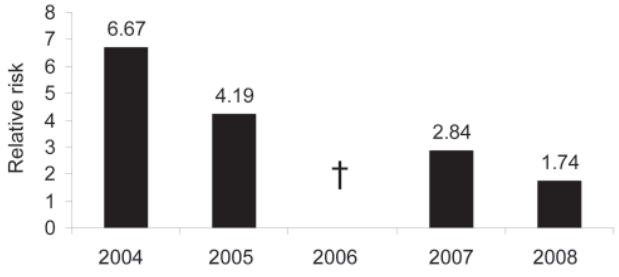

Figure. Association between illicit drug use and USA300 methicillin-resistant *Staphylococcus aureus* bacteremia among 300 veterans at 4 Veterans Affairs medical centers, USA, 2004–2008 (generalized linear model p value for trend over time = 0.23). †No illicit drug users had a bacteremic infection caused by USA300 MRSA in 2006.

This study has several strengths. First, the use of more than 1 study site helped improve the generalizability of these findings. For example, we evaluated whether any of the associations observed may have been due to differences between the 4 VAMCs (this analysis was in response to a finding that patients from the Buffalo VAMC were less likely to be illicit drug users and less likely to have an infection due to USA300 MRSA). After excluding all patients from the Buffalo VAMC from the analysis, we found no difference from the results of the entire cohort (data not shown); therefore, we chose to present the combined data. Additionally, the electronic medical record system used throughout the Veterans Health Administration is known to be a valuable asset because of the completeness of data and for the amount of time it has stored information. The use of this system for chart review helped decrease selection and information bias. Finally, the findings of this study are strengthened because we could provide molecular typing data for each of the isolates.

In conclusion, the data from this study showed that illicit drug users are more likely to acquire a bacteremic infection caused by USA300 MRSA than by all other *S. aureus* strains. The decrease observed in the association of illicit drug use and USA300 MRSA bacteremia over the study period suggests that the USA300 MRSA epidemic is now spreading from illicit drug users to other patient populations. Focusing infection control efforts on high-risk groups such as illicit drug users might slow the progression of the USA300 MRSA epidemic in areas of the country where the association between illicit drug use and USA300 is still high.

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Pneumococcal Serotypes in Children in 4 European Countries

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After heptavalent pneumococcal conjugate vaccine (PCV7) was marketed in France, Spain, Belgium, and England and Wales (United Kingdom), invasive disease from non-PCV7 serotypes (NVT) increased. Adjusted serotype-specific incidences among children <15 years of age were compared between 1999–2002 (prevaccine) and 2005–2006 (postmarketing). Vaccine coverage increased to ≈32%–48% in France, Spain, and Belgium but remained <1% in England and Wales. Serotype 1 incidence rose in all age groups and countries (incidence rate ratio [IRR] 1.3–4.2; p<0.004), independently of PCV7 use, but incidence of serotypes 7F and 19A increased most in France, Spain, and Belgium (IRR 1.9–16.9 in children <5 years; p<0.001), where PCV7 coverage was greater. Vaccine-induced replacement of PCV7 serotypes possibly contributed to NVT increases, as did secular trends. New vaccines targeting these serotypes are available, but serotype dynamics needs further exploration that accounts for underreporting and prevaccine trends.

Streptococcus pneumoniae is a leading cause of meningitis and septicemia worldwide. More than 90 serotypes have been identified for *S. pneumoniae*, but serotype distribution differs by area and changes over time (*1*–*3*). The heptavalent pneumococcal conjugate vaccine (PCV7) targets the 7 serotypes—4, 6B, 9V, 14, 18C, 19F, and 23F—that most commonly caused pediatric invasive pneumococcal disease (IPD) in the United States. Widespread PCV7 use in the United States since 2000 led to rapid and dramatic decreases in vaccine serotypes and an overall decrease of IPD incidence (*4*).

In Europe, PCV7 was licensed for pediatric use in 2001 and marketed in Spain and France in 2001, England and Wales in 2002, and Belgium in 2004; the 7 serotypes accounted for 68%–77% of IPD cases in children <2 or <5 years of age (*5*–*8*). Vaccination policies from marketing to introduction of PCV7 into the universal vaccination schedule differed among countries. In Spain, Belgium, and France, vaccination aimed to reach a progressively increasing proportion of children <2 years of age, resulting in low but increasing vaccine coverage (36%–50% of young children in 2005–2006) because PCV7 was not free for all of them (*6*,*7*). In England and Wales, until late 2006 PCV7 was recommended only for medical risk groups, and population-level vaccine coverage was negligible. PCV7 was introduced in the national universal program and delivered free in France and England and Wales in 2006 and Belgium in 2007. In Spain, free universal vaccination was limited to the Madrid region beginning in 2006.

Several PCV7 postlicensure studies in the United States and European countries have described substantial increases in non-PCV7 vaccine serotypes (NVTs) (*6*,*7*,*10*–*13*). These findings raised concern that vaccine use could lead to replacement of PCV7 serotypes by NVTs, as occurred with pneumococcal carriage (*14*).

In Spain, Belgium, France, and England and Wales, NVT disease increased substantially between marketing and introduction of PCV7 into the universal schedule, when vaccine use was moderate in Spain, Belgium, and France and negligible in England and Wales. Considering that a 10-valent vaccine and a 13-valent conjugate vaccine are licensed in the European Union (EU), a better understand-

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ing of the dynamics of the additional serotypes is needed to help decision making on future vaccine strategies. This study describes and compares temporal trends of PCV7 serotypes and NVTs among children in 4 European countries, taking into account the levels of PCV7 use. We focused on the emergence of serotypes 1, 7F, and 19A because they were responsible for most of the NVT increase.

Methods

This population-based study is based on surveillance data collected prospectively by the national reference laboratories (NRLs) of Spain, Belgium, France, and England and Wales during July 1996–June 2006. (In the United Kingdom, Scotland and Northern Ireland were not included in this study because they use separate surveillance systems.) IPD isolates were referred by laboratories throughout each country to the NRL. IPD surveillance among children was enhanced in England and Wales, France, and Belgium starting in 1996, 2002, and 2005, respectively, by encouraging microbiologists to systematically refer pneumococcal isolates to the NRL for typing.

De nitions and Inclusion Criteria

We included all IPD cases, defined by isolation of *S. pneumoniae* from a normally sterile fluid in children <15 years of age and referred to the NRL of their country. One isolate per disease episode was used in the analysis. Serotypes targeted by PCV7 were grouped as vaccine types (PCV7 types). All other serotypes were considered NVTs. Meningitis was defined as isolation of *S. pneumoniae* in cerebrospinal fluid (CSF).

An epidemiologic year was July through June. A prevaccine period was defined as the 3 epidemiologic years during July 1999–June 2002. The postmarketing period was 2005–2006.

Microbiologic Testing

Serotype and antimicrobial susceptibility were determined by each NRL as described (*9*,*15*–*17*). In Spain, all strains of serogroups 6 and 19 were subjected to PCR serotype identification (*18*). In Belgium during 1996–2004 (before PCV7 marketing), 36% of isolates had the serogroup but not the serotype determined (17% for serogroup 19). However, all serogroup 19 isolates from children <2 years of age were typed, and during the postmarketing period, the serotype was determined for all isolates received. Isolates with missing serotype were assumed to follow the same serotype distribution as isolates from the same serogroup, by year and patient age group.

Isolates were considered susceptible, intermediate, or resistant to antimicrobial drugs according to Clinical and Laboratory Standards Institute criteria (penicillin intermediate and resistant, MIC 0.12–1.0 mg/L and MIC >1 mg/L, respectively; erythromycin resistant, MIC ≥0.5 mg/L) (*19*). England and Wales data on antimicrobial drug resistance were not available for this study.

Vaccine Coverage and Macrolide Use

Because studies estimating vaccine coverage used different methods among countries, we used vaccine doses to estimate a proxy of vaccine coverage. Data on monthly vaccine doses sold or distributed were provided by the PCV7 manufacturer (Wyeth, Brussels, Belgium; Madrid, Spain; Maidenhead, UK; and Paris, France) and by the Health Protection Agency for doses distributed by the UK Department of Health. Assuming that all doses were administered to children <2 years of age at an average of 3 doses per child (allowing for missed doses and catch-up schedules), we calculated the proportion of children <2 years of age who should have received an average of 3 PCV7 doses. We also calculated the number of vaccine doses distributed per 1,000 children <5 years of age for comparison with serotype-specific incidences in children <5 years of age.

We collected data on use of antimicrobial drugs from the European Surveillance of Antimicrobial Consumption (*20*). We compared these data with the serotype-specific incidence of antimicrobial drug–resistant isolates.

Data Analysis

For all incidence calculations, we adjusted numbers of cases to the rate of underreporting to the NRL to estimate total numbers of cases and control for surveillance enhancement over time. Underreporting rates were calculated by country, year, and age group by dividing the respective number of cases with an isolate typed at the NRL by the total number of laboratory-confirmed IPD cases estimated in the country. Total numbers of IPD cases were estimated by different methods: in Belgium and France, through periodic capture–recapture studies and correction for laboratory coverage (*7*,*21*); in England and Wales, by reconciliation of 2 large datasets (*22*); in Spain, by calculation of hospital underreporting rates as a proxy (*23*). Age-specific incidence rates were computed by dividing adjusted numbers of cases by the respective midperiod population of each country and age group, by using population figures from Eurostat for Spain and from the national institutes for statistics of Belgium, France, and England and Wales. For serotype-specific incidence calculations, cases with missing serotype data were accounted for by multiplying the overall incidence by the yearly proportions of serotype-specific disease in each age group (*12*).

Trends in incidence over time were tested for linear model by the *t* test, except for France because of missing data points; this model showed an overall better fit for the 3 studied serotypes. Correlation between vaccine doses per 1,000 children <5 years of age and serotype-specific

incidence over years was tested by the Pearson correlation test, allowing for a 6-month lag between vaccine use and incidence. We also compared the annual average of serotype-specific incidence of the prevaccine period with the postmarketing period and computed incidence rate ratios (IRRs) and their exact 95% confidence intervals; p values were calculated by the Fisher exact test. We considered p values <0.05 significant. All statistical analyses were calculated by using STATA version 10.1 (StataCorp, College Station, TX, USA).

Results

PCV7 Coverage

PCV7 use began in Spain in 2000–2001, France in 2001–2002, Belgium in 2004–2005, and England and Wales in 2005–2006 and increased gradually in the first 3 countries (Figure 1). In 2005–2006, the proportion of children <2 years of age who had received an average of 3 PCV7 doses was ≈33% in Spain, ≈48% in France, ≈42% in Belgium, and <1% in England and Wales.

Overall Incidence

During July 1996–June 2006, the NRLs of the 4 countries reported 13,584 IPD cases among children <15 years of age: 3,170 cases in Spain, 2,862 in Belgium, 2,188 in France, and 5,364 in England and Wales. Data were not available from France from 1997–98 through 2000–2001 because NRL activities stopped in 1997 and began again in 2001. The proportion of confirmed IPD cases typed at the NRLs increased during the study period because of improving reporting of each NRL (Table 1). IPD incidence per 100,000 children <15 years of age increased during the 10-year period in Spain, Belgium, and England and Wales from 14.0 to 18.5, 20.1 to 28.2, and 6.1 to 10.6, respectively, but remained fairly stable in France, ≈7.0.

Isolates from CSF represented 15% of invasive isolates in patients <15 years in Spain, 11% in Belgium, 16% in England and Wales, and 32% in France. Because blood isolates were underrepresented in France, NRL data were adjusted to the CSF/blood distribution reported by national epidemiologic surveillance, by year and age group (*7*). Only adjusted data are presented here.

PCV7-Type and NVT IPD Cases

In children <5 years of age, incidence of PCV7-type disease started to decrease shortly after PCV7 introduction in Spain, Belgium, and France; the decrease was inversely related to increasing vaccine sales (Figure 1). Between the prevaccine period and the last study year (2005–2006),

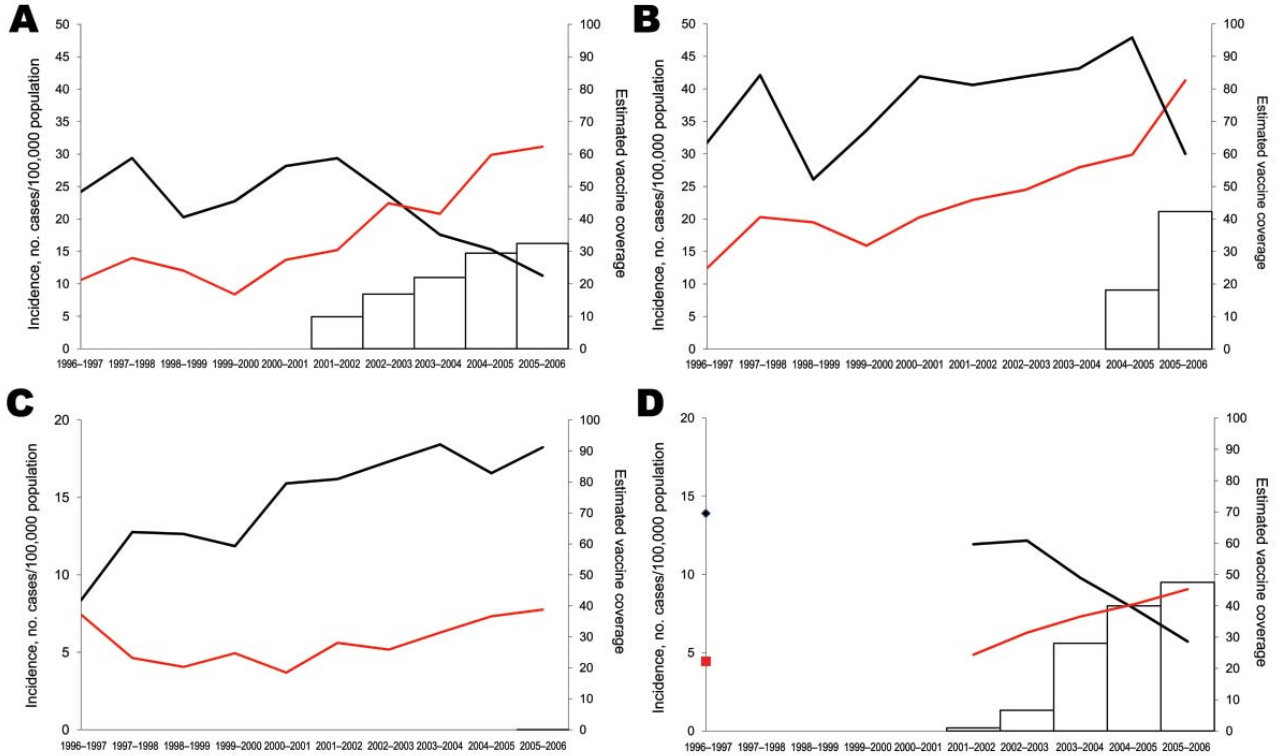

Figure 1. Incidence of pediatric invasive pneumococcal disease among children <5 years of age, by heptavalent pneumococcal conjugate vaccine (PCV7) (black lines) and non-PCV7 (red lines) serotypes, for A) Spain, B) Belgium, C) England and Wales, and D) France, 1996–2006. Estimated vaccine coverage is the annual number of PCV7 schedules per 100 children <2 years of age, assuming an average of 3 doses administered to each child. Vaccine coverage is not visible for England and Wales because it remains <1%.

PCV7-type IPD significantly declined by 58%, 22%, and 52%, respectively, in these 3 countries (Table 2). In England and Wales, where vaccine sales were negligible, PCV7-type IPD increased by 25%, but the proportion of IPD caused by PCV7-type decreased slightly, from 75% to 70% (p = 0.004). In older children, PCV7-type IPD showed no clear trend, except in Belgium, where it significantly decreased (Figure 2; Table 3).

In contrast, incidences of NVT significantly increased in all 4 countries during the 10-year period (p<0.001),

Table 1. Cases of invasive pneumococcal disease, underreporting rates, and numbers adjusted for underreporting among children <15 years of age, 4 European countries, 1996–2006*

Country/epidemiologic year	Total typed	Underreporting, %†	Total adjusted‡	Serotype				
				PCV7 types	NVT	1	7F	19A
Spain								
1996–97	155	18	869	544	325	62	0	28
1997–98	179	19	919	590	329	41	15	5
1998–99	191	26	740	415	326	81	16	31
1999–00	211	30	713	463	250	88	7	20
2000–01	252	28	901	580	322	107	11	54
2001–02	301	30	1,014	613	401	91	3	44
2002–03	426	39	1,092	528	564	131	31	97
2003–04	434	45	959	433	526	106	22	128
2004–05	528	43	1,228	367	860	207	70	198
2005–06	493	42	1,185	288	896	312	74	168
Belgium								
1996–97	160	44	363	225	138	34	7	11
1997–98	202	46	436	272	165	32	9	32
1998–99	185	55	339	179	159	31	7	29
1999–00	197	55	356	218	138	40	5	23
2000–01	256	56	455	293	162	52	11	32
2001–02	295	65	454	268	186	48	11	32
2002–03	370	75	497	283	214	66	15	42
2003–04	382	73	521	270	251	91	20	42
2004–05	437	76	578	315	263	103	22	45
2005–06	378	75	506	190	316	102	37	58
France§								
1996–97	258	33	773	547	226	52	0	40
1997–98	NA	NA	NA	NA	NA	NA	NA	NA
1998–99	NA	NA	NA	NA	NA	NA	NA	NA
1999–00	NA	NA	NA	NA	NA	NA	NA	NA
2000–01	NA	NA	NA	NA	NA	NA	NA	NA
2001–02	320	41	775	497	278	64	15	53
2002–03	399	46	864	531	333	92	32	87
2003–04	384	47	811	424	387	119	23	90
2004–05	438	54	806	359	447	159	41	71
2005–06	389	50	774	277	497	157	62	93
England and Wales								
1996–97	378	60	630	315	315	45	13	13
1997–98	429	62	689	462	226	43	14	26
1998–99	438	67	654	473	181	24	10	16
1999–00	429	64	666	453	213	36	8	37
2000–01	518	71	735	562	173	37	23	21
2001–02	557	72	770	549	221	37	14	28
2002–03	541	67	804	579	224	37	24	30
2003–04	627	71	889	628	261	67	16	37
2004–05	672	74	914	590	324	114	20	39
2005–06	775	76	1,014	640	374	139	38	38

*PCV7, heptavalent pneumococcal conjugate vaccine; PCV7 types, serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F; NVT, non-PCV7 vaccine serotypes, i.e., serotypes not included in the PCV7 vaccine; NA, not available.
†Underreporting rate = number of cases for which an isolate was typed at the national reference laboratory on the total number of laboratory-confirmed invasive pneumococcal cases estimated in the country (by capture–recapture or other methods) in children <15 years of age.
‡The sum of PCV7 types and NVT cases may slightly differ from the total adjusted because of rounding.
§Data have been adjusted for ascertainment and for the distribution of blood/cerebrospinal fluid isolates.

Table 2. Serotype-specific adjusted incidence rates of invasive pneumococcal disease in children <5 years of age before and after marketing of PCV7, Spain, Belgium, France, and England and Wales*

Country/serotypes	Incidence rate†		Incidence rate ratio (95% CI)	p value
	Prevaccine (1999–2002)	Postmarketing (2005–2006)		
Spain				
PCV7 types	26.8	11.3	0.4 (0.4–0.5)	<0.001
Non-PCV7 types	12.5	31.1	2.5 (2.2–2.8)	<0.001
1	2.7	8.1	3.0 (2.4– 3.7)	<0.001
7F	0.2	3.0	16.9 (8.6–37.7)	<0.001
19A	1.9	7.2	3.7 (2.9–4.8)	<0.001
Belgium				
PCV7 types	38.7	30.0	0.8 (0.7–0.9)	0.002
Non-PCV7 types	19.7	41.3	2.1 (1.8–2.5)	<0.001
1	3.4	9.5	2.8 (1.9– 4.1)	<0.001
7F	1.1	5.9	5.3 (2.9–9.8)	<0.001
19A	4.4	9.7	2.2 (1.5–3.1)	<0.001
France				
PCV7 types	11.9	5.7	0.5 (0.4–0.6)	<0.001
Non-PCV7 types	4.9	9.0	1.9 (1.5–2.2)	<0.001
1	0.6	1.5	2.7 (1.6–4.7)	<0.001
7F	0.3	1.3	4.2 (2.2–8.6)	<0.001
19A	1.3	2.4	1.9 (1.3–2.7)	<0.001
England and Wales				
PCV7 types	14.6	18.2	1.3 (1.1–1.4)	<0.001
Non-PCV7 types	4.7	7.8	1.6 (1.4–1.9)	<0.001
1	0.5	1.9	3.8 (2.6–5.8)	<0.001
7F	0.4	0.9	2.2 (1.3–3.7)	0.002
19A	0.8	1.0	1.3 (0.8–2.0)	0.271

*PCV7, heptavalent pneumococcal conjugate vaccine; CI, confidence interval. Marketing indicates that the vaccine was marketed and available for use in the country but not introduced in the vaccine schedule free of charge. Vaccine coverage differed by country during this period, ranging from 33% to 48% in Spain, Belgium, and France but <1% in England and Wales. PCV7 types include serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F.
†Cases per 100,000 children <5 years of age. Data for 1999–2002 are annual averages. Prevaccine period is 2001–2002 for France (data not available for previous years).

mainly from 2000–2002 onward (Figures 1, 2). From the prevaccine period to 2005–2006, NVT IPD increased significantly in both the <5-year and the 5–14-year age groups in all countries (Tables 2, 3). In each country, NVT IPD in the <5-year age group began to increase before PCV7 was introduced and gradually increased from year to year during 2002–2006. In children 5–14 years of age, NVT IPD fluctuated during the study period but mostly increased during 2002–2006. Serotypes 1, 7F, and 19A contributed most to this increase, representing 61% (range 57%–63%) of NVT IPD in children <15 years of age in 2005–2006. The dynamics of these 3 serotypes differed in terms of time trends and age groups affected.

Serotype 1

Serotype 1 disease increased significantly in each country during the 10-year period in both age groups (Figure 3). In children <5 years of age, the increase in serotype 1 began before PCV7 sales began. However, the largest increases occurred from 1999–2002 to 2005–2006, when incidence increased by 2–4-fold in both age groups in all countries (Tables 2, 3). Increases in the incidence of serotype 1 did not correlate significantly with PCV7 sales, except in France (Pearson r = 0.903, p = 0.036). In the 5–14-year age group, incidences were lower, but in 2005–2006, serotype 1 constituted in average 50% of IPD in that age group compared with 13% in children <5 years of age. All serotype 1 isolates were susceptible to penicillin. Incidence and proportion of erythromycin-resistant serotype 1 was low but increased in Belgium in 2004–2006.

Serotype 7F

Most (74%) serotype 7F cases occurred among children <5 years of age. In this group, IPD increased substantially in each country, mostly during 2004–2006, and correlated significantly with PCV7 sales, except in France (Pearson r = 0.901, p = 0.037 in Spain; r = 0.988, p = 0.002 in Belgium; r = 0.965, p = 0.008 in England and Wales; and r = 0.746, p = 0.148 in France) (Figure 4). From the prevaccine period to 2005–2006, incidence increased most in Spain and least in England and Wales (Table 2). In children 5–14 years of age, incidence rates also increased (Table 3), but numbers of cases were small. All serotype 7F isolates were susceptible to penicillin, and only 6/315 isolates in children <5 years of age were erythromycin resistant.

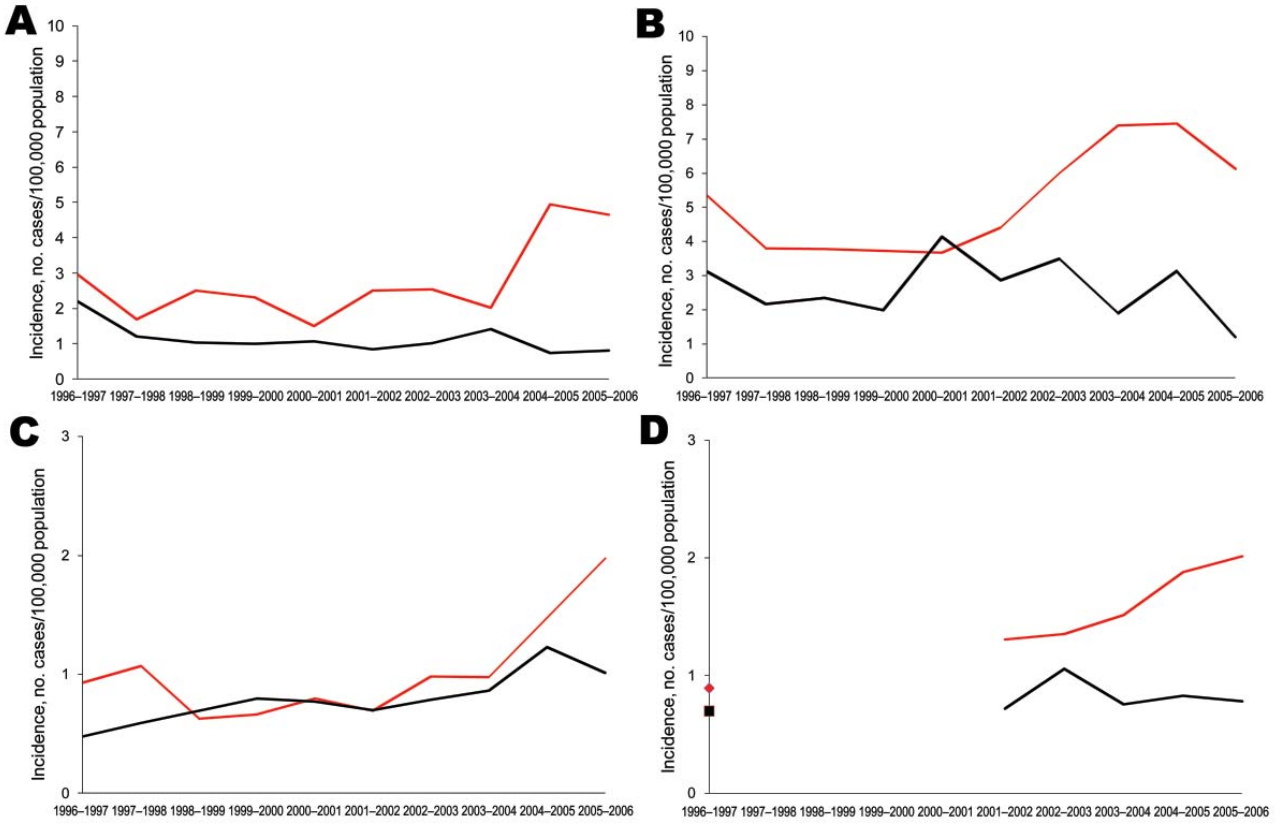

Figure 2. Incidence of pediatric invasive pneumococcal disease among children 5–14 years of age, by heptavalent pneumococcal conjugate vaccine (PCV7) (black lines) and non-PCV7 (red lines) serotypes, A) Spain, B) Belgium, C) England and Wales, and D) France, 1996–2006.

Serotype 19A

Serotype 19A disease affected predominantly children <5 years of age (94% of cases), for whom incidence rates more than doubled (IRR range 2.4–6.8) over the period (Figure 5). Incidence had already increased before PCV7 sales started in Belgium, Spain, and England and Wales, but the trend was significant only in Belgium (during 1996–2004). After PCV7 marketing, increases correlated significantly with vaccine sales in Spain and Belgium (Pearson r = 0.929 and 0.884, p = 0.022 and 0.047, respectively). From the prevaccine period to 2005–2006, 19A incidence significantly increased in Spain, France, and Belgium (Table 2); in England and Wales, the 27% increase was not significant. In children 5–14 years of age, numbers of cases were too small to identify any significant change.

Serotype 19A isolates showed high and increasing levels of antimicrobial drug resistance in Belgium, Spain, and particularly in France (data unavailable from England and Wales). In the <5-year age group, the prevalence of nonsusceptible strains ranged 0%–50% in 1996–1997 and increased in 2005–2006 to 21%, 48%, and 86% for penicillin and 67%, 61%, and 77% for macrolides in Belgium, Spain, and France, respectively. Full penicillin resistance was rare (0%–6% of isolates). Spain and Belgium shared similar patterns: incidence of penicillin-susceptible 19A increased more than resistant strains; incidence of erythromycin-resistant strains increased more than erythromycin-susceptible strains (Figure 6); in 1998–2006, the use of penicillin in ambulatory setting, calculated in defined daily doses per 1,000 inhabitants per day, increased slightly, and macrolide use declined by 31%–38%. In France, where nonsusceptible isolates predominated, use of penicillin and macrolides initially was much higher than that in the other countries but declined (–17% and –37%, respectively) until 2006. In England and Wales where use of antimicrobial drugs was initially lower, macrolide use became similar to Belgium and Spain from 2004 onward, but data on resistance were not available.

Evolution of Meningitis

Numbers of meningitis cases caused by individual serotypes were small, especially for serotype 1. In children <5 years of age, the incidence of meningitis from serotypes 19A and 7F combined also increased significantly in Spain,

Table 3. Serotype-specific adjusted incidence rates of invasive pneumococcal disease in children 5–14 years of age before and after marketing of PCV7, Spain, Belgium, France, and England and Wales*

Country/serotype	Incidence rate†		Relative risk (95% CI)	p value
	Prevaccine (1999–2002)	Postmarketing (2005–2006)		
Spain				
PCV7 types	1.0	0.8	0.8 (0.6–1.3)	0.387
Non-PCV7 types	2.1	4.6	2.2 (1.8–2.7)	<0.001
1	1.1	3.1	2.9 (2.3–3.7)	<0.001
7F	0.1	0.2	2.0 (0.7–5.6)	0.175
19A	0.06	0.12	2.1 (0.5–7.7)	0.226
Belgium				
PCV7 types	3.0	1.2	0.4 (0.2–0.7)	<0.001
Non-PCV7 types	3.9	6.1	1.6 (1.2–2.1)	0.003
1	2.2	3.8	1.8 (1.2–2.6)	0.003
7F	0.2	0.3	1.2 (0.2–5.2)	0.728
19A	0.3	0.1	0.2 (0.0–1.4)	0.094
France				
PCV7 types	0.7	0.8	1.1 (0.7–1.6)	0.664
Non-PCV7 types	1.3	2.0	1.5 (1.2–2.0)	0.001
1	0.6	1.3	2.3 (1.6–3.4)	<0.001
7F	0.0	0.1	3.6 (1.0–20.2)	0.038
19A	0.1	0.0	0.3 (0.0–1.6)	0.116
England and Wales				
PCV7 types	0.7	1.0	1.4 (1.0–1.9)	0.026
Non-PCV7 types	0.7	2.0	2.9 (2.3–3.6)	<0.001
1	0.3	1.3	4.2 (3.0–5.9)	<0.001
7F	0.0	0.2	5.5 (1.9–18.0)	0.001
19A	0.05	0.1	2.1 (0.7–5.9)	0.148

*PCV7, heptavalent pneumococcal conjugate vaccine; CI, confidence interval. Marketing indicates that the vaccine was marketed and available for use in the country but not introduced in the vaccine schedule free of charge. Vaccine coverage differed by country during this period, ranging from 33% to 48% in Spain, Belgium, and France but <1% in England and Wales. PCV7 types include serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F.
†Cases per 100,000 children 5–14 years of age. Data for 1999–2002 are annual averages. Prevaccine period is 2001–2002 for France (data not available for previous years).

Belgium, and France from the prevaccine period to 2005–06 (IRR 3.8, 4.9 and 2.3, respectively, p<0.001); in England and Wales, it did not increase significantly (IRR 1.3, p = 0.283).

Discussion

This study compared the dynamics of NVT disease in 4 countries before universal PCV7 vaccination. In Spain, Belgium and France, serotype 1, 7F and 19A incidence increased considerably under rising (though moderate) vaccine coverage. In England and Wales, where PCV7 use was negligible, serotype 1 disease increased substantially, 7F disease rose less than in the other countries, and 19A disease increased nonsignificantly. The proportion of IPD caused by serotype 19A remained stable in England and Wales, and the incidence of meningitis caused by 19A and 7F did not change significantly, suggesting that an increase in case detection caused part of the changes in 19A and 7F incidence. Indeed, a study showed that the 21% increase in IPD incidence in southwest England during 1996–2005 was no longer observed after adjustment for annual blood-culturing rates (*24*).

In Spain, the marked increase in NVT occurred concomitantly with PCV7 use, which led several studies to conclude that vaccine-induced replacement of serotypes had largely contributed to this increase (*6*,*11*). On the basis of our study findings, we suggest that vaccine use contributed to the increase in serotypes 7F and 19A. Both serotypes increased markedly under increasing PCV7 coverage and remained stable or increased less in the countries not using PCV7. Increases in 19A and 7F incidences correlated significantly with increasing vaccine sales in Spain and Belgium and were more pronounced in children <5 years of age, at whom PCV7 was aimed, than in older children. In Belgium and Spain, where both serotypes had already increased before PCV7 introduction, its slope escalated after PCV7 use. Although these data were observed under moderate PCV7 coverage, recent data from Belgium, England and Wales, and France indicate additional increases in the adjusted incidences of serotypes 7F and 19A under universal vaccination (2007–08) and high vaccine coverage (*25*–*27*).

However, we also suggest that vaccine-induced serotype replacement alone cannot explain the increase in NVT. First, serotype 1 rose well before PCV7 marketing in Belgium and Spain, affected predominantly older age groups, and increased in England and Wales in the absence of vaccine use. Second, serotype 19A increased in Belgium

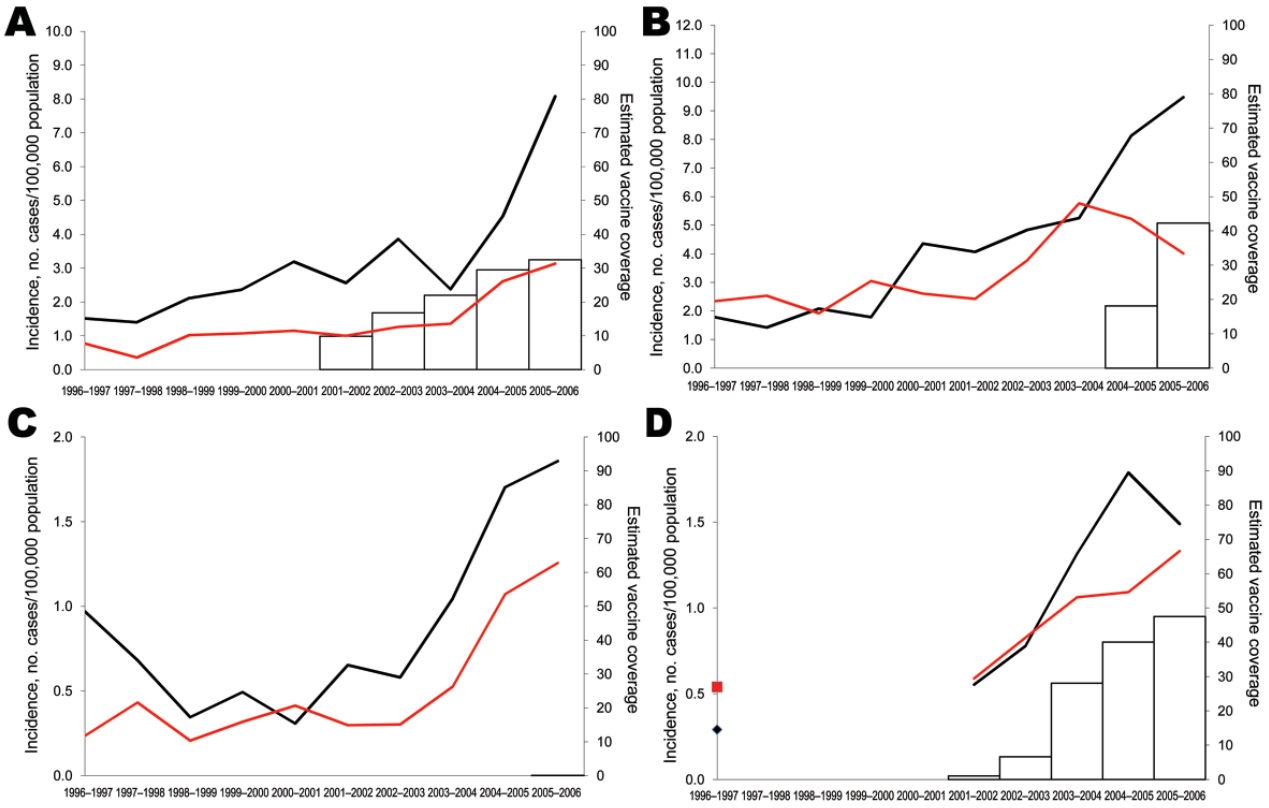

Figure 3. Incidence of invasive pneumococcal disease in children caused by serotype 1 for children <5 years of age (black lines) and 5–14 years of age (red lines), in A) Spain, B) Belgium, C) England and Wales, and D) France, 1996–2006. Estimated vaccine coverage is the annual number of PCV7 schedules per 100 children <2 years of age, assuming an average of 3 doses administered to each child. Vaccine coverage is not visible for England and Wales because it remains <1%.

and Spain before PCV7 use. Similarly, serotype 7F or 19A disease also increased in countries not using PCV7 (*28*–*30*). Third, some EU countries with widespread PCV7 use did not experience similar rises (*31*,*32*).

Other factors most likely contributed to the increases. Cyclical trends of serotype 1 were described in Scandinavian countries before any PCV7 use (*3*,*33*,*34*). A wave of serotype 1 (and possibly 7F) may have occurred in these 4 neighboring countries. Conversely, the high use of antimicrobial drugs, especially macrolides, allegedly favored the increase of nonsusceptible serotype 19A (*14*,*35*). A modeling study suggested that use of antimicrobial drugs played a larger role than did PCV7 use in the increase of resistant 19A in the United States (*36*). In 3 countries in our study where 19A incidence (and resistant strains) increased, use of antimicrobial drugs was higher than in England and Wales where 19A stayed stable. However, incidence of penicillin- and erythromycin-susceptible 19A strains also increased in Spain and Belgium. Macrolide use decreased 37%–41% during 1998–2006 in the 3 countries, whereas 19A incidence increased 80%–253%. Similarly, serotype 19A incidence increased in England and Wales after universal PCV7 vaccination despite stable macrolide use (*26*). The role of antimicrobial drugs is thus difficult to delineate and suggests a synergistic effect of antimicrobial drugs and PCV7. Other factors for replacement have been raised (high prevalence of NVT carriage and low vaccine coverage), but they conflict with current knowledge (*37*): 7F is a rarely carried serotype, and 19A and 7F increased further under higher PCV7 coverage. These conflicting views suggest that factors leading to replacement disease are still not fully understood; its cause is probably multifactorial and population dependant.

Our study has several limitations. First, enhancement of pediatric IPD surveillance and possible changes in blood culture practices could not be completely controlled by our methods of adjusting for underreporting. This limitation certainly applied to England and Wales, where reconciliation of 2 datasets could not totally adjust for the increase in case reporting and blood culturing, which most likely contributed to the increased incidence in nearly all serotypes studied, probably leading to overestimation of the NVT increase in England and Wales. Increase in blood culturing over time in the other countries is not suggested by the sharp

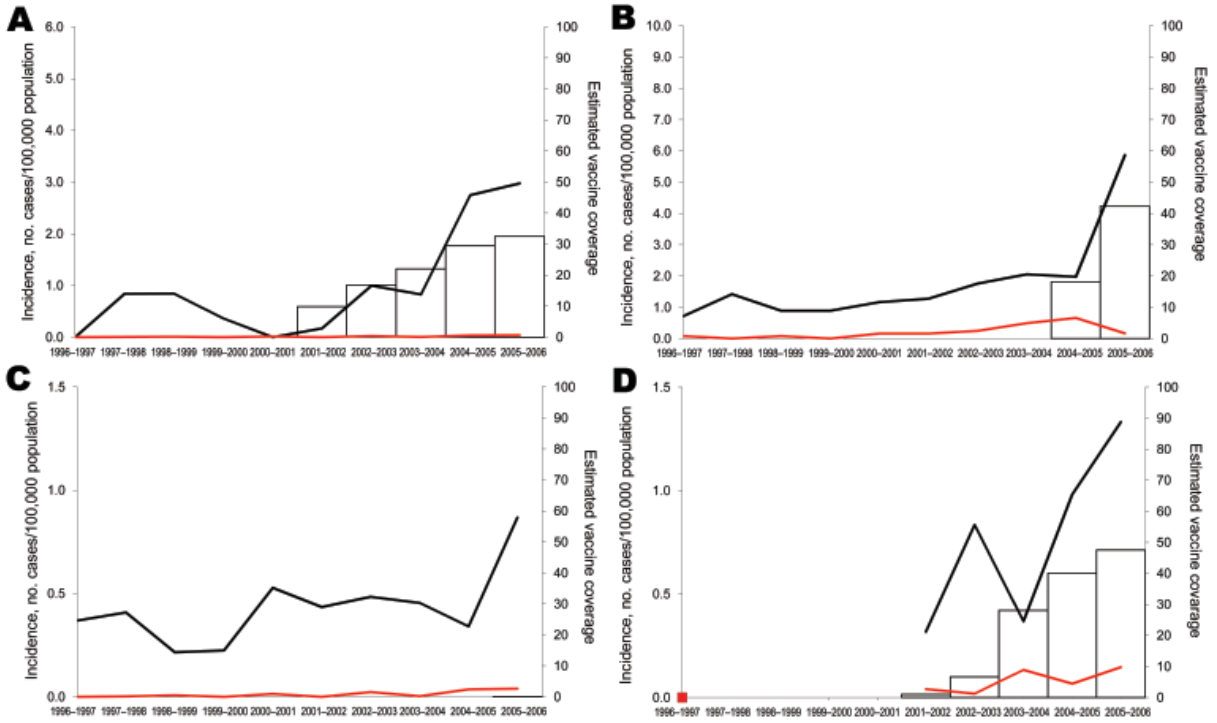

Figure 4. Incidence of invasive pneumococcal disease in children caused by serotype 7F for children <5 years of age (black lines) and 5–14 years of age (red lines), in A) Spain, B) Belgium, C) England and Wales, and D) France, 1996–2006. Estimated vaccine coverage is the annual number of PCV7 schedules per 100 children <2 years of age, assuming an average of 3 doses administered to each child. Vaccine coverage is not visible for England and Wales because it remains <1%.

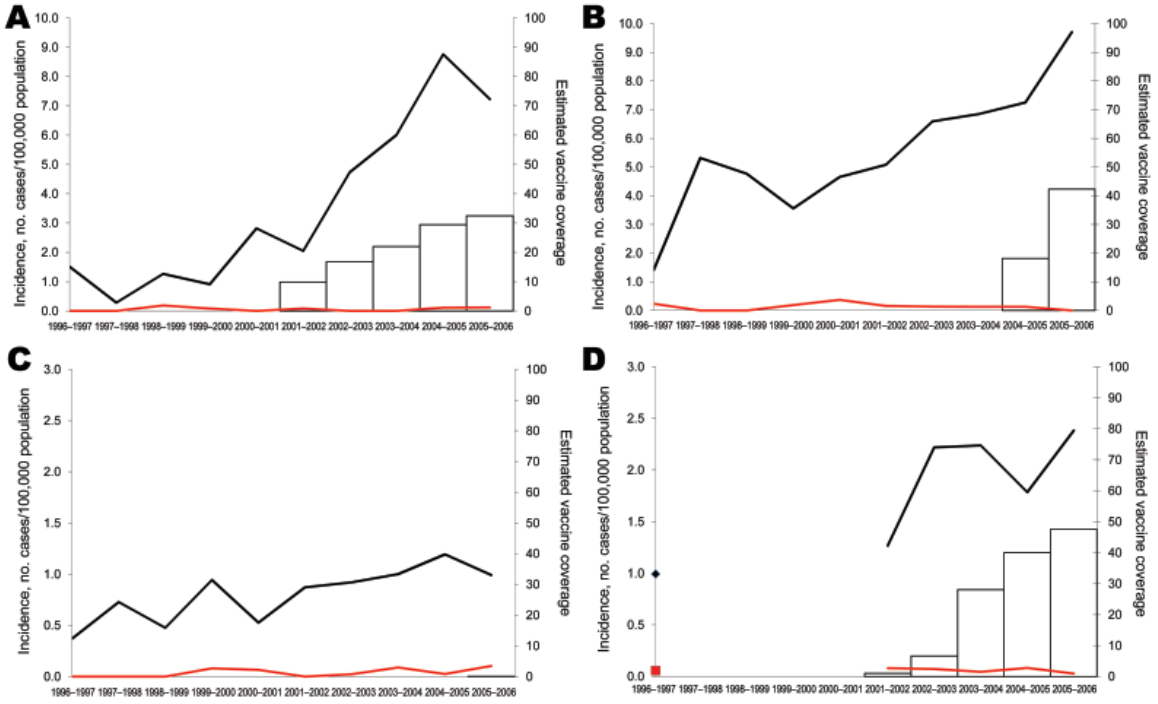

Figure 5. Incidence of invasive pneumococcal disease in children caused by serotype 19A for children <5 years of age (black lines) and 5–14 years of age (red lines), in A) Spain, B) Belgium, C) England and Wales, and D) France, 1996–2006. Estimated vaccine coverage is the annual number of PCV7 schedules per 100 children <2 years of age, assuming an average of 3 doses administered to each child. Vaccine coverage is not visible for England and Wales because it remains <1%.

decrease in PCV7-type IPD, the similar trends in serotype-specific meningitis incidence (based on CSF isolation), and data on blood cultures in hospitals in Belgium (+13% from 1999–2002 to 2005–2006 while NVT IPD increased 210%). Second, missing serotype data (more frequent in the prevaccine period) may have led to imprecision in serotype distributions; however, similar age-specific PCV7-type and NVT distributions and trends were observed in other studies in Belgium, France, Spain, and England (*5–8*,*24*), PCV7 serotype coverage did not vary with the geographic origin of pneumococcal strains in France (*7*), and the age and sample distribution of children for whom serotype information was available did not differ from that of other children in the Belgium dataset. Finally, estimation of vaccine coverage assumed that all PCV7 doses were administered at an average schedule of 3 doses for children <2 years of age. This method may overestimate PCV7 coverage because a proportion of children are likely to be incompletely vaccinated given the high cost of PCV7 paid for by parents (*38*), but it may also underestimate coverage because many children received fewer doses in catch-up vaccination. However, coverage values were close to those estimated by population surveys (*6*,*7*,*11*,*38*).

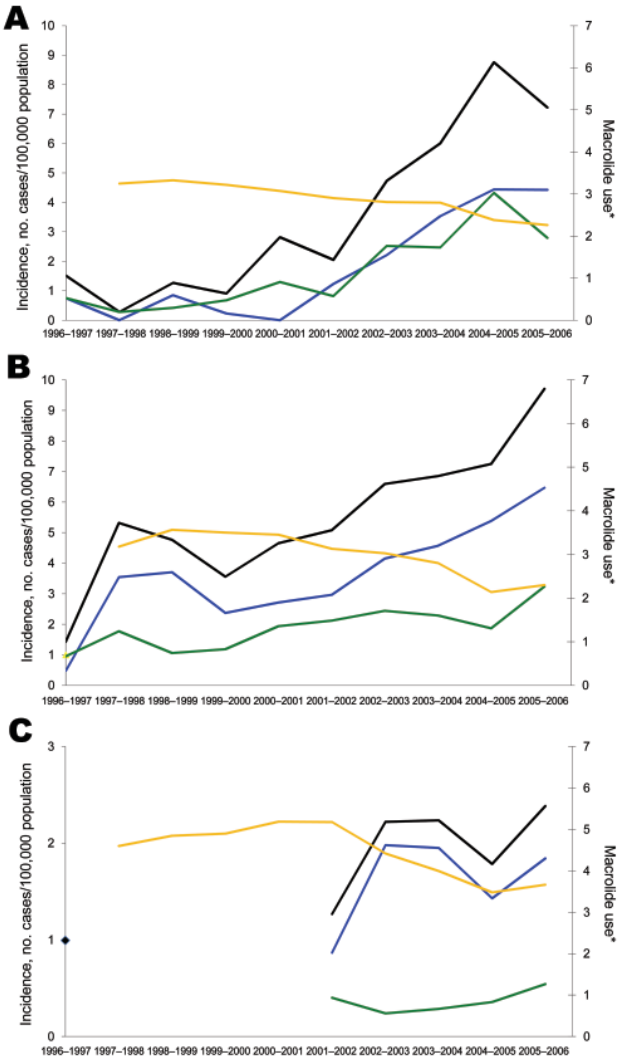

Figure 6. Incidence of serotype 19A invasive pneumococcal disease in children <5 years of age (black lines) showing breakdown of erythromycin-resistant (blue lines) versus -susceptible (green lines) infections and rate of macrolide use (gold line) in outpatient settings for A) Spain, B) Belgium, and C) France, 1996–2006. *Defined daily doses per 1,000 inhabitants per day.

Such an ecologic study cannot determine which rise in disease incidence is attributable to vaccine, secular trends, or use of antimicrobial drugs, and other possible factors may have contributed. However, the strength of this study is in the comparison of epidemiologic changes in 4 countries showing variations in serotype dynamics, vaccine use, and antimicrobial drug use.

The increase in incidence of serotypes 1, 7F, and 19A has partly countered the positive impact of PCV7 on overall IPD incidence in the first 2–5 years of nonuniversal vaccine use in Belgium, France, and Spain. The new 10-valent (1 and 7F) or 13-valent (1, 7F, and 19A) conjugate vaccines include these serotypes. However, a better understanding of serotype dynamics and contribution of vaccine and antimicrobial drug use is essential to guide decisions on the implementation of new vaccines and to assess their impact. Multicountry studies are useful for comparing serotype dynamics among population groups that have different levels of vaccine and antimicrobial drug use, but analyses should account for underreporting and prevaccine trends.

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Long-Term Health Risks for Children and Young Adults after Infective Gastroenteritis

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To quantify the risk and types of sequelae attributable to prior enteric infections, we undertook a population-based retrospective cohort study using linked administrative records. The risk for first-time hospitalization for sequelae was modeled by using Cox proportional regression analysis controlling for other health and sociodemographic factors. We identified a significant increase of 64% in the rate of first-time hospitalization for sequelae for persons with prior enteric infections: 52% for intragastrointestinal sequelae and 63% for extragastrointestinal sequelae compared with first-time hospitalization for those without prior infection. Extragastrointestinal sequelae occurred predominantly during the first 5 years after first-time enteric infection. In contrast, most intragastrointestinal sequelae occurred >10 years later. Infective gastroenteritis during childhood or adolescence increases the risk for first-time hospitalization for intragastrointestinal and extragastrointestinal disease over the 2 decades after first-time enteric infection, highlighting the importance of identifying ways of reducing the incidence of such infections.

Gastroenteritis is a common illness worldwide and has a considerable effect on the public health of communities and health systems that provide care. In developing countries, gastrointestinal infection is a major cause of death, claiming ≈2 million lives each year among children <5 years of age (*1*). By contrast, most episodes of gastroenteritis in industrialized nations do not cause serious, immediate, adverse sequelae but remain common, especially in the young (*2*,*3*). In addition to the immediate health concerns associated with gastroenteritis, subsequent medium- to long-term adverse sequelae have been described. A range of gastrointestinal, rheumatologic, neurologic, and skin and lung conditions have been associated with previous exposure to enteric infections (*4*–*13*).

Most of these data are from case reports and small-sample cross-sectional studies; however, several recent short-term longitudinal studies have provided estimates of the incidence of adverse health events after enteric infections (*8*,*14*,*15*). These studies suggest that the increase in risk for sequelae is considerable. For example, in a follow-up cohort study of a community exposed to a waterborne disease outbreak, the relative risks for chronic gastrointestinal symptoms, arthralgia, and psychiatric conditions were 2.4, 1.4, and 2.0, respectively (*16*). However, the long-term population-based extent of sequelae from prior enteric infection has remained unclear because previous studies have not adjusted for confounding variables, follow-up was short-term (*8*), evaluations were taken when populations were exposed to an outbreak of water-borne disease (*8*,*14*), and adverse events were either identified by self-reporting (*8*,*14*) or compared with expected, but not measured, rates of events in the general population (*15*).

The Western Australia Data Linkage System (WADLS) provided a unique opportunity to undertake a robust, long-term, longitudinal study of the sequelae associated with notifiable enteric infections in the general population. This system enables capture of health events in persons previously exposed and not exposed to an enteric infection. Our goal was to quantify the rate, risk, and type of sequelae attributable to previous childhood and adolescent exposure to enteric infections that lead to hospitalization, controlling for other health and sociodemographic factors.

Methods

This retrospective, population-based, longitudinal cohort study linked routinely collected administrative records from

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the Western Australian notifiable infectious diseases database (NIDD) with data contained in the Western Australian hospital morbidity data system (HMDS). Western Australian death notifications routinely collected under the Western Australian State government statute were also linked.

Study Participants and Sources of Data

The cohort comprised all persons having a Western Australia birth notification during January 1, 1985–December 31, 2000. For each person, the following records were extracted by the WADLS: 1) NIDD records of any enteric infection from birth through December 31, 2007, and including encrypted patient identification, sex, age at notification, date of notification or onset, species causing enteric infection, and residence recorded as postal code at notification; 2) HMDS records of all separations (discharge from hospital) from any Western Australia hospital (public and private) from December 31, 2007, and comprising encrypted patient identification, age at hospitalization, sex, Aboriginality, date of admission, and International Classification of Diseases, 9th Revision, code for principal and additional diagnoses; and 3) any death notification for the person, comprising encrypted patient identification, sex, age at death, and date of death.

De nition of Prior Infection

Each person was assigned to 1 of 2 mutually exclusive groups. The exposed group was defined as persons with any past enteric infection. More specifically, these were past infections notified on the NIDD or a past hospitalization for an enteric infection recorded on the HMDS where no NIDD notification was present. The unexposed group (those with no prior infection) comprised persons for whom the NIDD and HMDS had no notifications pertaining to any form of gastroenteritis.

For the exposed group, the date of past infection was the date of onset recorded on the NIDD notification or the date of admission recorded on the HMDS for a first-time enteric infection. Using a birth cohort methodology, the unexposed group (no prior infection) would be expected to be followed up for longer (birth to outcome) than the exposed group (prior infection to outcome). To reduce this bias, each person in the unexposed group was given a proxy date of prior infection achieved by randomly assigning the exposure dates observed in the exposed group to nonexposed persons.

De nition of Outcome

We were interested in outcomes (sequelae) that resulted in hospitalization. Therefore, the outcome was defined as a first-time hospitalization and a diagnosis of any sequelae of interest recorded as either the principal or co-diagnosis at any time from birth to death or end of follow-up. The sequelae of interest were further divided into 2 broad groups (intraintestinal and extraintestinal conditions) (online Appendix Table 1, www.cdc.gov/EID/content/16/9/1440-appT1.htm).

To differentiate persons with a first-time hospitalization for the sequelae of interest after a NIDD notification (incident cases) from those with a previous hospitalization for a sequelae before exposure (prevalent cases), we examined HMDS records dating back to birth. Prevalent cases were excluded from the study. For each person, the follow-up time, or time at risk for sequelae (years, or part thereof) was enumerated from the date of prior infection, or proxy date of prior infection for unexposed persons, to admission date for the sequelae of interest, date of death, or December 31, 2007 (end of follow-up), as appropriate.

Determination of Socioeconomic Status and Residence

Socioeconomic status and residence were also determined from existing records. Published Socio-Economic Indexes for Areas (Index of Relative Social Disadvantage) (*17*) and Accessibility/Remoteness Index of Australia scores (*18*) were mapped to the postal codes of persons at birth.

Presence of Preexisting Concurrent Conditions

The presence of preexisting concurrent conditions was identified by using the International Classification of Diseases, 9th Revision, codes for hospital separations. The Multipurpose Australian Comorbidity Scoring System (*19*) was used to determine any hospitalization for a concurrent illness for each person at the outcome, death, or end of follow-up.

Analysis

Data were analyzed by using SPSS software, version 14 (SPSS Inc., Chicago, IL, USA). Incidence rates for individual enteric infections and types of sequelae were calculated by using the number of events as the numerator and the follow-up time as the denominator.

After determining that the structure of the data was appropriate by testing the proportional hazards assumption (*20*), we used the Cox proportional hazards regression model to conduct survival analyses. We examined the risk for first-time hospitalization for any sequelae, intragastrointestinal sequelae, and extragastrointestinal sequelae over time by using Cox proportional hazards regression models, in which we compared the risk for first-time hospitalization among persons with prior enteric infection with that of those who had no prior infection. These models were adjusted for factors that may have influenced the probability of first-time hospitalization occurring, i.e., sex, indigenous status, year of birth, age at exposure or proxy, singleton versus multiple birth status, weight at birth, hospital birth

versus nonhospital birth, mother's region of birth, father's region of birth, socioeconomic status, accessibility to services, and previous hospitalization for comorbidity.

When possible, Cox proportional hazards models were also constructed separately by type of enteric infection. To determine which sociodemographic or disease factors were influential in sequelae development, separate survival models were also constructed for exposed and unexposed groups.

Attributable risk percent was used to estimate the proportion of sequelae for which prior exposure to an enteric infection was a component cause. This attributable risk was calculated as the adjusted rate ratio (obtained from the Cox proportional hazards regression model) minus 1, divided by the adjusted rate ratio, multiplied by 100. Thus, we estimated the percentage of first-time hospitalizations for sequelae in the exposed cohort that were attributable to being previously exposed to an enteric infection after controlling for known potential confounders.

Ethical Approval

The study was approved by The University of Western Australia's Human Research Ethics Committee. All data were deidentified before being provided to the researchers.

Results

Study Participants

Of the 336,401 persons who met the inclusion criteria, 23,477 (7%) had at least 1 notification for an enteric infection during the 22-year study period. Similar proportions (<3% difference) of male patients, nonindigenous persons, singleton births, and hospital births were found in both groups (Table 1). For those with prior enteric infection, small birth-weight babies ≤3,000 grams were overrepresented ($p \leq 0.0001$); normal birth-weight babies 3,001–4,000 grams were underrepresented; and disadvantaged persons (socioeconomic status and accessibility to services; $p \leq 0.0001$), persons having prior comorbidity ($p \leq 0.0001$), and persons having prior hospitalization ($p \leq 0.0001$) were overrepresented. The median year of birth for those with prior enteric infection was 1994, compared with 1992 for those with no prior enteric infection; however, the mean age at exposure or proxy was similar for both groups (2.9 years and 2.4 years, respectively). Mean survival to a first-time hospitalization for any sequelae, death, or end of follow-up for persons with and without prior enteric infection was 8.6 and 11 years, respectively ($p \leq 0.0001$).

Table 1. Sociodemographic characteristics and measures of preexisting health status for those with and without history of enteric infection, Western Australia, Australia, January 1, 1985–December 31, 2000

Characteristic	History, no. (%), n = 23,477	No history, no. (%), n = 312,924	% Difference*
Male sex	12,297 (52.4)	156,873 (50.1)	2.3
Not of indigenous status	19,872 (84.6)	272,226 (87.0)	–2.4
Singleton birth	22,867 (97.4)	305,028 (97.5)	–0.1
Hospital birth	22,382 (95.3)	298,919 (95.5)	–0.2
Weight at birth, g			
≤2,000	544 (2.3)	4,862 (1.6)	0.7
2,001–3,000	5,114 (21.8)	58,256 (18.6)	3.2†
3,001–4,000	14,550 (62.0)	205,289 (65.6)	–3.6†
4,001–5,000	2,005 (8.5)	31,705 (10.1)	–1.6
>5,001	43 (0.2)	469 (0.1)	0.1
Socioeconomic status			
Extremely advantaged	4,315 (18.4)	64,322 (20.6)	–2.2
Advantaged	3,365 (14.3)	43,709 (14.0)	0.3
Average	3,070 (13.1)	36,159 (11.6)	1.5
Disadvantaged	5,653 (24.1)	61,379 (19.6)	4.5†
Extremely disadvantaged	6,322 (26.9)	58,610 (18.7)	8.2†
Accessibility to services			
Highly accessible	14,926 (63.6)	199,090 (63.6)	0
Accessible	1,748 (7.4)	19,056 (6.1)	1.3
Moderately accessible	2,042 (8.7)	19,836 (6.3)	2.4
Remote	804 (3.4)	6,770 (2.2)	1.2
Very remote	2,812 (12.0)	13,808 (4.4)	7.6†
Ever hospitalized for a comorbidity‡	22,561 (96.1)	254,745 (81.4)	14.7†
Prior hospitalization‡	22,786 (97.0)	264,702 (84.6)	12.4†
Hospitalization in first year of life‡	18,361 (78.2)	189,959 (60.7)	17.5†
Hospitalization in first month of life‡	15,785 (67.2)	177,679 (56.8)	10.4†

*Percentage with history – percentage without history.
†$p \leq 0.0001$.
‡Excludes hospitalization for sequelae.

Distribution of Sequelae

The highest rates of sequelae were observed for extragastrointestinal conditions: 2,407 and 977 per 100,000 person-years for those with and without prior enteric infection, respectively (Table 2). Intragastrointestinal sequelae, in comparison, occurred less frequently (400 and 226 per 100,000 person-years for those with and without prior enteric infection, respectively).

Risk for Hospitalization for First-time Sequelae According to Exposure Status

The rate of first-time hospitalization increased significantly for all outcomes analyzed; a slightly larger increase was found in risk for extragastrointestinal sequelae compared with intragastrointestinal sequelae (Table 3). Some of the elevation in the crude rate ratios was reduced after adjustment for sociodemographic and preexisting health status, which indicates confounding by these variables (online Appendix Table 2, www.cdc.gov/EID/content/16/9/1440-appT2.htm), which indicates significant confounding for most of the variables assessed with respect to any and extragastrointestinal sequelae. However, for intragastrointestinal sequelae, only age, accessibility to services, born in hospital, mother's region of birth, and hospitalization for a prior comorbidity significantly affected the risk for hospitalization for sequelae (online Appendix Table 2). The adjusted rate ratios showed an increased rate of hospitalization for any sequela of 64%, intragastrointestinal sequelae of 52%, and extragastrointestinal sequelae of 63% for persons with prior enteric infection. The attributable risk fractions indicated that 39% of first-time hospitalizations for all sequelae (34% of intragastrointestinal and 39% of extragastrointestinal sequelae) were directly attributable to prior enteric infections.

Survivor Pro le

The survivor profiles for extragastrointestinal and intragastrointestinal sequelae differed (Figure). Extragastrointestinal sequelae occurred predominantly in the first 5 years after a first-time enteric infection; thereafter, the survivor function curves did not change significantly for the 2 groups. In contrast, the survivor function curves for intragastrointestinal sequelae indicate little difference between those with and without prior enteric infection over the first 10 years. After this time the survivor function curves deviate significantly, suggesting that these sequelae mostly occur later.

Table 2. Number and rates of first-time intragastrointestinal and extragastrointestinal sequelae for those with and without history of enteric infection, Western Australia, January 1, 1985–December 31, 2005*

	History		No history		
Category of first-time sequelae	No. (%)	Rate†	No. (%)	Rate†	Difference‡
Extragastroiintestinal sequelae	5,045 (100.0)	2,407.1	34,425 (100.0)	976.6	1,430.5
Respiratory infections	2,535 (50.2)	1,209.5	14,750 (42.8)	418.5	791.1
Infections of the middle ear and mastoid	1,544 (30.6)	736.7	13,164 (38.2)	373.5	363.2
Cellulitis, osteomyelitis, and myositis	364 (7.2)	173.7	2,327 (6.8)	66.0	107.7
Fever, unknown origin	303 (6.0)	144.6	1,409 (4.1)	40.0	104.6
Upper and lower urinary tract infections	78 (1.5)	37.2	715 (2.1)	20.3	16.9
Infections of lymphatic vessels	69 (1.4)	32.9	607 1.8)	17.2	15.7
Inflammatory diseases of the CNS	35 (0.7)	16.7	194 (0.6)	5.5	11.2
Arthropathies	39 (0.8)	18.6	275 (0.8)	7.8	10.8
Endometriosis	18 (0.4)	8.6	146 (0.4)	4.1	4.4
Orchitis and epididymitis	13 (0.3)	6.2	122 (0.4)	3.5	2.7
Bacteremia	8 (0.2)	3.8	55 (0.2)	1.6	2.3
Pilonidal cyst	38 (0.8)	18.1	600 (1.7)	17.0	1.1
Thyroiditis	1 (0.0)	0.5	2 (0.0)	0.1	0.4
Guillain-Barré syndrome	0	0.0	37 (0.1)	1.0	−1.0
Cardiac infections	0	0.0	22 (0.1)	0.6	−0.6
Intragastrointestinal sequelae	1,267 (100.0)	400.3	9,385 (100.0)	226.2	174.2
Intestinal obstruction, diverticular disease, irritable bowel syndrome/megacolon	278 (21.9)	87.8	1,536 (16.4)	37.0	50.8
Appendicitis	565 (44.6)	178.5	5,690 (60.6)	137.1	41.4
Infections of the oral cavity and esophagus	103 (8.1)	32.5	333 (3.5)	8.0	24.5
Enteritis, colitis and noninfective gastroenteritis	125 (9.9)	39.5	697 (7.4)	16.8	22.7
Infections of the stomach and duodenum	122 (9.6)	38.5	684 (7.3)	16.5	22.1
Peritonitis and ascites	16 (1.3)	5.1	25 (0.3)	0.6	4.5
Cholecystitis	40 (3.2)	12.6	346 (3.7)	8.3	4.3
Pancreatitis	18 (1.4)	5.7	74 (0.8)	1.8	3.9

*CNS, central nervous system.
†Per 100,000 person-years.
‡Rate for patients with history – rate for patients without history.

Type of Enteric Noti cations and Risk for Sequelae by Type of Infection

Of the 23,477 first-time notifications of enteric infection, the most frequently reported single defined causes were *Campylobacter* spp. (17%), viruses (17%), and *Salmonella* spp. (12%). Forty-two percent of notifications were identified solely by using hospitalization records as "Enteritis and gastroenteritis, not otherwise specified." An additional 12% of notifications were of various other specific organisms. The risk profile for intragastrointestinal and extragastrointestinal sequelae were similar for the most common infective pathogens (Table 4). The exception was for *Salmonella* infection, which did not confer an increased risk for intragastrointestinal sequelae.

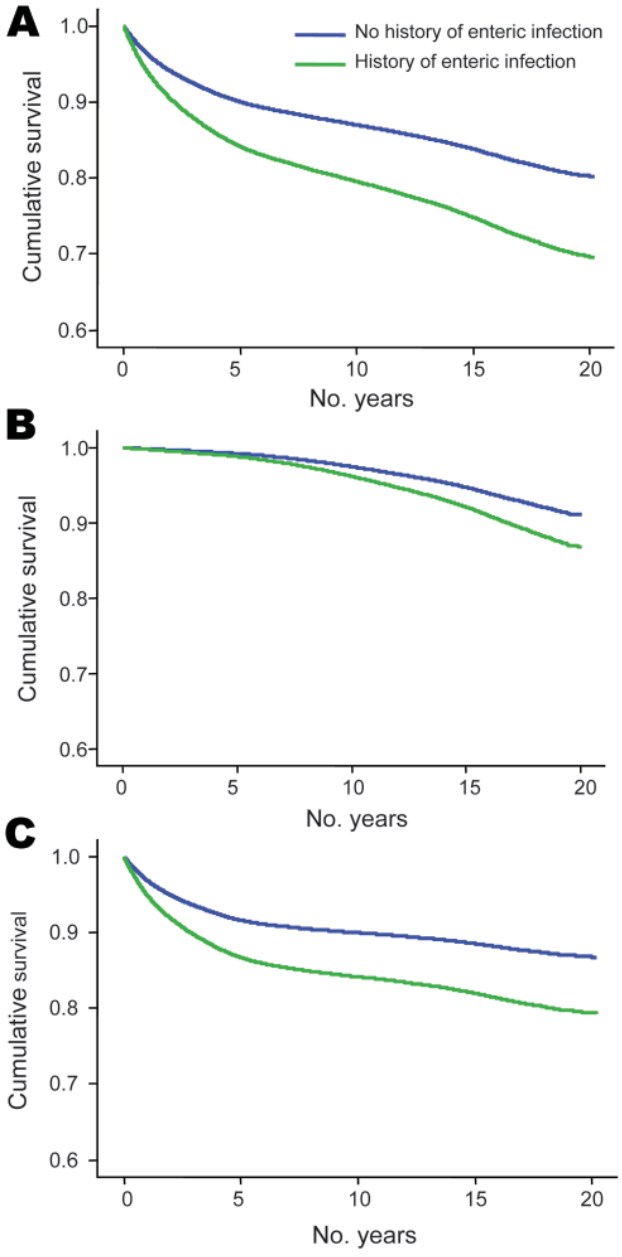

Figure. Kaplan-Meier estimates of the adjusted survivor function with respect to any sequelae (A), intragastrointestinal sequelae (B), and extragastrointestinal sequelae (C) for those with and without prior enteric infection, Western Australia, Australia, January 1, 1985–December 31, 2000.

Discussion

Our study showed that prior exposure to an enteric infection during childhood or adolescence increases the risk for a first-time hospitalization for a wide range of intragastrointestinal and extragastrointestinal illnesses by 64% over 22 years of follow-up. Furthermore, 39% of first-time hospitalizations for these illnesses were directly attributable to a previous enteric infection. The risk for extragastrointestinal sequelae was higher than that of intragastrointestinal sequelae, and the time of onset for the 2 categories of sequelae differed. Intragastrointestinal sequelae occurred much later after exposure than extragastrointestinal sequelae, a finding that has not been reported previously.

We found that respiratory and middle ear infections were the largest contributors to the excess rate of extragastrointestinal conditions. Appendicitis, the most common intragastrointestinal sequelae in this age-group, had a 23% increased risk in persons previously exposed to enteric infection. This association has previously been suggested for *Campylobacter* infection (*9*). We also found a 57% increase in risk for enteritis, colitis, and noninfective gastroenteritis, a diagnostic grouping that includes ulcerative colitis and Crohn disease; again, this association has previously been made (*7*,*8*). However, contrary to previous studies that reported an association between enteric infection and subsequent disease (*14*,*21*–*26*), the findings in our study suggest that risk increases over time, especially with regards to intragastrointestinal sequelae where the risk becomes greatest >10 years after onset of gastroenteritis infections in children and adolescents.

Our findings indicate that being male, indigenous, of low birthweight, and socioeconomically disadvantaged and being born outside the metropolitan area increases risk for developing the measured sequelae in exposed and unexposed persons (data not shown). However, we confirmed that previous exposure to an enteric infection is an important risk factor because after these factors are adjusted for, a 64% increase in risk for sequelae remains. We found that salmonellosis afforded no additional risk for intragastrointestinal sequelae but increased the risk for extragastrointestinal sequelae by 43%. With this exception, all common causes of infective gastroenteritis were equally represented as increasing the risk for sequelae.

This study has several strengths and limitations that warrant consideration when interpreting our results. A major strength of the research was the use of linked birth, hos-

Table 3. Number and rate of first-time hospitalizations, rate ratios, and attributable risk for sequelae for those with and without history of enteric infection, Western Australia, Australia, January 1,1985–December 31, 2000*

Type of sequelae	First-time hospitalizations				Crude rate ratio, RR (95% CI)	Adjusted† rate ratio, RR (95% CI)	Adjusted AR, %‡	Goodness of fit§
	With history		No history					
	No.	Rate	No.	Rate				
Any	5,634	27.8	41,054	11.8	2.36 (2.28–2.41)	1.64 (1.59–1.67)	39	0.05
Intragastrointestinal	1,267	4.0	9,385	2.3	1.77 (1.67–1.88)	1.52 (1.42–1.62)	34	0.04
Extragastrointestinal	5,045	24.1	34,425	9.8	2.46 (2.39–2.54)	1.63 (1.57–1.68)	39	0.08

*Rates are per 100,000 person-years. RR, relative risk; CI, confidence interval; AR, attributable risk.
†Multivariate Cox regression estimating the adjusted rate ratio of first-time hospitalization for any, intragastrointestinal, and extragastrointestinal sequelae. Adjusted for gender, indigenous status, year of birth, age at exposure or proxy, singleton, weight at birth, hospital birth, mother's region of birth, father's region of birth, socioeconomic status, accessibility to services and previous hospitalization for comorbidity.
‡Proportion of first-time hospitalizations for sequelae where previous exposure to an enteric infection was a component cause.
§Pseudo R^2. As explained by Hosmer and Lemeshow (*20*), a measure analogous to R^2 would be useful as a measure of Cox regression model performance; however, although a pseudo R^2 can be calculated the values obtained are often low because of the censored nature of the data even though the model is adequate. In our models the R^2 values were 0.05, 0.04, and 0.08 for the 3 models (any, intragastrointestinal, and extragastrointestinal), respectively. The models generated were population-based descriptive models, which aimed to evaluate the average effect on survival to first-time hospitalization with the outcome of interest adjusted for known and measurable confounders, rather than predict the probability of survival for a specified individual. Thus, the most important assessment criteria for evaluating the appropriateness of a descriptive Cox regression model is that the proportional hazards assumption is not violated and the overall model is significant. In all of our models the proportional hazards assumption was tested and found not to be violated and the overall model significance was Prob > χ^2 <0.00005.

pital, death, and communicable disease notifications data obtained over a long time, which made available a comprehensive patient-based longitudinal dataset, as opposed to an events-based dataset. Data were of a routine administrative nature, and there was no likelihood of a Hawthorne effect (changes caused by participants being observed) or recall bias. The study covered the entire population of those born in Western Australia and therefore avoided challenges to external validity that arise when patient series are reported from selected institutions.

Our study incorporated an extensive amount of sociodemographic and other health-related data, thus enabling adjustment of the models for a range of measurable confounding variables. We recognize that a variety of unmeasurable, potentially confounding, sociodemographic factors may not have been completely captured by this study. However, given the magnitude of the increase in risk for sequelae observed for those exposed, all of this increase in risk would be unlikely to be removed if these potential confounders could be adjusted for. One way to have ad-

Table 4. Crude and adjusted rate ratio and attributable risk for first-time hospitalization for sequelae, by type of prior infection and classification of sequelae, Western Australia, Australia, January 1,1985–December 31, 2000*

Type of sequelae and type of prior infection	Crude rate ratio, RR (95% CI)	Adjusted† rate ratio, RR (95% CI)	Adjusted AR, %‡	Goodness of fit§
Any				0.05
Campylobacteriosis	1.56 (1.43–1.69)	1.52 (1.39–1.66)	34	
Giardiasis	2.10 (1.91–2.31)	1.51 (1.36–1.68)	34	
Salmonellosis	1.86 (1.71–2.03)	1.39 (1.26–1.53)	28	
Viral enteritis	2.10 (1.96–2.24)	1.68 (1.56–1.81)	40	
Enteritis/gastroenteritis NOS	2.60 (2.51–2.70)	1.76 (1.70–1.84)	43	
Extragastrointestinal				0.07
Campylobacteriosis	1.50 (1.37–1.64)	1.45 (1.32–1.60)	31	
Giardiasis	2.26 (2.04–2.49)	1.54 (1.38–1.73)	35	
Salmonellosis	2.01 (1.85–2.21)	1.43 (1.29–1.58)	31	
Viral enteritis	2.12 (1.98–2.27)	1.63 (1.51–1.76)	39	
Enteritis/gastroenteritis NOS	2.69 (2.58–2.80)	1.74 (1.66–1.82)	43	
Intragastrointestinal				0.02
Campylobacteriosis	1.76 (1.51–2.05)	1.64 (1.40–1.93)	39	
Giardiasis	1.37 (1.10–1.68)	1.29 (1.03–1.61)	23	
Salmonellosis	1.09 (0.87–1.34)	1.00 (0.79–1.25)	0	
Viral enteritis	1.99 (1.72–2.29)	1.56 (1.34–1.85)	36	
Enteritis/gastroenteritis NOS	2.03 (1.88–2.20)	1.66 (1.52–1.81)	40	

*RR, relative risk; CI, confidence interval; AR, attributable risk; NOS, not otherwise specified.
†Multivariate Cox regression estimating the adjusted rate ratio of first-time hospitalization for any, intragastrointestinal, and extragastrointestinal sequelae. Adjusted for gender,indigenous status, year of birth, age at exposure or proxy, singleton, weight at birth, hospital birth, mother's region of birth, father's region of birth, socioeconomic status, accessibility to services, and previous hospitalization for comorbid conditions.
‡Proportion of first-time hospitalizations for sequelae where previous exposure to the specified enteric infection was a component cause.
§Pseudo R^2. In our models, the R^2 values were 0.05, 0.07, and 0.02 for the 3 sets of models (any, extragastrointestinal, and intragastrointestinal) respectively. In all models the proportional hazards assumption was tested and found not to be violated, and the overall model significance was Prob > χ^2 <0.00005.

justed for unmeasurable factors would have been to restrict the study population to those hospitalized for a condition unrelated to the exposure (prior enteric infection) and the outcome, such as injury. However, this adjustment would have dramatically reduced the sample size, and hence the power of the study, and would have substantially limited generalizability.

Substantial errors in our dataset are unlikely because classification regimens were applied consistently throughout; validation research on the WADLS (27) has shown that missing data items are uncommon (<1%) and that the technical performance of the linkage between records is high (>99% specificity and sensitivity). Accordingly, our data are a highly robust representation of the population studied, based on usual hospital admission practices and outcomes of persons after notification of an enteric infection within the Australian healthcare system.

Our study relied on the use of notifications or hospitalization for enteric infections; not all enteric infections are notified. Enteric infections that cause only mild symptoms may go unreported either because the person may not seek medical advice or because a medical practitioner may not undertake confirmatory testing (*28*). Our conclusions are based on an assumption that a similar proportion of underreporting of enteric infection occurred for those classified with and without prior infection. Because notification of enteric infection is more likely for moderate to severe illness, our results pertain to this group rather than all enteric infections. This restriction limits generalizability (the range of persons not studied directly to whom the results can be applied), although it does not affect the validity of our results.

Sequelae in this study were captured as a first-time hospitalization for the condition of interest. Thus, some sequelae that produced only mild symptoms not requiring hospitalization were not captured, producing an underestimation of first-time sequelae. We evaluated the risk for first-time hospitalization for sequelae rather than incidence of sequelae per se. Although this type of evaluation is a limitation, the sequelae that have been measured are of clinical importance by virtue of the need for hospitalization.

We used the Western Australian birth register as the method of identification. These data enabled comprehensive inclusion of all persons born in the state, enabling complete capture of first-time enteric infections and outcome, within the definitions discussed above. The birth register also provided information about parents' place of birth, enabling these potential confounding variables to be evaluated when constructing the models. However, the use of a birth cohort did not permit the inclusion of international or interstate migrants. Again, this exclusion potentially limits the generalizability of our findings because migrants may have different risks of developing sequelae than nonmigrants. Inclusion of migrants in this study would have created difficulty capturing exposure and outcome data because their health records were not available before individuals became residents of Western Australia.

Although our study estimated the magnitude of the increased risk for illness attributable to prior enteric infections, we cannot explain why enteric infection during childhood or adolescence subsequently increases the risk for illness. Genetic susceptibility for disease, combined with an environmental trigger, has previously formed the basis for explaining this risk for conditions with an autoimmune basis, such as inflammatory bowel disease (*15*,*21*). Our finding of an increased risk for nongastrointestinal infections is more difficult to account for by autoimmune mechanisms. We speculate that increased risk for nongastrointestinal infections may result from dysregulated immunity, particularly in the short to medium term, after gastroenteritis, brought about by the influence of enteric infection on the gastrointestinal immune system.

Medium- to long-term adverse health implications of gastroenteritis must be accurately assessed so that appropriate risk-management strategies can be developed for those exposed to enteric infections. Recent reports have described the effect of acute gastrointestinal infections in industrialized countries, such as Australia (*2*,*28*) and Canada (*29*). These investigations have focused on identifying rates of, and risk factors for, infectious gastrointestinal illness with the goal of informing public health policy and planning. The need to investigate the impact of long-term sequelae of infectious gastrointestinal illness has also been highlighted (*29*). According to our data, sequelae are clearly substantial and provide another reason for trying to reduce the incidence of acute gastrointestinal infection worldwide.

Our study shows that enteric infection during childhood or adolescence increases the risk for first-time hospitalization for a range of intragastrointestinal and extragastrointestinal disease for 2 decades after onset of infection. This risk is greater, and occurs earlier, for extragastrointestinal sequelae than for intragastrointestinal sequelae. Our results highlight the importance of identifying ways of reducing such infections.

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Typhoid Fever and Invasive Nontyphoid Salmonellosis, Malawi and South Africa

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To determine the prevalence of invasive nontyphoid salmonellosis and typhoid fever in Malawi and South Africa, we compared case frequency and patient age distribution. Invasive nontyphoid salmonellosis showed a clear bimodal age distribution; the infection developed in women at a younger age than in men. Case frequency for typhoid fever was lower than for salmonellosis.

Invasive nontyphoid salmonellosis (iNTS) was first described as an AIDS-related illness in Africa and the United States in the 1980s. Although incidence in industrialized countries declined, nontyphoid *Salmonella* (NTS) spp. serovars (predominantly *S. enterica* serovars Typhimurium and Enteritidis) remain a common cause of bloodstream and focal infection in sub-Saharan Africa for adults with HIV infection and children with HIV, malaria, and malnutrition. iNTS has a strong seasonal pattern in adults and children. In addition, epidemics of iNTS have been described as associated with the emergence of multidrug resistance in Malawi (*1*). Similarly, multidrug resistance is well recognized in iNTS in South Africa (www.nicd.ac.za/pubs/survbull/2010/CommDisBullMay10_Vol0802.pdf). Death rates are 20%–25% among adults and children (*1*). In sub-Saharan Africa, transmission is thought most likely to be between humans, and no food or animal source has been found, although epidemiologic data remain sparse (*2*).

In comparison to iNTS, *S. enterica* serovar Typhi is a highly adapted, invasive, human-restricted pathogen that in the 19th century caused considerable illness and death in the United States and Europe but now has the greatest impact in developing countries. In sub-Saharan Africa, perhaps surprisingly, typhoid fever is not associated with HIV among adults (*3*).

Regional data on the demography and prevalence of both iNTS and *S.* Typhi for sub-Saharan Africa are incomplete (*4*). Estimates of incidence of iNTS among children, 175–388/100,000 (*5–7*), and among adult HIV-prevalent cohorts, 2,000–8,500/100,000 (*8–10*), have been made separately, in different locations, giving no overall demographic picture. Estimates of the incidence of typhoid fever have relied on limited available data from sub-Saharan Africa (*11*). Although typhoid is usually regarded as an illness of school-age (>5 years of age) children and young adults, there is considerable heterogeneity; some sites in Asia report high incidences of typhoid fever among children <5 years of age (*12*). We compared case frequency and patient age distribution for the predominant types of invasive salmonellosis among febrile patients of all ages treated at our 2 centers in 2 regions in sub-Saharan Africa, Malawi and South Africa, before 2004.

The Study

In Malawi, Queen Elizabeth Central Hospital is the government-funded hospital for Blantyre District, serving ≈1 million persons. From January 1998 through December 2004, persons from the community who came to the hospital with fever (adults >14 years, axillary temperature >37°C; and children ≤14 years and >1 month, temperature >37.5°C, and negative malaria test result) had venous blood taken for routine culture, as previously described (*1*). During 1998–2000, a manual culture system was used. From December 2000 onward, the same volume of blood was cultured by using the BacT/Alert 3D automated system (bioMérieux, Marcy l'Étoile, France). All isolates were identified by using standard diagnostic techniques. Outbreaks caused by individual NTS serovars were observed during this period, occurred simultaneously among adults and children, and showed an identical age distribution to baseline data (*1*).

In South Africa, active laboratory-based surveillance for *Salmonella* spp. was introduced nationally in 2003. Data from January 2003 through December 2004 were collected by the Enteric Disease Reference Unit, the national reference center representing data from >250 diagnostic laboratories across South Africa, serving ≈46 million persons (www.nhls.ac.za). Samples from normally sterile sites (bloodstream, cerebrospinal fluid, pleural fluid) from patients admitted to hospitals in South Africa were collected according to the clinician's judgment, and isolates were submitted to the local diagnostic laboratory, where they were identified by using standard diagnostic techniques before being sent to Enteric Disease Reference Unit

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for confirmation of identification and serotype. No major outbreaks of typhoid fever or iNTS were observed during this period.

Conclusions

In South Africa during 2003 and 2004, 1,318 cases of iNTS were microbiologically confirmed (67% *S.* Typhimurium, 10% *S.* Enteritidis, 7% *S.* Isangi, and 6% *S.* Dublin), and 105 cases of *S.* Typhi were identified under surveillance that included demographic data. In Malawi during 1998–2004, 62,778 blood samples were taken, of which 10,628 yielded pathogens. Information included 4,956 cases of iNTS (75% *S.* Typhimurium, 21% *S.* Enteritidis), for which demographic data were available for 4,044, and 105 cases of *S.* Typhi bacteremia, for which demographic data were available for 75.

Age distribution of patients with of iNTS and typhoid fever in Malawi and South Africa are shown in Figure 1. Despite the potential differences in sampling and surveillance intensity between sites, the data show a similar pattern of age distribution for iNTS at both sites, with a clear bimodal distribution. A peak was seen during the first 2 years of life, which rapidly declined thereafter until a second peak at ≈30 years. This age distribution was the same for all individual NTS serovars apart from *S.* Isangi and reflects the well-described risk factors for NTS infection: malaria, malnutrition, and HIV among children, and HIV among adults. A relative paucity of iNTS in the first few months of life has been reported from the Malawi center (*13*). We note that 32% (425/1,318) compared with 54% (2176/4044) of iNTS cases were in children <15 years of age for South Africa and Malawi, respectively. It is, however, not possible to say whether this observed difference may be explained by sampling bias, differences in population demographics, or iNTS specific risk factors in the 2 countries. Notably, patients from whom *S.* Isangi was isolated in South Africa were significantly younger (median age 5 years; p<0.001) than patients infected with other serovars.

There was a significant gender difference in the age at which adults acquire iNTS (Figure 2). iNTS occurred in women at a younger age than men in South Africa (median age 30 years for women vs. 35 years for men; p<0.001) and Malawi (median age 33 years for women vs. 37 years for men; p<0.001). The principal risk factor for iNTS among adults is HIV (*3*), and this finding is consistent with the observation that women acquire HIV infection at a younger age than men. The HIV prevalence in those 15–24 years of age is 3× greater among women than men across sub-Saharan Africa. In South Africa the difference is even more marked, and HIV prevalence peaks in women 25–29 years of age and in men 30–34 years of age (*14*)

The relative frequency of iNTS cases detected by both centers was much higher than that of *S.* Typhi, which suggests a substantially higher number of cases. However, the different sampling protocols do not permit comment on absolute incidence rates.

Typhoid fever patients showed a markedly contrasting age distribution from that seen in iNTS, affecting mainly school-age children and younger adults, but differed slight-

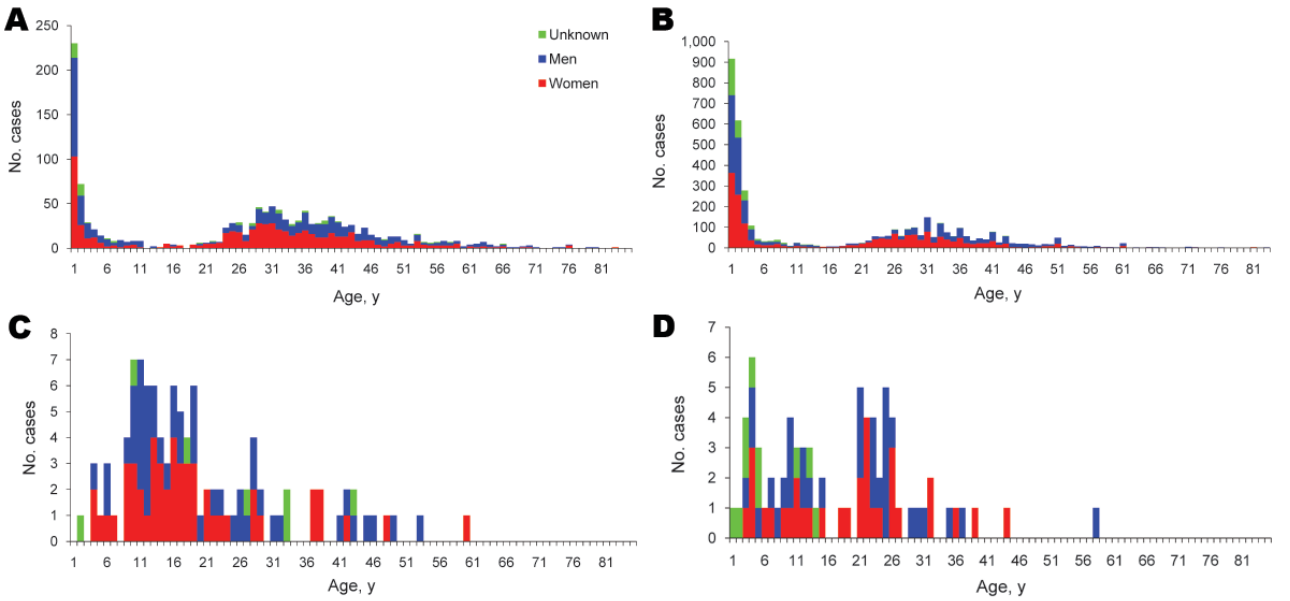

Figure 1. Age and gender distribution of patients with invasive nontyphoid *Salmonella* spp. infections in A) South Africa, 2003–2004, and B) Blantyre, Malawi, 1998–2004; and age and gender distribution of patients with *Salmonella enterica* serovar Typhi infection in C) South Africa, 2003–2004, and D) Blantyre, Malawi, 1998–2004.

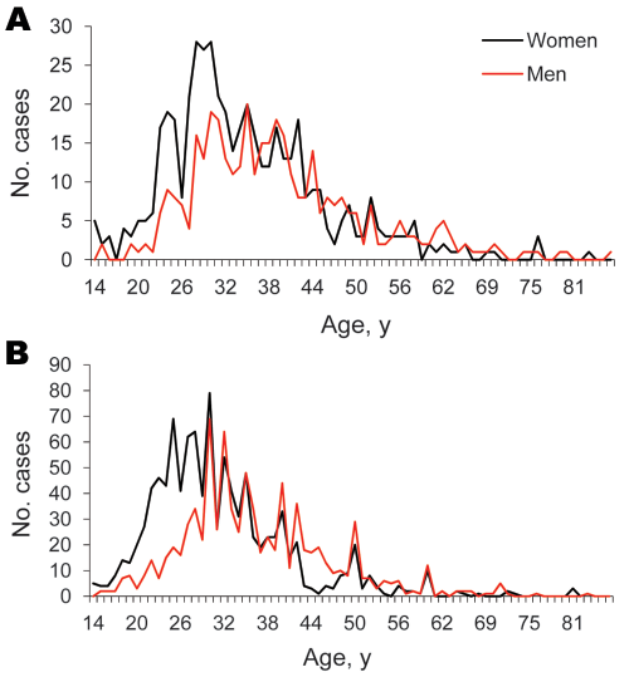

Figure 2. Age and gender distribution of adult patients (>14 years of age) with invasive nontyphoid *Salmonella* spp. infection in A) South Africa, 2003–2004, and B) Blantyre, Malawi, 1998–2004.

ly between the 2 sites. In Malawi, 15 of 75 typhoid cases were in preschool-age children, compared with only 5 of 105 of cases in South Africa. Again, the reasons for this cannot be determined.

It is noteworthy that the relative case frequencies and age distributions of iNTS and typhoid are so contrasting. Unlike in industrialized countries, NTS in sub-Saharan Africa is thought to be transmitted person to person, and a unique pathovar, *Salmonella* Typhimurium ST313, has emerged that shows genomic degradation similar to that seen in the human-restricted *S.* Typhi (*15*). Despite this evidence of convergent evolution, these data strongly suggest that the epidemiology and transmission routes of *S.* Typhi and NTS may be distinct. It remains to be seen what effects the wide availability of antiretroviral drugs or enhanced malaria eradication programs in sub-Saharan Africa will have on the demography of iNTS.

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Novel Hepatitis E Virus Genotype in Norway Rats, Germany

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Human hepatitis E virus infections may be caused by zoonotic transmission of virus genotypes 3 and 4. To determine whether rodents are a reservoir, we analyzed the complete nucleotide sequence of a hepatitis E–like virus from 2 Norway rats in Germany. The sequence suggests a separate genotype for this hepatotropic virus.

Hepatitis E virus (HEV) is a nonenveloped virus, diameter 30–34 nm, that belongs to the genus *Hepevirus*. Its single-stranded, positive-polarity RNA genome of 6.6–7.3 kb harbors 3 major open reading frames (ORFs) flanked by a capped 5′ end and a poly A at the 3′ end. ORF1 at the 5′ end of the genome codes for several nonstructural proteins, ORF2 encodes the immunodominant capsid protein, and the partially overlapping ORF3 codes for a cytoskeleton-associated phosphoprotein with multiple functions (*1*).

Hepatitis E, an acute self-limiting disease, occurs worldwide; large outbreaks have occurred in developing countries, as was recently reported from Uganda (*2*). Initially, hepatitis E was believed to be endemic only to developing countries in Asia, Africa, and Central America, but recent studies have demonstrated autochthonous infections in industrialized countries (Europe, Japan) (*3*). In contrast to the fecal–oral transmission of HEV that occurs in developing countries, it is suspected that these human infections result from zoonotic transmission of HEV genotypes 3 and 4; domestic pigs, wild boars, and deer represent major reservoir hosts (*1*,*4*). However, rodents, especially commensal rodents, may represent an additional HEV reservoir and may play a role in the epidemiology of hepatitis E. HEV-reactive antibodies have been detected in several rat species (*Rattus norvegicus*, *R. rattus, R. exulans)* but also in some noncommensal wild rodent species (*5*–*8*). By using broad-spectrum, nested, reverse transcription–PCR (RT-PCR), we recently detected HEV-like sequences in fecal samples of Norway rats (*R. norvegicus*) trapped as part of the Rodent-borne Pathogens network (which coordinates activities with regard to rodent trapping during outbreaks) (*9*,*10*). These sequence fragments had high nucleotide sequence divergence to genotypes 1–4 and to avian HEV strains.

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The Study

During July 8–16, 2009, a total of 6 Norway rats, 3 male and 3 female, 65–432 g, were trapped in manholes of the sewer system of Hamburg, northern Germany, at the same locations where ≈12 months before HEV RNA had been detected in rat feces (*10*). Standardized necropsy (*9*) found no morphologic abnormalities. Initial serologic screening with a commercial genotype 1–based ELISA (Axiom, Bürstadt, Germany) detected no reactive antibod-

Table. Nucleotide and deduced amino acid sequence identities between human, rabbit, and avian HEV strains compared with HEV isolated from 2 Norway rats, Germany, July 2009*

Strain, GenBank accession no.	Rat no., GenBank accession no.							
	63, GU345042				68, GU345043			
	Genome, nt	ORF1, aa	ORF2, aa	ORF3, aa	Genome, nt	ORF1, aa	ORF2, aa	ORF3, aa
Genotype 1, F076239	55.9	47.6	56.2	27.5	55.7	47.4	56.4	30.4
Genotype 2, M74506	55.3	48.7	55.4	28.4	55.2	48.6	55.5	29.4
Genotype 3, F060668	55.7	48.0	57.2	24.8	55.7	47.7	57.3	26.7
Genotype 4, J272108	55.5	48.2	55.9	27.5	55.3	47.8	56.1	26.5
Rabbit HEV, J906895	55.1	48.7	56.7	23.5	55.1	48.6	56.8	25.5
Avian HEV/Hungary, AM943646	50.2	46.5	45.9	26.9	49.9	46.4	46.3	26.9
Avian HEV/Australia, AM943647	49.9	46.6	46.1	26.9	49.3	46.5	46.5	26.9
Avian HEV/USA, AY535004	49.5	46.7	46.1	26.9	49.8	46.7	46.5	26.9

*HEV, hepatitis E virus; ORF, open reading frame.

ies in transudates of any of the 6 rats. Liver RNA from 1 female (no. 68, 311 g) and 1 male (no. 63, 313 g) rat yielded an amplification product of the expected size (331–334 nt) and a sequence identity of 83.8%–94.6 % with the HEV sequences recently obtained from rat feces (data not shown). Using a strategy according to Schielke et al. (*4*), we determined the entire rat HEV genome sequences from each sample to be 6,945 nt and 6,948 nt; the sequences differed by an insertion–deletion polymorphism in the 3′ noncoding region. The sequence identity between each complete sequence was 95.3% and reached 55.1%–55.9% to HEV genotypes 1–4 and 49.3%–50.2% to avian HEV strains (Table). Using prediction software, we identified the major ORFs 1, 2, and 3 in the new genomes in an organization typical for HEV (Figure 1, panel A). In contrast to HEV genotypes 1–3, rat HEV ORFs 1 and 3 do not overlap. Three additional putative ORFs of 280–600 nt that overlap with ORFs 1 or 2 were predicted for each rat HEV genome (Figure 1, panel A). However, before the meaning of these findings can be verified, sequence information from additional rat HEV strains and experimental proof are needed. Phylogenetic analyses of a 1,576-nt segment available for all published rat HEV sequences demonstrated clear separation from mammalian genotypes 1–4 and avian strains (Figure 1, panel B). The same 3 phylogenetic clusters were obtained when the complete genomes were analyzed (Figure 1, panel C) and when the nucleotide and deduced amino acid sequences of ORF1, ORF2, and ORF3 were investigated separately (data not shown).

To compare viral load in different tissues of the 2 HEV–positive rats, we developed a real-time RT-PCR selective for a region in the ORF2 of rat HEV. Parallel analysis of RNA isolated from 10 mg of each tissue or 10 μL of blood reproducibly showed the highest viral load to be in the liver; cycle threshold values for liver were 20.5 and 21.6 for each animal and lower for all other tissues (online Appendix Figure, www.cdc.gov/EID/content/16/9/1452-appF.htm). Further, immunohistochemical analysis, using anti-HEV serum, detected viral antigen in the cytoplasm of a few hepatocytes from each HEV-positive rat. Antigen

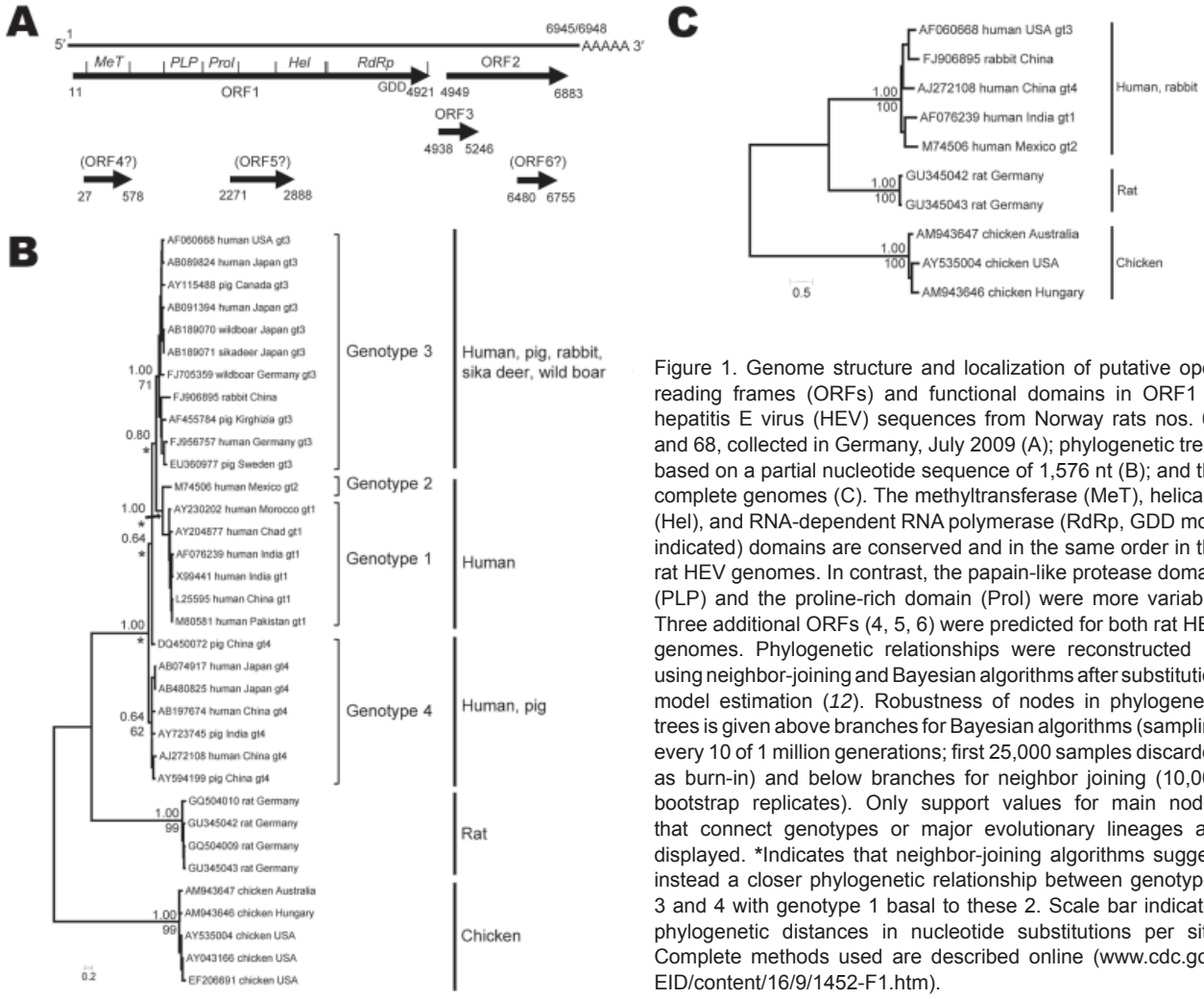

Figure 1. Genome structure and localization of putative open reading frames (ORFs) and functional domains in ORF1 of hepatitis E virus (HEV) sequences from Norway rats nos. 63 and 68, collected in Germany, July 2009 (A); phylogenetic trees based on a partial nucleotide sequence of 1,576 nt (B); and the complete genomes (C). The methyltransferase (MeT), helicase (Hel), and RNA-dependent RNA polymerase (RdRp, GDD motif indicated) domains are conserved and in the same order in the rat HEV genomes. In contrast, the papain-like protease domain (PLP) and the proline-rich domain (Prol) were more variable. Three additional ORFs (4, 5, 6) were predicted for both rat HEV genomes. Phylogenetic relationships were reconstructed by using neighbor-joining and Bayesian algorithms after substitution model estimation (*12*). Robustness of nodes in phylogenetic trees is given above branches for Bayesian algorithms (sampling every 10 of 1 million generations; first 25,000 samples discarded as burn-in) and below branches for neighbor joining (10,000 bootstrap replicates). Only support values for main nodes that connect genotypes or major evolutionary lineages are displayed. *Indicates that neighbor-joining algorithms suggest instead a closer phylogenetic relationship between genotypes 3 and 4 with genotype 1 basal to these 2. Scale bar indicates phylogenetic distances in nucleotide substitutions per site. Complete methods used are described online (www.cdc.gov/EID/content/16/9/1452-F1.htm).

was also observed in some activated hepatic stellate cells (Figure 2). Hematoxylin-eosin staining showed a marginally increased number of monocytes and granulocytes in sinusoids as well as a moderately increased number of lymphocytes and plasma cells in some Glisson triads of the livers (data not shown).

Conclusions

Phylogenetic analyses and nucleotide and amino acid sequence comparisons demonstrated that the complete rat HEV genome sequences were consistently well separated from those of mammalian genotypes 1–4 and the tentative avian genotype. This finding suggests that these sequences represent an additional genotype (Figure 1, Table). In our analyses, the recently described HEV strain found in domestic rabbits, proposed to represent a separate genotype (*13*), clustered with human HEV genotypes irrespective of the genome part, nucleotide, or deduced amino acid sequences analyzed (Figure 1, panels B, C, and data not shown). Therefore, this strain may represent the consequence of recent spillover rather than the result of long-term virus–host coevolution. In contrast, the nonzoonotic avian HEV strains strongly differ from the mammalian HEV genotypes 1–4 (Figure 1, panels B, C). Although in the genus *Hepevirus* no species demarcation criteria have been defined, the marked sequence diversities suggest that the rat HEV represents an additional virus species other than HEV-1, HEV-2, HEV-3, HEV-4, and the tentative species avian HEV, which are currently classified in this genus (*14*).

Detection of rat HEV RNA and antigen in the liver cells of the infected Norway rats may indicate hepatotropism of this virus. Therefore, regarding its organ and cell-type tropism, this virus seems to be similar to the human and pig HEV genotypes (*15*). Because the virus was also detected in the intestine and, in the previous study, in feces (*10*), fecal–oral transmission as for genotypes 1–4 is plausible. The common properties of this virus and the human HEV genotypes suggest the usefulness of developing an HEV model in laboratory rats. In addition, the detection of rat HEV in animals from an urban region in Germany raises questions about the putative epidemiologic role of rat HEV for hepatitis E in humans.

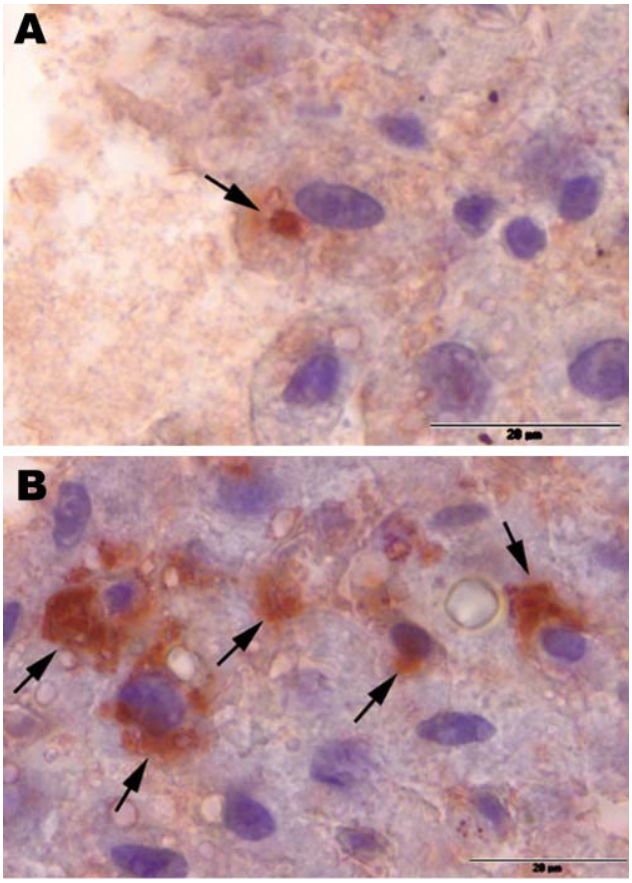

Figure 2. Immunohistochemical staining (peroxidase-antiperoxidase (PAP) technique) of liver samples from 2 rat-hepatitis E virus (HEV)–positive Norway rats from Germany, July 2009. Arrows indicate immunohistochemical positive reactions in the cytoplasm of single hepatocytes (A) and in a few foci in hepatocytes and stellate cells (B). For PAP staining, deparaffinized slides of liver samples were incubated with anti-HEV–positive human serum, which had been previously used to detect rat HEV by using solid phase immunoelectron microscopy (*10*), for 1 h at 37°C with protein A (Sigma-Aldrich, Steinheim, Germany) at a dilution of 1:100 for 45 min at 37°C and finally with PAP complexes from rabbits (Sigma, St. Louis, MO, USA) at a dilution 1:200 for 45 min at 37°C. AEC (3-amino-9-ethylcarbazol; Sigma Chemie GmbH, Deisenhofen, Germany) was used as the substrate chromogene. The slides were counterstained with hematoxylin and subsequently analyzed by light microscopy. Scale bars = 20 µm.

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Increasing Incidence of Mucormycosis in University Hospital, Belgium

Veroniek Saegeman, Johan Maertens, Wouter Meersseman, Isabel Spriet, Eric Verbeken, and Katrien Lagrou

To determine why incidence of mucormycosis infections was increasing in a large university hospital in Belgium, we examined case data from 2000–2009. We found the increase was not related to voriconazole use but most probably to an increase in high-risk patients, particularly those with underlying hematologic malignancies.

In September 2009, Bitar et al. reported an increasing incidence of mucormycosis in France from 1997 through 2006 (*1*). Their epidemiologic study was based on International Classification of Diseases, 10th Revision (ICD-10), codes extracted from hospital information systems from an estimated 95% of public and private hospitals in France. This study is particularly interesting because population-based estimates of the incidence of mucormycosis are scarce (*2*). However, as the authors state, ICD code–based evaluations have limitations; such limitations are mainly related to difficulties encountered with the diagnosis of mucormycosis in clinical practice. Conventional diagnostic tools lack sensitivity. Moreover, distinction between colonization and infection is problematic in the absence of invasive procedures or autopsy data. Thus, epidemiologic studies on mucormycosis are often hampered by the limited number of documented cases.

Several surveys have been conducted in large US transplant centers. Based on these studies, the increasing incidence of mucormycosis has been linked to the widespread use of voriconazole prophylaxis in high-risk patients (*3*,*4*). In contrast with the study in France, these surveys have the limitation of focusing on particular risk groups, mainly cancer patients, and do not provide a general estimate on the incidence of mucormycosis. In our study, we determined the incidence rate of invasive mucormycosis in a large university hospital in Belgium over a 10-year period and reviewed the clinical data of the patients with proven or probable disease.

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The Study

Case finding was based on culture and pathology data and not on ICD codes. Culture data from all patients hospitalized at the University Hospitals of Leuven (Belgium) from 2000 through 2009 were retrieved from the Sirscan 2000 system (i2a, Montpelier, France). Computer queries were run to retrieve all reports from histologic examinations of tissue specimens to which the Leiden code "fungus" had been assigned (*5*). Medical records of all patients with culture or histopathologic evidence for *Mucorales* spp. organisms were reviewed, and all patients were classified as having proven or probable invasive mucormycosis according to European Organization for Research and Treatment of Cancer and Mycoses Study Group (EORTC-MSG) criteria (*6*).

Over this 10-year time period, 31 patients with mucormycosis were identified: 21 with proven and 10 with probable disease. The annual incidence increased from 0.019 cases/10,000 patient-days in 2000 to 0.148 cases/10,000 patient-days in 2009 (Spearman correlation coefficient 0.75; p = 0.01) (Figure 1). The incidence increased mainly from 2005–2009. Averaged over the 10 years, the incidence was 0.058/10,000 patient-days. The sex ratio (M/F) was 16/15; mean age was 54 years (median 59 years, range 12–79 years). Nineteen (61%) of 31 mucormycosis patients had a hematologic disorder; 8 patients underwent a hematopoietic stem cell transplantation. Other underlying conditions were diabetes mellitus (n = 2), solid organ transplantation (n = 4), surgery/trauma (n = 4), autoimmune disorder (n = 1), and HIV infection (n = 1).

The number of patients belonging to the risk groups diabetes mellitus, solid organ transplantation, hematologic disorder/malignancy, and hematopoietic stem cell transplantation was determined based on International Classification of Diseases, 9th Revision, Clinical Modification (ICD-9-CM), codes entered in the hospital information system (available up to the end of 2008). An increase in the number of diabetes mellitus (p<0.01) and hematologic disorder/malignancy (p<0.01) patients (Spearman correlation; Table) was seen

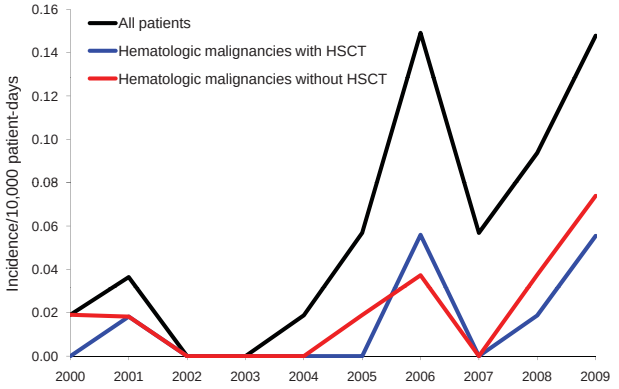

Figure 1. Incidence of mucormycosis cases in a hospital in Belgium, 2000–2009. HSCT, hematopoietic stem cell transplantation.

Table. Specific annual incidence rate of mucormycosis cases per risk group in a hospital in Belgium, 2000–2008*

Year	Diabetes mellitus	Solid organ transplantation	Hematologic disorder/ malignancy	HSCT
2000	0/3,087	0/235	1/684	0/96
2001	0/3,328	0/217	2/557	1/86
2002	0/3,319	0/207	0/650	0/87
2003	0/3,662	0/245	0/775	0/118
2004	1/3,845	0/217	0/787	0/126
2005	1/4,121	1/191	1/853	0/103
2006	0/4,235	2/251	5/1,061	3/91
2007	0/4,328	0/279	0/942	0/121
2008	0/4,477	1/267	3/859	1/116
p value for the increase in				
No. patients per risk group	<0.01	0.12	<0.01	0.19
Specific annual incidence rate	0.81	0.11	0.91	0.55

*Values are no. cases per risk group/no. patients admitted per year with that risk factor, unless otherwise indicated. Data for risk group patients are based on codes from the International Classification of Diseases, 9th Revision, Clinical Modification, available 2000–2008. HSCT, hematopoietic stem cell transplantation.

from 2000 through 2008. The specific annual incidence rate (defined as the number of mucormycosis cases per risk group over the number of risk patients per year) for these 4 risk groups did not increase significantly during this period (Spearman correlation; p>0.01) (Table; Figure 2).

None of the 31 patients had received voriconazole prophylaxis (per institutional policy). Mucormycosis developed in 1 patient while that patient was receiving voriconazole therapy for invasive aspergillosis.

Clinical signs included pulmonary (51.5%), disseminated (23%), cutaneous (13%), sinus (6.5%), cerebral (3%), and mycetoma (3%) mucormycosis. A high percentage (45%) of co-infections with *Aspergillus* spp. was recorded. Five of the 6 patients showing a halo sign on chest computed tomography scan were co-infected with *Aspergillus* spp.

The death rate was 65%. For 48% of the patients, death was directly related to mucormycosis infection.

Conclusions

We report an increasing incidence rate of invasive mucormycosis not related to the use of voriconazole, either prophylactically or therapeutically, in a university hospital in Belgium. Pongas et al. calculated that 49% of mucormycosis cases were encountered in the setting of voriconazole prophylaxis (*3*). A case–control observational study identified voriconazole prophylaxis as an independent risk factor for the development of mucormycosis (*7*). However, in our series, none of the 31 patients had received voriconazole prophylaxis, and mucormycosis developed in only 1 patient who was receiving voriconazole therapy for invasive aspergillosis.

Compared with the study from France (6.8%), our study reported a notably high percentage (23%) of cases of disseminated disease (*1*). However, high incidences, up to 17% of cases, were also reported from other recent clinical registries (*8*,*9*). In our hospital, the number of patients with disseminated mucormycosis infection may be increasing; 1 case was diagnosed in 2000, 1 in 2005, 2 in 2008, and 3 in 2009. This finding possibly reflects the growing population of severely immunosuppressed patients.

The death rate of 65% is consistent with death rates reported in recent clinical registries (≈50%) but appears to be much higher than rates reported in the study in France. However, this discrepancy might be merely a reflection of differences in case definitions (*1*,*8*,*9*). We expect death rates to be higher in studies or surveillances that apply more stringent case definitions; cases with only fungal colonization, not invasive disease, are less likely to be included in these studies.

During the 10-year period, the most important risk group, namely patients with a hematologic disorder/malignancy, expanded considerably. The population of patients with diabetes also increased, but mucormycosis in this host population is quite rare in our institution (only 2 cases in 10 years). A decreasing number of published mucormycosis

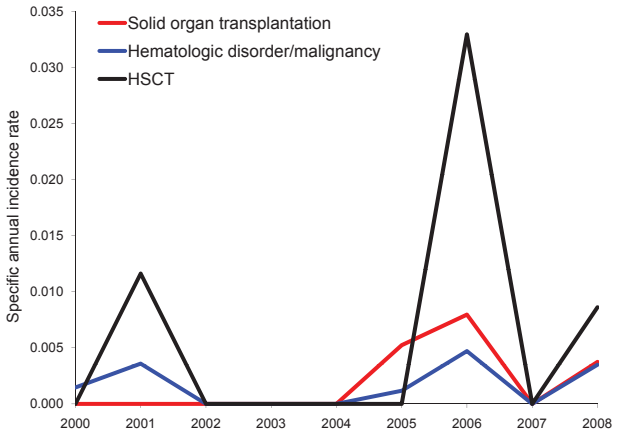

Figure 2. Specific annual incidence rate for the risk groups solid organ transplantation, hematologic disorder/malignancy, and hematopoietic stem cell transplantation (HSCT) in a hospital in Belgium, 2000–2008.

cases since the 1990s in patients with diabetes mellitus as underlying illness was reported in a large review of the literature (*10*). This finding could be a consequence of better glycemic control and decreasing rates of diabetic ketoacidosis and of the widespread use of statins in patients with diabetes (*11*).

Several limitations of our study can be mentioned. First, 5 cases of mucormycosis were diagnosed on the basis of histopathologic findings only. Second, the numbers of the risk populations were derived from ICD-9-CM codes and are thus subject to all inaccuracies associated with discharge coding of patients. Third, we used the EORTC-MSG criteria for classification of cases, but, unlike other filamentous fungal pathogens that target immunocompromised hosts, *Mucorales* spp. organisms infect a broader and more heterogeneous population. Thus, invasive mucormycosis also occurs in immunocompetent patients without any host factor as defined by these criteria. As such, invasive procedures are necessary in immunocompetent patients to enable the diagnosis of proven invasive mucormycosis according to the EORTC-MSG criteria. However, we estimate the effect of this limitation to be minor because in immunocompetent patients, biopsy procedures are generally feasible, particularly because mucormycosis is most often seen as a cutaneous infection in these patients. Finally, our results are single-institution data and are not necessarily representative of other institutions or other geographic regions.

In conclusion, the increasing incidence of mucormycosis shown in our study was not related to the prior use of voriconazole. Rather, the increasing incidence was most probably associated with an increasing number of patients who had an underlying hematologic malignancy.

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Ms Saegeman is a microbiologist in training at Catholic University of Leuven, Belgium. Her clinical and research interests focus on fungal infections.

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Human Herpesvirus 8 Genotype E in Patients with Kaposi Sarcoma, Peru

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To determine human herpesvirus 8 (HHV-8) K1 genotypes in patients with Kaposi sarcoma (KS) from Peru, we characterized HHV-8 in 25 KS biopsy samples. Our findings of 8 A, 1 B, 14 C, and 2 E subtypes showed high HHV-8 diversity in these patients and association between E genotype and KS development.

Human herpesvirus 8 (HHV-8; also known as Kaposi sarcoma–associated herpesvirus) is the etiologic agent of all forms of Kaposi sarcoma (KS) (*1*,*2*). In 2002, the number of KS cases worldwide was ≈65,000, nearly 1% of all diagnosed cancer cases (*3*). KS occurs commonly during HIV-1 infection (AIDS-KS); in transplant recipients; and in persons not infected with HIV, predominantly elderly men of Mediterranean and Middle Eastern origin (classic KS) or in children and adult men from eastern and Central Africa (endemic KS).

Sequence analysis of the highly variable open reading frame (ORF) K1 of HHV-8 has enabled the identification of 5 main HHV-8 molecular subtypes, A–E (*4*). A and C subtypes are prevalent in Europe, Mediterranean countries, the United States, northwestern People's Republic of China, and southern Siberia; subtype B, in sub-Saharan Africa; and subtype D, in Japan and Oceania. Subtype E is found among Native Americans (*5*–*9*). To our knowledge, KS has been reported in patients infected by all HHV-8 subtypes, except E.

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Recent studies demonstrated that classic KS is common in Peru and that AIDS-KS incidence is increasing because of the spread of HIV infection (*10*,*11*). Classic and epidemic KS occurred in patients of Amerindian origin (Quechuas) and in mestizos, reflecting the multiethnic origin of the Peruvian population.

A goal of our study was to determine the HHV-8 genotypes for a series of classic KS or AIDS-KS cases in Peru. We also aimed to report KS in patients infected by an E subtype.

The Study

We studied a series of 36 KS tumors diagnosed during 1989–2002 at the Hospital Nacional Cayetano Heredia in Lima. All these formalin-fixed, paraffin-embedded biopsy samples were stained by using hematoxylin–eosin stain and Perl methods. Immunohistochemistry was performed on deparaffinized sections by using monoclonal antibodies directed against CD34 and latent nuclear antigen (LANA-1) (*12*).

DNA was extracted from paraffin blocks by using the QIAamp DNA Mini Kit (QIAGEN GmbH, Hilden, Germany). HHV-8 infection was determined by nested PCR to obtain a 220-bp (variable region [VR] 1–inner fragment) and a 240–300-bp (VR2-inner) fragment of the ORF-K1 (*13*). Phylogenetic trees were generated with the neighbor-joining method (PAUP* version 4.0b10; http://paup.csit.fsu.edu) on fragments of either 309 bp (VR1-outer) or 165 bp (VR1-inner) of the VR1 (K1 gene) by using different sequence prototypes of the 4 major HHV-8 genotypes (*13*,*14*).

Histopathologic analysis was originally conducted on 36 biopsy samples, mostly from skin, diagnosed as KS. Three patterns were observed by hematoxylin–eosin stained specimens. The first was characterized by dilated, irregular, and angulated blood vessels in the dermis, associated with a variable number of lymphocytes. In the second pattern, dermal vascular channels lined by plump spindle cells were seen; some of these spindle cells coalesced to form aggregates, which were poorly delineated and often located around blood vessels. The third pattern (nearly half of all biopsy samples) was characterized by well-delineated sheets and bundles of spindle cells, which coalesced to form nodules. The proportion of spindle cells labeled with the monoclonal antibody LANA-1 varied according to the histopathologic pattern; the bundles and sheets of spindle cells in the third pattern displayed the strongest signal (Figure 1, panel A).

DNA was extracted from the 36 formalin-fixed, paraffin-embedded biopsy samples; DNA quantity and quality were appropriate in 30 biopsy samples. A faint PCR signal of the expected size was seen after single PCR in

[1]These authors contributed equally to this article.

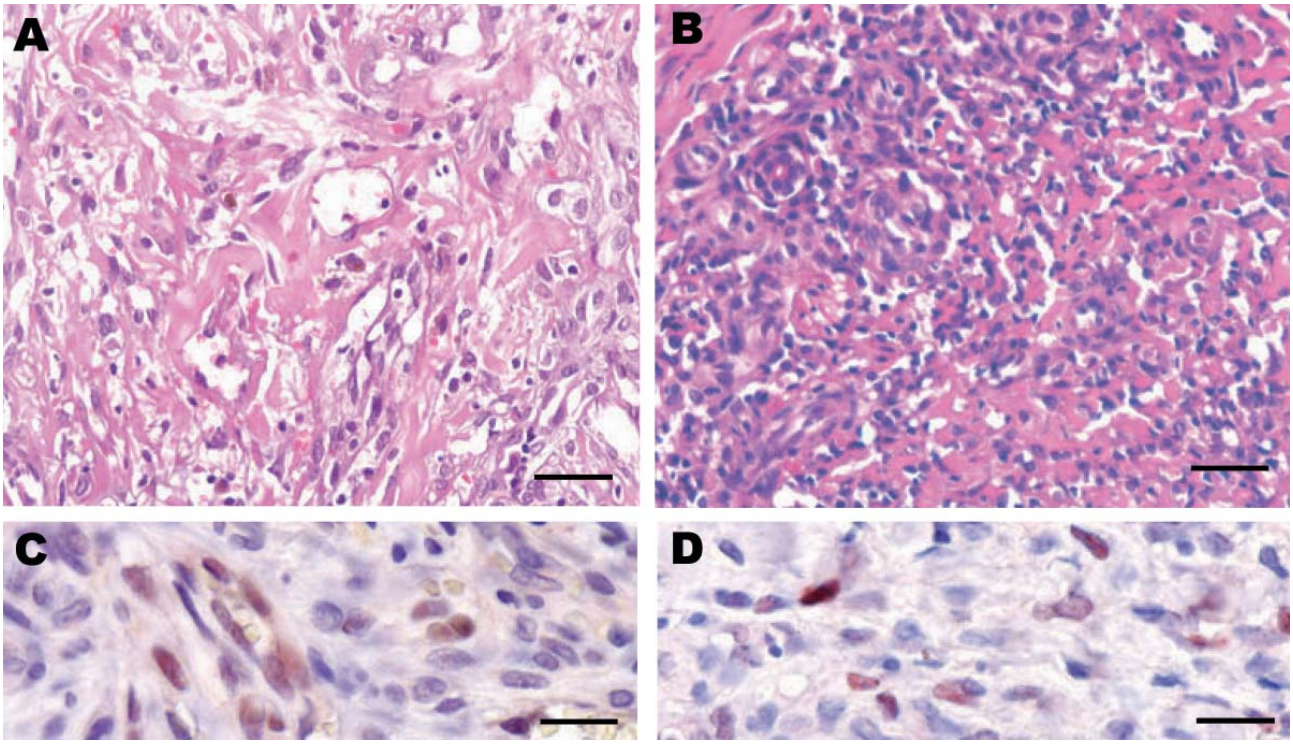

Figure 1. Histologic patterns of cutaneous Kaposi sarcoma (KS) associated with a human herpesvirus 8 (HHV-8) type E infection. Patient 1: A) The spindle cells were organized as bundles, forming vascular slit-like spaces containing erythrocytes. Some macrophages containing hemosiderin were observed (data not shown). C) Immunohistochemical testing showed a positive signal for HHV-8 infection (latent nuclear antigen [LANA-1]) and CD34 (data not shown). The Perls staining also gave highly positive results (data not shown). (Patient 1 corresponds to the first patient [04/0480] in the online Appendix Table (www.cdc.gov/eid/content/16/9/1459-appT.htm), a 51-year-old mestizo man who had HIV-1 infection. Patient 2: B) Spindle cells forming rare vascular channels, with numerous lymphocytes, plasma cells, and macrophages. D) Immunohistochemistry showed a lower positive signal for HHV-8 infection (LANA-1) and CD34 (data not shown). Few cells displayed a positive Perls staining (data not shown). (Patient 2 corresponds to the tenth patient [06/0772] in the online Appendix Table, a 24-year-old mestizo man with HIV-1 infection.)

12 samples for VR1 (VR1-outer) and in 4 cases for VR2 (VR2-outer) amplification. After nested PCR, a signal was obtained in 25 cases for VR1-inner and in 17 cases for VR2-inner (online Appendix Table, www.cdc.gov/eid/content/16/9/1459-TechApp.htm). After cloning and sequencing procedures, the HHV-8 genotype was obtained for 25 different KS cases, of which 8 genotypes belonged to the A subtype, including an A5, and 14 belonged to the C subtype. An E subtype was identified for 2 patients. In 1 case, a B subtype was determined. Among these 25 sequences, 16 were unique and 9 formed 3 groups of identical sequences. The 25 VR1-inner sequences exhibited 0%–24.4% nt divergence and 0%–43.1% aa acid divergence among pairwise comparisons. When the VR1-outer and VR2-inner sequences (536 bp) of the 2 E subtype strains in Peru were combined, the nucleotide divergence was ≈7% and reached 10% at the amino acid level. The 04480 and 06772 E subtype strains were closer to the Brazilian Amerindian strains (Kat, Sio, Wai, Tir, Tupi) than to the Ecuadorian (Hua1, Hua2, Hua3) or French Guianan Amerindian (Wagu) strains.

Phylogenetic analyses were performed by using 2 sets of sequences; 25 VR1-inner (Figure 2) and 11 VR1-outer sequences (data not shown). Forty-seven prototype strain sequences were added. The main molecular HHV-8 subtypes, A–E, were identified on the basis of consistent topology and bootstrap values obtained (Figure 2; data are not shown for other phylogenetic analyses performed, for example with the 11 VR1-outer sequences obtained after the first round of PCR). Among the 25 VR1-inner sequences, 22/25 were located in the large A/C subtype, and 8 strains belong to the A subtype, with strains scattered among 3 different subgroups (Figure 2). The 06758 strain belongs to the typical sub-Saharan A5 group. Fourteen strains are distributed among different groups in the C subtype, and the remaining sequences clustered in the sub-Saharan African B (04489) and Amerindian E (06772 and 04480) subtypes.

Two AIDS-KS mestizo patients (Figure 1) were thus found to be infected by typical E subtype HHV-8 strains: a 51-year-old man with a tumor on his neck and a 24-year-old man with multiple tumors on the upper limbs. These

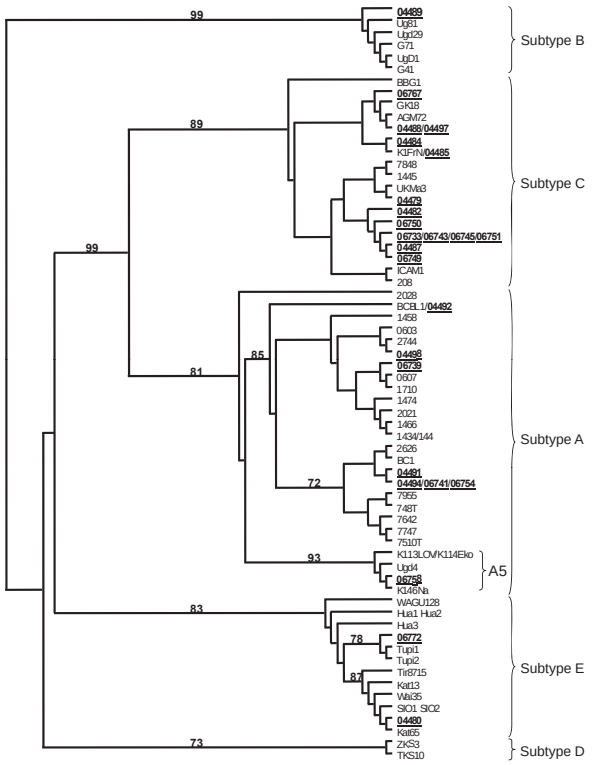

Figure 2. Unrooted phylogenetic tree generated with the neighbor-joining method (PAUP* version 4.0b10; http://paup.csit.fsu.edu) on the best 165-bp alignment of the variable region [VR] 1 comprising 79 human herpesvirus 8 nt sequences, including 25 novel sequences generated (GenBank accession nos. GU827339–GU827363). The strains were aligned with Data Analysis in Molecular Biology software (http://dambe.bio.uottawa.ca/software.asp), and the final alignment was submitted to the ModelTest software version 3.6 (http://darwin.uvigo.es/software/modeltest.html) to select, according to the Akaike Information Criterion, the best model to apply to phylogenetic analyses. The selected model was the general time reversible model. The reliability of the inferred tree was evaluated by bootstrap analysis on 1,000 replicates. Bootstrap support is noted on the branches of the tree.

tumors were mostly macroscopic nodules but displayed major histologic differences (Figure 1). CD34 (data not shown) and LANA-1–positive cells (Figure 1) were noted in both KS biopsy samples.

Conclusions

HHV-8 K1 gene characterization of KS tumor biopsy samples demonstrated high molecular genotype diversity, including 4 of the 5 main known molecular subtypes. The most frequent were the A and C subtypes, typically of European origin. Other patients were infected by a sub-Saharan African HHV-8 genotype (1 A5 and 1 B) or by HHV-8 genotype E strains of Amerindian origin.

Such findings were not unexpected because persons in Peru are of many ethnicities. Indeed, the genetic background of the Peruvian population is diverse, reflecting the multiple waves or populations that colonized the country during the last millennium. Schematically, this began with different Amerindians groups in the Lithic period (infected possibly with E subtype), followed by the Spanish colonization of the Americas mainly from southern Europe (infected possibly by A and C subtypes) and later slave trade from Africa (infected by A5 or B subtypes). Such HHV-8 strain diversity has been previously observed in French Guiana (*6*,*14*).

HHV-8 genotype E previously has been found exclusively in Amerindians from Central and South America, including Brazil (*5*,*9*), Ecuador (*7*), and French Guiana (*6*). In each instance, HHV-8 was detected in blood samples, and there was some debate about the presence/development of KS in such highly infected populations (*15*).

We demonstrated that KS can occur in HHV-8 subtype E–infected persons. Indeed, in 2 AIDS-KS patients, an E genotype was characterized in the tumor lesions. Further studies are ongoing to provide new insights into the distribution and genetic epidemiology of such HHV-8 infection in Amerindian populations.

Acknowledgments

We dedicate this study to the memory of Juan Carlos Ferrufino, who was chief of the Pathology Department at Hospital Nacional Cayetano Heredia and founding member of the Human Herpesvirus-8 and Kaposi Sarcoma Working Group.

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Rhinovirus Outbreaks in Long-Term Care Facilities, Ontario, Canada

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Diagnostic difficulties may have led to underestimation of rhinovirus infections in long-term care facilities. Using surveillance data, we found that rhinovirus caused 59% (174/297) of respiratory outbreaks in these facilities during 6 months in 2009. Disease was sometimes severe. Molecular diagnostic testing can differentiate these outbreaks from other infections such as influenza.

Respiratory tract illnesses are a major cause of illness and death among elderly persons, especially those in long-term care facilities. Although the most commonly identified viruses have been influenza virus and respiratory syncytial virus (RSV) (*1*), human rhinovirus (HRV) is being increasingly associated with severe respiratory disease and outbreaks in these facilities (*2*–*6*). Clinical diagnosis of HRV by immunofluorescence and virus culture has been difficult because these methods are unreliable (*7*,*8*). Moreover, because multiple serotypes of HRV exist, retrospective serologic testing cannot be used to evaluate the prevalence of HRV disease (*5*). As a result, the number of outbreaks caused by HRV in long-term care facilities, and the associated illness and death, may be substantially underestimated. We therefore used 2009 surveillance data to estimate prevalence of HRV disease in long-term care facilities.

The Study

Using data from an active surveillance network, we investigated all respiratory outbreaks (as defined by the Ministry of Health) (*9*), in long-term care facilities, reported from July 1 through December 31, 2009, in the province of Ontario, Canada. The number and timing of specimens collected was left to the discretion of the attending physicians. The regional clinical laboratories cultured specimens (blood, urine, and sputum) for bacteria and performed rapid viral antigen testing for influenza A/B and RSV. The Ontario Public Health Laboratory performed reference microbiology testing that included *Legionella* culture, *Mycoplasma/Chlamydophila* nucleic acid testing (NAT), and virus cultures. Multiplex NAT (Luminex xTAG Respiratory Viral Panel; Luminex Diagnostics, Toronto, Ontario, Canada) was used according to the manufacturer's recommendations to test nasopharyngeal swabs for viral pathogens (adenovirus, influenza A/B, parainfluenzae 1–4, RSV A/B, enterovirus [ENT]/HRV, coronavirus OC43/229E/NL63/HKU1, and metapneumovirus). An assay specific for pandemic (H1N1) 2009 virus was also performed.

To facilitate turnaround time during periods of higher demand, we used an alternate multiplex NAT kit (Seeplex RV; Seegene USA, Rockville, MD, USA) in conjunction with the Luminex assay. Because the Luminex assay cannot differentiate between ENT and HRV, we used the Seeplex RV kit, which can identify HRV, to confirm results in a random subset of ENT/HRV-positive samples. To type the HRV implicated in outbreaks during which deaths occurred, we amplified and sequenced the hypervariable region of the 5′ noncoding region, the entire viral capsid protein (VP) 4 gene, and the 5′ terminus of the VP2 gene; we then constructed phylogenetic trees as described (*10*,*11*).

During the surveillance period, 297 respiratory disease outbreaks in long-term care facilities were reported to the Ontario Public Health Laboratory; we received samples from 269 facilities (Table 1). A total of 987 specimens were tested (average 3.7 samples/outbreak). Of the 234 (79%) outbreaks for which a pathogen was identified, 174 (59%) pathogens were determined to be ENT/HRV (representing 531 positive samples) and were temporally spread throughout the surveillance period. Pandemic (H1N1) 2009 virus and parainfluenza-1 virus represented 7% and 6%, respectively, of identified pathogens. Other viruses were identified for <2% of outbreaks. Viral co-infection was identified in 7 samples from 7 outbreaks. A subset of 66 samples, representing 15% of ENT/HRV outbreaks, were randomly selected and subsequently tested with the Seeplex RV kit to further differentiate ENT from HRV; HRV was detected in 100% of these specimens.

Deaths were potentially associated with ENT/HRV in 4 facilities (outbreaks A–D; Table 2). Samples from patients involved in these outbreaks were confirmed to contain HRV; no other causative bacteria or viruses were identified. Clinical data were available for 7 of 13 of the patients who died; 6 deaths were attributed to pneumonia/

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Table 1. Viruses identified in 297 respiratory illness outbreaks in long-term care facilities, Ontario, Canada, July 1–December 31, 2009*

Virus	Outbreaks, no. (%)
Enterovirus/rhinovirus	174 (59.0)
Influenza A	22 (7.0)
Parainfluenza 1	18 (6)
Parainfluenza 2	3 (1.0)
Parainfluenza 3	3 (1.0)
Parainfluenza 4	2 (0.7)
Metapneumovirus	2 (0.7)
Influenza B	1 (0.3)
Respiratory syncytial virus A	1 (0.3)
Respiratory syncytial virus B	1 (0.3)
Adenovirus	0
No specimens received	28 (9.0)
Negative	63 (21.0)

*In 187 outbreaks, 1 virus was detected; in 17 outbreaks, 2 viruses were detected; and in 3 outbreaks, 3 viruses were detected.

respiratory infection. Of the 7 patients who died, 5 (71%) had osteoarthritis, 4 (57%) had cardiovascular conditions, 4 (57%) had dementia, 2 (29%) had diabetes, and 1 (14%) had cancer. The only postmortem lung tissue specimen collected was positive for HRV-C (outbreak D).

Nucleotide sequences obtained from isolates from outbreaks A, B, C, and D showed homology to HRV-A 31 (92%), HRV-A 33 (93%), HRV-A 82 (91%), and HRV-C N7 (90%), respectively. We performed multiple sequence alignments of the 410 bp of the 5′ untranslated region, VP4/VP2, and VP1 and compared them with 66 published representative HRV sequences. We could not obtain a VP1 sequence from strains isolated during outbreak D. Phylogenic trees were constructed, and the VP4/VP2 region tree showed better discriminatory power than did that of the 5′ untranslated region (Figure). VP4/VP2 sequence identity was >98% within each outbreak. Sequences were deposited in GenBank under accession nos. GU477323–GU477344.

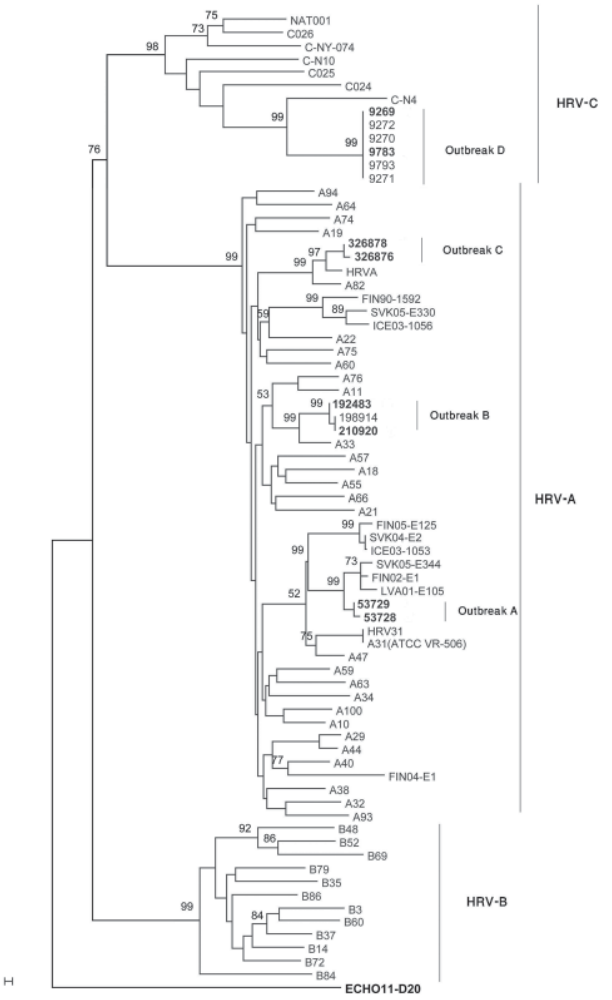

Figure. Neighbor-joining phylogenetic tree of human rhinoviruses (HRV) isolated from 4 respiratory disease outbreaks with associated deaths in long-term care facilities, Ontario, Canada. Tree was constructed by using a 549-bp nt region encoding viral capsid protein (VP) 4/VP2, along with strains representative of HRV species A, B, and C. Echo 11 is the outgroup. Bootstrap analysis used 1,000 pseudoreplicate datasets. Scale bar represents 0.1% of nucleotide changes between close relatives. **Boldface** indicates sequences deposited in GenBank.

Conclusions

We cautiously assume that HRV was the causative organism for 174 (59%) of the 297 respiratory outbreaks in long-term care facilities in Ontario during the surveillance period. Multiplex molecular methods were crucial for rapid identification of the pathogens involved in these outbreaks. We were able to provide results in a timely fashion for every outbreak. However, the cost and expertise associated with such technology might be beyond the reach of some clinical laboratories. Because of the limitations of the sur-

Table 2. Epidemiologic data for rhinovirus outbreaks associated with deaths in long-term care facilities, Ontario, Canada, July 1–December 31, 2009

Outbreak	No. sick residents/total no. residents (%)	No. deaths	No. sick staff members/total no. staff members (%)	Outbreak duration, d	HRV species and strain*
A	28/59 (47)	1	16/80 (20)	38	HRV-A 31
B	32/60 (53)	7	21/100 (21)	43	HRV-A 33
C	19/158 (12)	3	1/200 (0.5)	20	HRV-A 82
D	23/115 (20)	2	3/134 (2)	12	HRV-C N7

*HRV, human rhinovirus.

veillance program, we were unable to assess whether such testing is cost-effective in terms of patient care.

Of the 4 outbreaks with associated deaths, 3 were attributed to HRV-A and 1 to HRV-C. The link between respiratory disease severity and HRV-C speciation is debatable (*12*). In a study from Hong Kong, 8 (62%) of 13 adults with HRV-C infection had pneumonia compared with 6 (27%) of 22 adults with HRV-A infection (*13*). However, in the cases we studied, most deaths were associated with HRV-A; a recent study found that HRV-C disease had the same indistinct clinical presentation as did other HRV diseases (*14*).

Viruses isolated from nasopharyngeal swabs by sensitive NAT may represent asymptomatic colonization or nonliving organisms. Although postmortem specimens were available for analysis from only 1 outbreak-related case, we identified HRV in the postmortem lung specimen. Because we do not know whether HRV was present in the lower respiratory tract of the remaining patients who died, a causal association between HRV and severe disease must be made cautiously. We used the 2 NAT assays interchangeably because their reported specificity is >96% for all targets (*15*). Sensitivity for each assay differs according to target; compared with the Luminex assay, Seeplex RV is more sensitive for parainfluenza, RSV, coronavirus, and adenovirus but less sensitive for HRV (*15*). However, despite limitations for epidemiologic data collection, no pathogens other than HRV could explain these outbreaks and associated deaths. Our testing panel did not include human bocavirus or influenza C virus, which could be involved in the remaining 21% of outbreaks that had no identified cause and could even represent confounding factors in the causal relationship of a supposed pathogen.

In conclusion, using data from a routine surveillance network, we found high prevalence of HRV during a period that encompassed the first and second waves of pandemic (H1N1) 2009. These findings are in accordance with the increasing knowledge that HRV outbreaks cause severe and fatal disease.

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Tuberculosis Acquired Outside of Households, Rural Vietnam

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Using population-based data from rural Vietnam, we assessed tuberculosis (TB) transmission within and outside of households. Eighty-three percent of persons with recent household TB were infected by different strains of *Mycobacterium tuberculosis* than were their household members. This result argues against the effectiveness of active TB case finding among household members.

Because of airborne transmission of *Mycobacterium tuberculosis*, persons who share a household with persons who have tuberculosis (TB) are at high risk for infection (*1*). In urban settings in South Africa, studies using DNA fingerprinting that found high TB transmission and HIV prevalence up to 5% suggested that more TB transmission occurs outside households than previously assumed (*2*,*3*). Few data exist for other settings that have a high incidence of TB, particularly rural areas in Asia where HIV prevalence is low.

In Vietnam, TB incidence is high; 70% of the population live in rural areas where the average HIV prevalence in adults is <0.5% (*4*). A recent survey showed TB prevalence to be higher than assumed, which suggests that case finding is inadequate (*5*). Improving TB case finding is thus a priority for TB control in Vietnam. To assess the value of active case finding among household contacts of patients with infectious TB, we studied within- and outside-household TB transmission, using data collected in a population-based study in rural southern Vietnam. The characteristics of the study site have been described elsewhere (*6*).

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The Study

We prospectively collected data on all patients who had TB sputum smear–positive samples and for whom TB had been diagnosed during January 1, 2003–December 31, 2006, by the National TB Program by microscopic examination of ≥1 Ziehl-Neelsen–stained sputum smears (*7*). *M. tuberculosis* isolates were typed by spoligotyping, 15-loci variable number of tandem repeats (VNTR) typing, and partly by IS*6110*-based restriction fragment-length polymorphism (RFLP) typing (*8*). We collected sociodemographic data through structured interviews with individual patients about their households (defined as all persons who share the same floor and the same food).

Index case-patients were defined as all persons for whom TB had been diagnosed through December 31, 2004. From our database, we identified as household case-patients their household members for whom TB was diagnosed within 24 months after enrollment of the index case-patient. We compared the genotypes and DNA fingerprints of strains isolated from household case-patients with strains isolated from index case-patients in the same household. TB was diagnosed passively, i.e., at the time persons sought care at the study clinics because of symptoms.

Isolated strains of *M. tuberculosis* were defined as identical if spoligotype and RFLP and/or VNTR patterns, as applicable, were the same (differing by ≤1 locus) in the household and index case-patients. Mixed infections were defined as RFLP types with discordant spoligotypes or VNTR patterns with multiple alleles on >1 locus in 1 patient isolate. Genotypes were defined by spoligotyping as described by Brudey et al.; the Beijing genotype was defined as any isolate without direct repeat spacers 1–34 and ≥3 of the spacers 35–43 (*9*,*10*).

Data were entered into Epi Info version 6.04 (Centers for Disease Control and Prevention, Atlanta, GA, USA). Analyses were performed in Stata version 8 (StataCorp, College Station, TX, USA). Patients with negative cultures or cultures that grew nontuberculous mycobacteria were excluded.

Through December 31, 2004, a total of 1,589 patients were registered. We excluded 36 patients because of mismatching and 20 because of laboratory/technical errors; therefore, sputum specimens of 1,533 patients were cultured. After excluding 57 negative cultures and 34 cultures that grew nontuberculous mycobacteria, we included 1,442 (90.8%) index case-patients in the analyses. These patients had 4,141 household contacts ≥15 years of age. During 24 months of follow-up, ≥1 household case-patients were identified for each of 12 (0.8%) index case-patients, including 1 with 2 household case-patients. Household case-patients were not significantly associated with sex, age, family size, *M. tuberculosis* genotype, or smear grade of the index case-patient (data not shown).

None of the 12 index and 13 household case-patients had mixed infections.

Of the 13 household case-patients, 1 (a 72-year-old man) had recurrent infection; 3 had infections were caused by Beijing genotype (23%). For 2 (17%) household case-patients (95% confidence interval [CI] 1.9%–45.4%), the infecting strain was similar to that from the index case-patient. The interval between diagnoses for index and household case-patients was (mean ± SD) 13.0 ± 2.8 weeks for identical strains, and 47.1 ± 33.1 weeks for different strains (p = 0.114).

Conclusions

Of TB case-patients who had had exposure to a TB patient in their household during the preceding 2 years, 17% harbored the same strain as the index case-patient. Even if we classified as similar 1 case pair with only 2 loci differences according to VNTR (no RFLP type available: patient 4 in the Table), i.e., if we assumed that this difference was caused by evolution of the VNTR pattern or a processing error, the proportion of infections acquired outside the household still would be 77% (10/13). Although we cannot exclude the possibility of transmission from another person in the same household for whom TB had not been diagnosed by the TB Program and included in our database, this finding suggests that in our study population, most TB cases resulted from transmission outside the household. These results differ from observations in low-incidence settings but are similar to those in high-incidence settings in South Africa, Gambia, and Malawi (*11*–*14*), even though the follow-up periods of these studies differed. The high proportion of cases resulting from transmission outside the household in those studies and ours may be explained by high exposure to different *M. tuberculosis* strains circulating in the community. Having common factors that determine the risk for breakdown of TB infection to disease also may play a role but is less likely in our study because HIV prevalence is lower in rural Vietnam than in South Africa. Alternatively, the large proportion of nonmatching genotypes within households could reflect specimen mislabeling. Although we excluded specimens that had been mislabeled at collection, additional mislabeling may have occurred in the laboratory. However, because mislabeling is expected to occur at random, for most of the nonmatches to be caused by errors, nearly half of all specimens must have been mislabeled, which seems unlikely.

We identified household TB cases for ≈1% of the index cases, which is less than the 6%–7% reported in studies of active case finding (*1*,*14*). This finding could reflect studies that included household case-patients of all ages with all forms of TB, whereas we included only persons ≥15 years of age who had smear-positive TB. In addition, we relied on self-reporting rather than on active TB screening. Although self-reporting may have limited the number of household cases we identified, it was unlikely to have affected the proportion resulting from household transmission. An exception should probably be made for young children, who because of less social mixing, may have higher probability of being infected within than outside the household. The shorter interval between diagnoses of index and household case-patients with identical strains, as well as the observation that the average interval for case-patients with different strains was approximately half the 2-year study period (i.e., consistent with random occurrence over time) supports our interpretation that the strains that differed between household and index cases were from another source.

Table. Characteristics of case-patients with exposure to tuberculosis within household, rural Vietnam, 2003–2006*

Patient no.	Age, y/sex	Previous treatment	Relationship to index case-patient	Genotype		Comparison of strain type with that of index case-patient			
				Index case-patient	Household case-patient	Spoligotype (no. different spacers)†	RFLP	VNTR‡	Final classification
1	40/M	No	Brother	Beijing	EAI2-Manila	Different (26)	Different	12	Different
2	55/M	No	Brother	NR	Beijing	Different (27)	Different	10	Different
3	49/M	No	Brother	EAI4-VNM	EAI4-VNM	Same (0)	Different	4	Different
4	51/F	No	Spouse	EAI4-VNM	EAI4-VNM	Same (0)	ND	2	Different
5	48/M	No	Spouse	Zero	Zero	Same (0)	Same	0	Same
6	36/M	No	Brother	Beijing	Beijing	Same (0)	ND	1	Same
7	41/M	No	Grandson	EAI5	EAI5	Same (1)	ND	5	Different
8	41/M	No	Brother	U	EAI4-VNM	Different (17)	ND	2	Different
9	31/M	No	Brother	EAI4-VNM	NR	Different (4)	ND	4	Different
10	29/M	No	Brother	EAI5	NR	Different (5)	ND	7	Different
11	72/M	Yes	Grandson	EAI4-VNM	Beijing	Different (27)	Different	13	Different
12	37/M	No	Son	Beijing	EAI5	Different (29)	ND	7	Different
13	38/F	No	Spouse	EAI4-VNM	EAI2-NTB	Different (16)	Different	3	Different

*RFLP, restriction fragment-length polymorphism; VNTR, variable number of tandem repeats; EAI4-VNM: East African–Indian family, Vietnam genotype; NR, not represented in spoligotype database (*10*); ND, RFLP typing not done.
†Difference in the number of direct repeat spacers between the spoligotypes of the index case-patient and that of the household case-patients. Genotypes based on spoligotyping in accordance with classification by Brudey et al. (*10*).
‡No. loci with different alleles.

In rural Vietnam, where HIV prevalence is low, most persons with secondary TB are infected by a source case-patient outside of their households. This finding argues against active TB case finding among household members as an effective method for improving case finding.

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Analysis of Avian Hepatitis E Virus from Chickens, China

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Avian hepatitis E virus (HEV) has been identified in chickens; however, only 4 complete or near-complete genomic sequences have been reported. We found that the near-complete genomic sequence of avian HEV in chickens from China shared the highest identity (98.3%) with avian HEV from Europe and belonged to avian HEV genotype 3.

Hepatitis E virus (HEV) is a nonenveloped, positive-sense, single-stranded RNA virus. It has 3 open reading frames (ORFs) and a genome size of 7.2 kb (*1*). So far, HEV strains are classified into 4 major genotypes, and genotypes 3 and 4 are probably zoonotic.

Avian HEVs have been identified from chickens with big liver syndrome and hepatitis–splenomegaly syndrome. Each syndrome mainly causes increased deaths, reduced egg production, and enlarged liver and spleen (*2*); hepatitis–splenomegaly syndrome also causes accumulation of bloody fluid in the abdomen and vasculitis and amyloidosis in the liver (*3*,*4*). Molecular epidemiologic investigations have shown that avian HEV infection in chickens is endemic to the United States and Spain (*5*,*6*). Because propagating avian HEV in cell culture or embryonated eggs is difficult (*2*,*3*), avian HEV is primarily detected by reverse transcription–PCR (RT-PCR). However, only 4 complete or near-complete genomic sequences have been reported to GenBank (*7*–*9*). We identified and analyzed the near-complete genomic sequence of avian HEV in a chicken flock from the People's Republic of China.

The Study

In May 2009, hepatitis–splenomegaly syndrome affected a flock of 37-week-old broiler breeder hens in Shandong, China. This flock had a history of decreased egg production. Affected chickens had regressive ovaries, extensive necrosis and hemorrhage of the liver, and enlarged liver and spleen. Antibodies against avian HEV ORF2 were detected in 80 of 94 serum samples from the same chicken flock, according to ELISA (*5*,*10*) with the truncated ORF2 protein used by Guo et al (*10*) and chicken serum diluted 1:100 in 0.5% Tween-20 phosphate-buffered saline containing 2.5% nonfat dry milk and 10% *Escherichia coli* lysate. On the basis of previous results, we used a cutoff optical density of 0.43 (*11*). Using a published method (*12*), we detected an avian HEV ORF2 RNA gene with 242 bp in 7 of 10 fecal and 5 of 8 bile samples.

From the bile samples that were positive for the avian HEV ORF2 gene, we used nested RT-PCR with 5 overlapping fragments to amplify the near-complete genomic sequence of avian HEV. Primers were designed on the basis of the other 4 avian HEV near-complete sequences in GenBank (Table 1). The RT-PCR conditions and reaction mixture were designed according to the SuperScript II One-Step RT-PCR System instructions (Invitrogen, Carlsbad, CA, USA). To identify the extreme 3′ genomic sequence, we used a modified RACE (3′ rapid amplification of cDNA ends) technique. The sense primer F5 (Table 1) was chosen from the ORF2 region, and the antisense primers included a commercially available anchored adaptor primer and an amplification primer (Invitrogen). Using inner PCR prim-

Table 1. Primers used for PCR amplification of the China avian hepatitis E virus genome

Primer*	Sequence, 5′ → 3′†	Position, nt‡
F1-1	CCATGCCAGGGTAAGAATG	9–27
R1-1	AAAACAGCAAGGACCTCC	1872–1889
F1-2	CCAGGGTAAGAATGGACG	14–31
R1-2	TAATCCAGGTGGCGAGC	1308–1324
F2-1	CACTGTGGGTAACATTGTGGC	1071–1091
R2-1	GTTCGACTGCTTAGCCACCTG	2935–2955
F2-2	AGGCGGAACACGCACAGCA	1214–1232
R2-2	TCGTCCACAATGACCCTGC	2624–2642
F3-1	GGCTGTGTGGCATGTTCCA	1985–2003
R3-1	GGTAAAGAGCCACCATCCAAT	4010–4030
F3-2	CCGTGATGGTGACTTGTTGGTTGT	2262–2285
R3-2	GGCACATCTCCGCATACTC	3586–3604
F4-1	CCCTTCAACATTGGAGTATGC	3573–3593
R4-1	ATCTGGTACCGTGCGAGT	4899–4916
F4-2	ACATTGGAGTATGCGGAGATG	3580–3600
R4-2	TTGAGCGCTCCACTGGGCT	4820–4838
F5	GACAATTCAGCCCAGTGGA G	4809–4828
AUAP§	GACTCGAGTCGACATCG A	Nonviral
AP§	GACTCGAGTCGACATCGA $(T)_{17}$	Nonviral

*Primers F1-1 to R1-2, F2-1 to R2-2, F3-1 to R3-2, and F4-1 to R4-2 were used to amplify the first, second, third, and fourth fragment of the near-complete avian hepatitis E virus (HEV) genome. Primers F5, amplification primer (AUAP), and adapter primer (AP) were used to amplify the extreme 3′ genomic sequence. Primers R1-1, R2-1, R3-1, R4-1, and AP are also reverse transcription primers.
†Sequences of primers were designed according to the sequences of 4 other known avian HEV strains.
‡Positions of primers located in the complete genome are shown according to the Europe avian HEV isolate.
§Commercial primer (Invitrogen, Carlsbad, CA, USA) of nonviral origin.

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ers, we sequenced the PCR products of 5 fragments in both directions (Table 1); the sequence data were collected by an ABI3730 Genetic Analyzer (JinSiTe Biotech Co., Nanjing, China).

We assembled the near-complete genome of avian HEV, which was 6,660 nt long including the 3′ poly A tail, by using 5 overlapping fragments sequences and Lasergene 7.0 EditSeq computer programs (DNAStar, Madison, WI, USA) and designated it China avian HEV (CaHEV). CaHEV contained a complete ORF1 gene encoding a nonstructural protein of 1,522 aa, an ORF2 gene encoding a capsid protein of 606 aa, an ORF3 gene encoding a cytoskeleton-associated phosphoprotein of 87 aa, and a 3′ noncoding region of 121 nt. The sequences of CaHEV were deposited into GenBank under accession no. GU954430.

The near-complete genomic and different region sequence analyses performed by using ClustalW (www.clustal.org) and Lasergene 7.0 MegAlign software indicated that CaHEV shared the highest identity (98.3%) with European avian HEV isolate (EaHEV) and 82.0%–82.6% with 3 other avian HEV isolates (Table 2). Moreover, compared with the different regions of 4 other avian HEV strains, the ORF1 gene of CaHEV shared 80.7%–98.3% nt and 92.7%–98.8% aa sequence identities, the ORF2 gene shared 84.1%–98.5% nt and 98.3%–99.7% aa sequence identities, the ORF3 gene shared 93.9%–98.9% nt and 88.6%– 97.7% aa identities, and the 3′ noncoding region shared 78.9%–97.6% nt identities (Table 2).

ORF1 of CaHEV contained most mutations compared with prototype avian HEV (prototype aHEV); 5, 16, and 29 nonsilent mutations occurred in the methyltransferase, helicase, and RNA-dependent RNA polymerase (RdRp) functional domains, respectively (data not shown). However, only 2 mutations occurred in motif VII of RdRp domain (Figure 1, panel A), which contains 8 motifs responsible for virus replication (*13*). The 2 mutations in motif VII of the CaHEV RdRp domain are L(1432)M and I(1434)V. Australian avian HEV isolate (AaHEV) also has the mutation in the latter position and was a transition from I(1433) to T (Figure 1, panel A). This position is well conserved among mammalian HEV isolates by the presence of V, which is the same as CaHEV (Figure 1, panel A).

Table 2. Percentage identities among avian HEV strains in nucleotide/amino acid sequences*

Sequence and strain	"Avirulent aHEV"	Prototype aHEV	AaHEV	EaHEV	CaHEV
Near-complete genome sequence					
"Avirulent aHEV"		90.1	82.7	82.9	82.6
Prototype aHEV			82.5	82.2	82.0
AaHEV				82.5	82.4
EaHEV					98.3
CaHEV					
ORF1					
"Avirulent aHEV"		89.6	82.1	81.8	81.7
Prototype aHEV	**97.4**		81.6	81.0	80.7
AaHEV	**93.9**	**93.7**		81.7	81.6
EaHEV	**92.9**	**93.0**	**93.1**		98.3
CaHEV	**92.7**	**92.8**	**93.0**	**98.8**	
ORF2					
"Avirulent aHEV"		90.7	84.5	84.0	84.1
Prototype aHEV	**99.0**		84.3	84.4	84.5
AaHEV	**98.5**	**98.8**		84.1	84.4
EaHEV	**98.2**	**98.7**	**98.8**		98.5
CaHEV	**98.3**	**99.0**	**98.8**	**99.7**	
ORF3					
"Avirulent aHEV"		97.0	95.4	93.6	93.9
Prototype aHEV	**99.0**		95.4	93.6	93.9
AaHEV	**94.3**	**96.6**		93.5	93.9
EaHEV	**88.6**	**88.6**	**92.0**		98.9
CaHEV	**88.6**	**88.6**	**92.0**	**97.7**	
3′ NCR					
"Avirulent aHEV"		92.8	82.8	88.6	89.4
Prototype aHEV			83.6	85.5	86.3
AaHEV				80.5	78.9
EaHEV					97.6
CaHEV					

*HEV, hepatitis E virus; ORF, open reading frame; NCR, noncoding region. **Boldface** indicates percentage identities of amino acid sequences. "Avirulent aHEV" and prototype aHEV are avian HEV isolates from the United States, GenBank accession nos. EF206691 and AM535004, respectively. AaHEV, EaHEV, and CaHEV are avian HEV isolates from Australia, Europe, and China, GenBank accession nos. AM943647, AM943646, and GU954430, respectively.

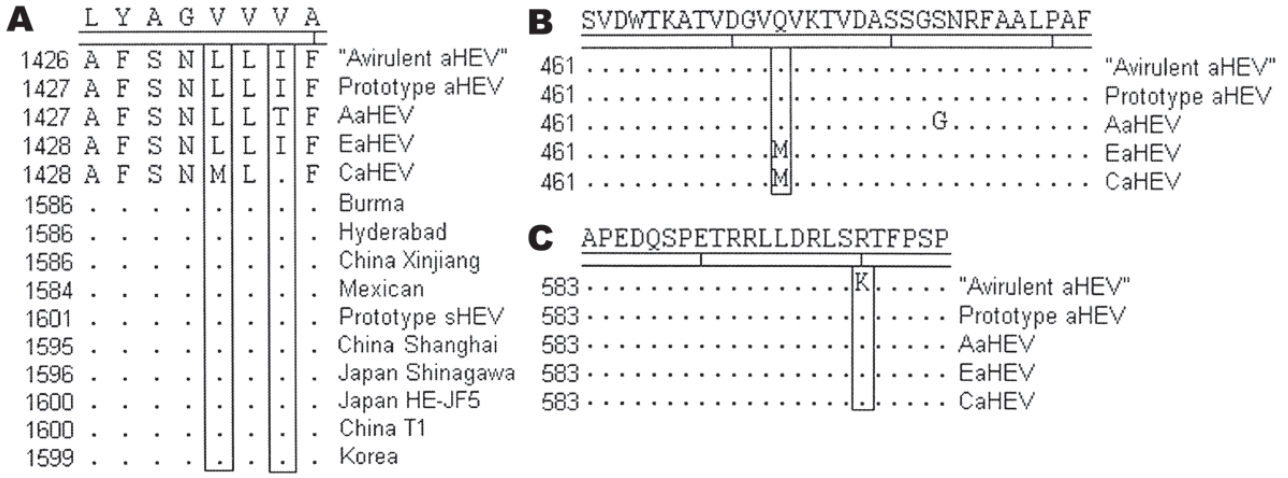

Figure 1. Amino acid sequence comparison of motif VII in the open reading frame (ORF) 1 RNA-dependent RNA polymerase (RdRp) region of avian, human, and swine hepatitis E viruses (HEVs) (A), antigenic domain II (B), and antigenic domain IV (C) in the ORF2 region of avian HEV (aHEV). Residues that are conserved among aHEV isolates are shown as the consensus above the sequences; residues that are conserved in the HEV strains are not shown. GenBank accession numbers of human and swine HEV (sHEV) strains are M73218 (Burma), AF076239 (Hyderabad), D11092 (China Xingjiang), M74506 (Mexico), AF082843 (prototype sHEV), FJ527832 (China Shanghai), AB291955 (Japan Shinagawa), AJ272108 (China T1), AB480825 (Japan HE-JF5), and FJ763142 (Korea). GenBank accession numbers of avian HEV strains are EF206691 ("avirulent aHEV" from the United States), AM535004 (prototype aHEV from the United States), AM943647 (aHEV from Australia [AaHEV]), AM943646 (aHEV from Europe [EaHEV]), and GU954430 (aHEV from China [CaHEV]). Boxes indicate mutations of CaHEV compared with different HEV strains.

In the ORF2 region, 6 nonsilent mutations (C4R, R5G, G27S, T42A, T303V, and Q473M) were determined for CaHEV and compared with prototype aHEV. One mutation of Q(473)M, in the antigenic domain II, was seen in EaHEV and in CaHEV (Figure 1, panel B). Because this domain is unique to avian HEV, as predicted by Haqshenas et al. (*14*) and Guo et al. (*10*), this point mutation may change the antigenicity of the epitopes in domain II of the capsid protein. In antigenic domain IV, a mutation of R(600)K occurred in the "avirulent aHEV" compared with other 4 avian HEV strains, including CaHEV, from the sick chickens (Figure 1, panel C). This mutation may affect the virulence of avian HEV as speculated by Billam et al. (*8*) and Billic et al. (*9*). The 3 putative N-linked glycosylation sites (^{255}NLS [1], ^{510}NST [2], and ^{522}NGS [3]) are shared between prototype aHEV and CaHEV (data not shown). However, the second site is ^{510}NNT in "avirulent aHEV" and AaHEV strains and is eliminated in the EaHEV strain (*9*). In human and swine HEV strains, these sites are ^{137}NLS (1), ^{310}NLT (2), and ^{562}NLS (3). Recently, the potential N-linked glycosylation in ORF2 was shown to prevent formation of infectious particles, but its role in other functions of HEV, e.g., virus virulence and cell tropism, remain to be elucidated (*15*). In the ORF3 gene, including only 83 aa, 10 nonsilent mutations were found compared with the prototype aHEV, and 9 mutations were the same as EaHEV (data not shown).

Phylogenetic trees of the near full-length sequence of avian and mammalian HEV strains were constructed by using the neighbor-joining distance method and Lasergene

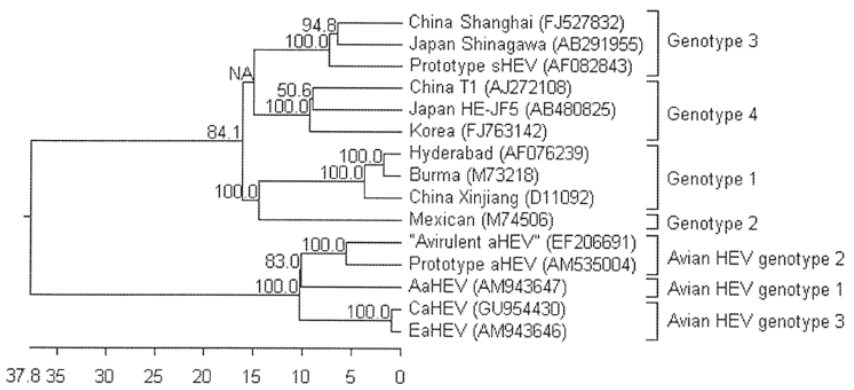

Figure 2. Phylogenetic trees based on the near-complete genomic sequences of avian hepatitis E virus (HEV) and 10 human and swine HEV isolates. GenBank accession numbers follow the name of HEV strains. The trees were constructed by the neighbor-joining method with 1,000 bootstrap replicates using Lasergene 7.0 (DNAStar, Madison, WI, USA). The length of each pair of branches represents the distance between sequence pairs; the units at the bottom of the tree indicate the number of substitution events.

7.0 software. A bootstrap test of 1,000 replicates was used to evaluate the reliability of the groups. Avian HEV was segregated into a distinct branch separate from mammalian HEV; according to the genotype separation corresponding to their geographic origin suggested by Bilic et al. (*9*), Ca-HEV belongs to avian HEV genotype 3 (Figure 2).

Conclusions

Avian HEV infection of a chicken flock in Shandong, China, was identified by detection of avian HEV ORF2 antibodies and viral RNA. A near-complete avian HEV genome from the flock was determined, and sequence analysis indicated that this avian HEV strain displayed the highest identity (98.3%) with EaHEV and belonged to avian HEV genotype 3.

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Extensively Drug-Resistant Tuberculosis, Pakistan

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Frequency of extensively drug-resistant tuberculosis in Pakistan increased from 1.5% in 2006 to 4.5% in 2009 (p<0.01). To understand the epidemiology, we genotyped selected strains by using spoligotyping, mycobacterial interspersed repetitive units–variable number of tandem repeats, and IS*6110* restriction fragment length polymorphism analysis.

Emergence and spread of multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis (TB) are facilitated by inadequate detection and treatment (*1*). TB detection and treatment are more difficult in countries, like Pakistan, that are facing complex emergencies, including humanitarian crises and conflicts (*2*). Published data report an increasing prevalence of MDR TB and emergence of XDR TB strains in Pakistan (*3*).

This study documents an increasing trend of XDR resistance within MDR TB isolates in Pakistan. To obtain better insight into the epidemiology of these strains, we genotyped selected XDR TB strains by using spoligotyping, mycobacterial interspersed repetitive units–variable number of tandem repeats (MIRU-VNTR), and IS*6110* restriction fragment length polymorphism (RFLP) analysis. This study was approved by the Ethics Review Committee of the Aga Khan University and conducted at the Aga Khan University Hospital, Karachi, Pakistan.

The Study

The Aga Khan University Hospital laboratory receives specimens from >180 collection units located in major cities and towns in Pakistan. Specimens for TB cultures are requested by physicians as required and received through passive specimen collection. Past treatment history is usually not available and thus could not be included in this study. *Mycobacterium tuberculosis* was isolated from the specimens by using Lowenstein-Jensen and MGIT (Becton Dickinson, Franklin Lakes, NJ, USA) media. It was then identified by using the BACTEC NAP TB differentiation test (Becton Dickinson), growth in para-nitrobenzoic acid–containing media, nitrate reduction, and niacin accumulation (*4*).

We performed susceptibility testing by using an agar proportion method on enriched Middlebrook 7H10 medium (BBL Microbiology Systems, Cockeysville, MD, USA) at the following concentrations: rifampicin 1 μg/mL, isoniazid 0.2 μg/mL, streptomycin 2 μg/mL and 10 μg/mL, and ethambutol 5 μg/mL. Pyrazinamide sensitivity was determined by using BACTEC 7H12 medium, pH 6.0, at 100 μg/mL (BACTEC PZA test medium, Becton Dickinson). MDR TB strains were further tested with capreomycin 10 μg/mL, ciprofloxacin 2 μg/mL, ethionamide 5 μg/mL, amikacin 5μg/mL, and kanamycin 6 μg/mL. Reference strain *M. tuberculosis* H37Rv was used as a control with each susceptibility testing batch (*5*). *M. tuberculosis* drug-susceptibility testing is validated by the Supranational Laboratory Network of the World Health Organization (www.who.int/drugresistance/tb/en/).

MDR TB was defined as resistance to at least isoniazid and rifampicin. XDR TB was defined as resistance to quinolones and to 1 of the injectable second-line drugs in addition to MDR. Randomly selected isolates were confirmed as XDR TB by using the GenoType MTBDRsl assay (HAIN Lifesciences, Nehren, Germany). XDR TB isolates were stored at –80°C. Stored XDR TB strains were revived for genotyping; 57 isolates that grew were used for spoligotyping and MIRU-VNTR typing (3 isolates from 2006, 5 from 2007, 19 from 2008, and 30 from 2009).

DNA was extracted by using the cetyltrimethylammonium bromide method (*6*). Spoligotyping was performed by using a commercially available kit provided by Isogen Life Science (De Meern, the Netherlands). Spoligotyping based on the 43 spacers of the DR region of *M. tuberculosis* complex was performed by using primers DRa 5′-GGTTTTGGGTCTGACGAC-3′ and DRb 5′-CCGGAGAGGGGACGGAAAC-3′ as described (*7*). Negative and positive controls, including template-free PCR–amplified reaction mixture and *M. tuberculosis* H37Rv DNA, were used with each spoligotype blot.

Data extracted from the computerized information system of the hospital were transferred to the statistical software SPSS version 14.0 (SPSS, Chicago, IL, USA). Frequencies with percentages were computed for each year. We used χ^2 for trend analysis to assess resistance trends over the study period. A p value <5% was considered significant.

The spoligotyping results were entered in the BioNumerics Software version 3.5 (Applied Maths Program, Biosystematica, Ceredigion, UK). A dendrogram was gen-

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Table 1. Frequency of multidrug-resistance and extensively drug-resistance among *Mycobacterium tuberculosis* isolates, Pakistan, 2006–2009*

Year	No. *M. tuberculosis* isolates	MDR isolates, no. (%)	MDR + ciprofloxacin-resistant isolates, no. (% of total MDR)	Total no. XDR isolates	XDR as % of total MDR
2006	1,917	728 (38)	131 (18)	11	1.5
2007	2,019	782 (39)	168 (21.5)	17	2.2
2008	2,584	991 (38)	356 (36)	32	3.2
2009	3,003	1,181 (39)	512 (43)	53†	4.5

*MDR, multidrug resistant; XDR, extensively drug resistant.
†By trend analysis (p value for trend <0.01).

erated by using the unweighted pair–group method with arithmetic averages calculation. The spoligotypes were compared with the most prevalent *M. tuberculosis* subfamilies as identified by the World Spoligotyping Database SpolDB4.0 of Pasteur Institute of Guadeloupe (www.pasteur-guadeloupe.fr/tb/bd_myco.html) (*8*), which includes >40,000 isolates split into 1,030 shared types and >3,530 orphan profiles.

The isolates were genotyped by PCR amplification of 15 MIRU-VNTR loci by using standard methods as described (*9*). Sizes of the PCR fragments and assignment of the various VNTR alleles were also determined by using standard protocol for gel electrophoresis (www.genoscreen.com). All reactions were performed in duplicate by using standard positive and negative controls supplied in the MIRU-VNTR validation kit.

IS*6110* RFLP of *M. tuberculosis* strains were performed by standardized methods (*10*). Briefly, XDR TB strains were recovered on LJ medium. DNA was extracted from the strains by standard methods (*6*,*10*). *Pvu*II-digested DNA was subjected to agarose gel electrophoresis and Southern blotting. DNA fingerprinting was performed by hybridization with the IS*6110* by using the enhanced chemiluminescence method (Amersham Biosciences, Piscataway, NJ, USA).

During 2006–2009, a total of 9,523 *M. tuberculosis* strains were isolated, including 3,682 (38.7%) MDR TB strains. Although the MDR TB rate remained constant (Table 1), the XDR TB rate (expressed as a percentage of MDR TB isolated in a year) showed a significant increase. The XDR TB strains were from specimens received from 23 cities; the largest numbers were from Karachi (22), Hyderabad (13), and Peshawar (12). Mean ± SD age of patients with XDR TB was 37 ± 14 (range 16–80) years; 57.15% were men and 42.85% women.

Diversity of XDR TB strains among the 57 isolates genotyped is shown by spoligotyping (Table 2) and MIRU-VNTR analysis. Spoligotyping data points to the predominance of strains belonging to the Central Asian Strain (CAS) 1 family (n = 24 [42.1%]). Overall CAS strains included CAS1_KILI ST21 (n = 2); CAS1_DEHLI ST25 (n = 1); CAS1, ST26 (n = 24); CAS1_DEHLI ST428 (n = 1); CAS1_DEHLI ST794 (n = 1); CAS1_DEHLI ST1198 (n = 3); and CAS1_DEHLI ST1401 (n = 1). The second largest family comprised the Beijing (ST1) genogroup (n = 5 [8.8%]). Four (7%) strains of T family (ST53 and ST804) and 2 strains of U family (ST346) were also identified. Additional XDR TB strains included 1 (2%) strain each of EAI (ST11) and X (ST200). Furthermore, 1 new unmatched cluster and 9 unmatched orphan types were also recognized. MIRU-VNTR patterns of all XDR TB strains were different, indicating a lack of clustering among the strains studied. Randomly selected strains present in CAS1 (ST26, n = 6) and Beijing (ST1, n = 3) clusters were subjected to IS*6110* RFLP typing. RFLP patterns of each of these strains also differed, further confirming a lack of clustering among the XDR TB strains studied.

Conclusions

This study demonstrated a rising XDR-TB trend in Pakistan and raises concerns despite the fact that Pakistan's 2009 XDR rate (4.5%) of MDR TB is below the global av-

Table 2. Spoligotype distribution of 57 extensively drug-resistant strains of *Mycobacterium tuberculosis*, Pakistan, 2006–2009*

Genogroup and shared type	No. (%) isolates	Overall %
CAS		57.9
ST21	2 (3.5)	
ST25	1 (1.8)	
ST26	24 (42.1)	
ST428	1 (1.8)	
ST794	1 (1.8)	
ST1198	3 (5.3)	
ST1401	1 (1.8)	
Beijing		8.8
ST1	5 (8.8)	
EAI3		1.8
ST11	1 (1.8)	
U		1.8
ST346	2 (3.5)	
T1		7.0
ST53	1 (1.8)	
ST804	3 (5.3)	
X3		1.8
ST200	1 (1.8)	
Unmatched cluster	2 (3.5)	3.5
Orphan types	9 (15.8)	15.8

*ST, spoligotype; CAS, Central Asian strain; EAI, East African Indian strain.

erage (6.6%–23.7%) (*11*). Genotyping data are comparable with those from earlier studies (*12*,*13*), suggesting dominance of CAS1 strains. The fact that Beijing family strains were 9% of XDR TB isolates vs. 3% prevalence in the overall MDR TB reported in this population (*13*) suggests that Beijing strains are associated with drug resistance in Pakistan and adjacent countries (*14*).

Furthermore, the finding that the XDR TB strains in our study were genetically diverse argues against dissemination of 1 particular genogroup responsible for drug resistance and supports the concept that XDR TB in Pakistanis is likely to be a consequence of inadequate treatment of TB. The challenging sociopolitical situation in Pakistan is likely to exacerbate this public health problem. Emergency measures are required to avoid an exponential rise in drug-resistant TB in the country and the region. We recommend that increased XDR TB rates in this area be considered not just of national concern but also be recognized as a regional public health issue requiring introduction of cooperative and support measures aimed at limiting the spread of drug-resistant TB within southern Asia.

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Co-infections with *Plasmodium knowlesi* and Other Malaria Parasites, Myanmar

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To determine the frequency of co-infections with *Plasmodium* species in southern Myanmar, we investigated the prevalence of *P. knowlesi*. More than 20% of patients with malaria had *P. knowlesi* infection, which occurred predominantly as a co-infection with either *P. falciparum* or *P. vivax*.

Plasmodium species are co-endemic to regions of Southeast Asia (*1*,*2*). This finding is believed to be underestimated because of insufficient sensitivity of microscopic detection of parasites. The prevalence of mixed infections with malaria parasites in the border regions between Thailand and Myanmar was recently found to be ≤24% (*3*). Identification of *P. knowlesi* as the fifth human malaria pathogen, which is prevalent in countries in Southeast Asia, has complicated this situation. *P. knowlesi* is a parasite that infects mainly long-tailed macaques (*Macaca fascicularis*) and pig-tailed macaques (*M. nemestrina*) in Southeast Asia (*4*). The parasite has developed the capacity to naturally infect humans, and infections in some persons have been life-threatening (*5*,*6*). Furthermore, infections with *P. knowlesi* in travelers to this region have been increasing (*7*,*8*).

P. knowlesi isolates obtained from humans have been frequently misidentified as *P. falciparum* or *P. malariae* because of the morphologic similarities of these parasites (*2*). Use of PCRs specific for 18S small subunit (SSU) rRNA genes of malaria parasites has identified suspected cases (*2*,*9*). *P. knowlesi* infection in humans in the border area between the People's Republic of China and Myanmar has been reported (*10*), but the prevalence is unknown. We investigated the frequency of co-infections with *P. knowlesi* and other *Plasmodium* spp. in this region.

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The Study

The study was reviewed and approved by the Ethic Committee of the Institute for Parasitic Disease Control of Yunnan Province and local administration authority in Myanmar. One hundred forty-six blood samples were obtained in 2008 from randomly selected patients with uncomplicated malaria in southern Myanmar near Yunnan Province of China, where pig-tailed macaques are also present. Written consent was obtained from each person before blood samples were obtained. A drop (20 μL–50 μL) of fingerprick blood was placed directly on premarked filter paper. Malaria infection was identified by microscopic analysis of Giemsa-stained blood films made from blood spotted on the paper.

DNA templates for a nested PCR were prepared from whole blood spots on filter paper according to a previously reported method (*11*). Genomic DNA of *P. falciparum* was obtained from in vitro–proliferated 3D7 clone. Genomic DNA of *P. vivax* was obtained from a patient from the study region previously identified by using PCR and the primer sets used in this study. As reported (*2*,*12*), DNA (3 μL/sample and 1 ng of each positive control DNA) from each sample was amplified with the *Plasmodium* genus–specific primer pair rPLU1 and rPLU5.

Two microliters of PCR product from each amplification was subjected to a second PCR amplification with species-specific primer pairs rFAL1 and rFAL2 for *P. falciparum*, rMAL1 and rMAL2 for *P. malariae*, rVIV1 and rVIV2 for *P. vivax*, rOVA1 and rOVA2 for *P. ovale*, and Pmk8 and Pmkr9 for *P. knowlesi*. PCR products amplified with nested primers were analyzed by agarose gel electrophoresis. DNA bands were removed from the gel, purified by using the QIAquick Gel Extraction Kit (QIAGEN, Valencia, CA, USA) and ligated to T-cloning vector (Invitrogen, Carlsbad, CA, USA) according to protocols provided by the manufacturers. Plasmid inserts were then sequenced.

Sequence identity was confirmed by random basic local alignment search tool analysis of sequences in GenBank (http://blast.ncbi.nlm.nih.gov/). Novel sequences were deposited in GenBank with accession nos. GU816242–GU816250. Phylogenetic relationships of unique sequences amplified by using nested primers with corresponding reference sequences were constructed by using the neighbor-joining method in MEGA version 4.0 (*13*). All sequences clustered with reference sequences of *P. falciparum*, *P. vivax*, or *P. knowlesi*, which suggested that all sequences were species specific (Figure).

Conclusions

Three parasite species (*P. falciparum*, *P. vivax*, and *P. knowlesi*) were identified in 146 infected persons. Phylo-

[1]These authors contributed equally to this article.

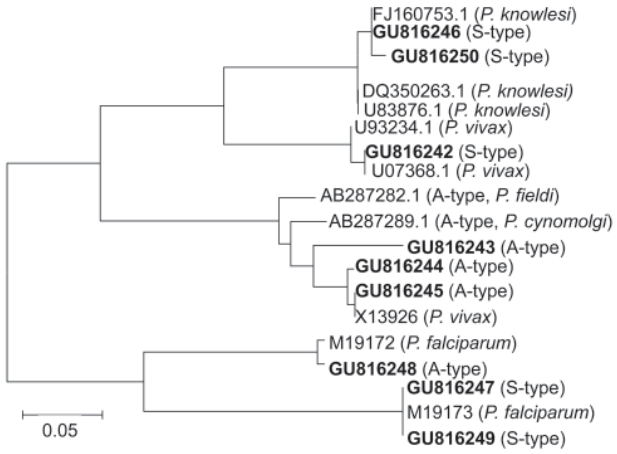

Figure. Phylogenetic analysis of A-type and S-type 18S small subunit (SSU) rRNA gene sequences of *Plasmodium* spp., Myanmar, 2008. Fragments of 18S SSU rRNA gene sequences of samples were analyzed by aligning with published homologous sequences of *P. falciparum*, *P. vivax,* and *P. knowlesi.* A phylogenetic tree was constructed on the basis of similarities by using MEGA version 4.1. (*13*). Novel sequences identified in this study are indicated in **boldface**. Scale bar indicates nucleotide substitutions per site.

genetic analysis showed that amplified products were species specific (Figure). Monoinfection with *P. falciparum*, *P. vivax*, and *P. knowlesi* accounted for 34.9% (51/146), 36.3% (53/146), and 2.7% (4/146), respectively, of the infections. Mixed infections of *P. knowlesi* with *P. falciparum* or *P. vivax* accounted for 6.9% (10/146) of the infections, and mixed infections with *P. knowlesi* and either *P. falciparum* or *P. vivax* accounted for 8.9% (n = 13 in both groups) of the infections. Only 2 samples (1.4%) had mixed infections with *P. falciparum*, *P. vivax*, and *P. knowlesi* (Table). Thus, the prevalence of mixed infections in southern Myanmar was lower than that in northern Myanmar near the border with Thailand (*3*). The prevalence of *P. knowlesi* was 21.9%. In most cases, this parasite showed co-infection with either *P. falciparum* or *P. vivax*, which indicated that *P. knowlesi* may have not fully adapted to the human host or that humans who were infected with other malaria parasites may be more vulnerable to *P. knowlesi* infection.

Table. *Plasmodium* species identified in 146 persons, Myanmar, 2008

Parasite	No. (%) persons
Plasmodium knowlesi	4 (2.7)
P. knowlesi/*P. falciparum*	13 (8.9)
P. knowlesi/*P. vivax*	13(8.9)
P. knowlesi/*P. falciparum*/*P. vivax*	2(1.4)
P. falciparum	51 (34.9)
P. vivax	53 (36.3)
P. falciparum/*P. vivax*	10 (6.9)

Our study also emphasizes the need for improvement of current methods for detecting *P. knowlesi* infection (*14*,*15*). A recent report found that the primer pair Pmk8 and Pmkr9, which is specific for the 18S SSU rRNA gene of *P. knowlesi*, can cross-hybridize with the corresponding sequence of *P. vivax* (*14*). We observed weak amplifications in 16 samples (11%); all were from *P. vivax*–infected blood. All amplicons made by using Pmk8 and Pmkr9 primers were sequenced and compared with the homologous sequences. Because of the high similarity of 18S SSU rRNA gene sequences among these parasites, more specific sequences are needed for establishing a reliable PCR-based method for routine diagnosis of *P. knowlesi* infection (*14*).

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Exposure of Dentists to *Mycobacterium tuberculosis*, Ibadan, Nigeria

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To determine the prevalence of *Mycobacterium tuberculosis* infection among dental patients and to assess dentists' risk for exposure, we conducted a study among dental patients at a large tertiary hospital in Nigeria, a country where tuberculosis is endemic. Ten (13%) of 78 sputum samples obtained were positive for *M. tuberculosis*.

Tuberculosis (TB) is a serious public health concern globally, and almost half of new infections are undetected (*1*). The infection is spread by airborne droplet nuclei that contain *Mycobacterium tuberculosis*, which remain airborne for minutes to hours after expectoration through coughing, sneezing, or talking by persons with pulmonary TB (*2*).

Acquisition of TB in healthcare facilities is a well-recognized hazard for healthcare workers and patients. Dental practitioners may be at increased risk because they work in close proximity to potentially infectious secretions (*3*). The risk is higher in areas like Nigeria, where TB is endemic; control practices are poor; and compliance with guidelines for preventing TB in healthcare facilities, such as those issued by the US Centers for Disease Control and Prevention (*4*), is limited.

We initiated this study to assess the risk for exposure of dentists to *M. tuberculosis* infection and to determine its prevalence among dental patients. Participants were patients in the dental clinic of University College Hospital, Ibadan, Nigeria, the largest hospital in the country.

The Study

This study was conducted in the dental center of University College Hospital, a tertiary hospital located in Ibadan, Oyo State, southwestern Nigeria. Nigeria, which has a population of >140 million, is rated as fourth among TB-endemic nations, with a prevalence of 521 cases per 100,000 population during 2007 (*5*). Ethical clearance for the study was obtained from the Oyo State Ethical Review Board as part of a multifocused study of TB in the state.

The dental center comprises 7 units that provide care to ≈1,800 patients annually. This study was carried out in the Oral Surgery Clinic, one of the units in the center where a member of the study team is a consultant. Patients were recruited during February 2006 through July 2007. A total of 312 consecutive patients who came to the clinic for treatment were invited to participate; 101 gave consent (23/101 patients were excluded because they could not produce a sputum sample). Thus, 78 patients were evaluated.

Each participant completed a structured questionnaire that requested information about age, sex, occupation, bacille Calmette-Guérin (BCG) vaccination, history of chronic cough or TB, HIV status, and contact with a patient who had chronic cough or TB. A single expectorated sputum sample was collected from each participant and placed in a sterile universal container. Collection was done through the support of medical house officers/registrars posted to the unit; a consultant (1 of the study group members) was responsible for overall supervision.

The sputum specimen and culture were processed by using a standard procedure as described by Cadmus et al. (*6*). Using the Ziehl-Neelsen technique, we performed smear microscopy. Isolates were harvested for molecular typing analysis by scraping the growth from a slope into 200 μL of distilled water and heating the product at 80ºC for 1 h.

Molecular identification of isolates was conducted in the Veterinary Laboratories Agency, Addlestone, Surrey, UK, by spoligotyping as described by Kamerbeek et al. (*7*) with minor modifications and repeated at the Center for Infectious Disease Control (RIVM) in the Netherlands. Furthermore, the Hain Genotype *Mycobacterium tuberculosis* complex (MTBC) test (Hain Lifescience, Nehren, Germany) was applied to determine whether an isolate was a member of the MTBC, after which the Hain line probe assay identification kit for subspecies of MTBC was applied (Hain Lifescience).

Seventy-eight dental patients who could give a sputum sample consented to participate in the study. Forty-one (52%) participants were women; median age was 32 years. A total of 20 participants (26%) reported having received BCG vaccine, and 10 reported prior exposure to a person with chronic cough or with TB. None of the participants reported a personal history of chronic cough or TB. Ten (13%) of the 78 participants had culture-confirmed TB, 7 of whom had initial positive acid-fast bacilli (AFB) smears through concentrated sputum samples. The

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Table. Demographic characteristics, BCG vaccination status, and TB exposure history of participants with positive sputum culture results, University College Hospital, Ibadan, Nigeria, 2006–2007*

Participant ID	Age, y/sex	Occupation	BCG vaccination	Exposure to TB patients	Positive AFB smear
1	49/F	Hairdresser	No	No	Yes
2	60/M	Trader	Yes	No	Yes
3	24/F	None	Yes	No	No
4	30/F	Student	No	No	Yes
5	45/M	Businessman	Yes	No	Yes
6	77/M	Retired	Yes	No	No
7	44/M	Clergyman	Yes	No	No
8	40/F	Trader	No	Yes	Yes
9	63/M	Retired	Yes	No	Yes
10	54/F	Civil servant	Yes	No	Yes

*BCG, bacille Calmette-Guérin; TB, tuberculosis; ID, identification; AFB, acid-fast bacilli.

HIV status of 70 respondents (including those with TB) was unavailable, and 1 of the remaining 8 respondents reported being positive for HIV during questionnaire/sample collection. The demographic characteristics, AFB smear results, BCG vaccination history, and known TB exposure of the participants with mycobacterial growth are presented in the Table.

Spoligotyping in the United Kingdom and repeated molecular identification in the Netherlands identified the strains as *M. tuberculosis*. In spoligotyping, different genotypic patterns were visualized (*8*), which limited the possibility of laboratory cross-contamination.

Conclusions

In patients who sought treatment at the dental clinic of a tertiary hospital in Nigeria, we found an unexpectedly high rate of unrecognized infection with pathogenic mycobacteria. Ten (13%) of 78 study participants who provided sputum samples were infected with *M. tuberculosis*. Our findings corroborate other studies of TB cases and show that AFB smears alone would miss some infected patients. *M. tuberculosis* is transmitted with high efficiency to household contacts (*9*), and transmission of mycobacteria, including multidrug-resistant *M. tuberculosis* strains, from dental patients to dental practitioners, probably occurs (*10*). Accordingly, infected participants in this study, especially those who had positive AFB smears, were, in principle, capable of infecting dental practitioners and other patients. Reports from other settings suggest that the threat of TB transmission from clients to dentists is not only theoretical (*11*). This extremely high percentage of undiagnosed AFB positivity is of public health concern because dental clinics in TB-endemic areas are not usually considered a place of high risk for TB transmission; therefore, preventive measures are not routinely implemented.

This study has several limitations. First, patients were recruited from only 1 of the 7 units in the dental center, constricting the pool of patients invited to participate. However, all patients visiting the center were exposed to similar conditions, and the patients who came to the oral surgery clinic had often been referred from other units in the dental center. Second, only 1 sputum sample was collected from each participant. The yield possibly would have been higher if we had collected more samples. Third, dental problems (*12*) and TB (*13*) are major comorbidities among persons infected with the HIV, and the synergism between *M. tuberculosis* and HIV is well documented**.** Because we did not collect complete histories on the HIV status of participants, we are unable to delineate the role of HIV with respect to our observations. However, in 2003, the same clinic recorded a 2.3% HIV prevalence among 300 patients screened for oral surgery (*14*); the 2008 prevalence data for the state was 2.2% (*15*). Fourth, our study was conducted in the dental clinic of the major tertiary hospital in 1 of the largest cities in Nigeria; thus, the findings may not be generalizable to all facilities where dental care is provided. Nevertheless, a surprisingly high proportion of dental patients in this study had potentially infectious TB. Therefore, evidence-based strategies should be implemented to quickly diagnose TB and prevent its transmission in dental clinics. Of particular importance in preventing nosocomial TB in dental clinics will be the adoption of infection control measures that may include the use of appropriate masks. Finally, our study also emphasizes the view that active case finding in high prevalence settings could be beneficial.

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KI and WU Polyomaviruses and CD4+ Cell Counts in HIV-1–infected Patients, Italy

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To investigate an association between KI and WU polyomavirus (KIPyV and WUPyV) infections and CD4+ cell counts, we tested HIV-1–positive patients and blood donors. No association was found between cell counts and virus infections in HIV-1–positive patients. Frequency of KIPyV infection was similar for both groups. WUPyV was more frequent in HIV-1–positive patients.

BK and JC polyomaviruses are known to infect humans (*1*,*2*). Recently, the novel KI polyomavirus (KIPyV) and WU polyomavirus (WUPyV) have been identified in respiratory secretions of children with signs of acute respiratory disease (*3*,*4*). However, there is little evidence that these viruses are the causative agents of respiratory disease. The pathogenic role of these viruses in immunocompromised patients is also unclear.

In a study that investigated human polyomaviruses in autopsy lymphoid tissue samples from patients who were positive for HIV, KIPyV was detected in 7.1% of immunocompromised patients with AIDS and in 1.8% of non-immunocompromised controls; WUPyV was detected in 9.5% of patients with AIDS but not in controls (*5*). We detected KIPyV and WUPyV in 3.2% and 1.6%, respectively, of plasma samples from HIV-1–infected patients (*6*). To determine an association between infection with KIPyV and WUPyV and CD4+ cell counts, we obtained plasma samples from HIV-1–positive patients having high and low CD4+ cell counts and a group of healthy controls and tested them for these 2 polyomaviruses.

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The Study

Plasma specimens from 153 HIV-1–infected persons (75% male patients, median age 41.9 years, interquartile range 33.8–47.3 years) with high (110 persons) and low (43 persons) CD4+ counts and from 130 blood donors (80% male donors, median age 41 years, interquartile range 32–47.5 years) were obtained at the Foundation Polyclinic Tor Vergata in Rome, Italy, during 2004–2009. Of 153 HIV-1–infected patients, 74 were receiving highly active antiretroviral therapy: a nucleoside reverse transcriptase inhibitor (NRTI) and a protease inhibitor (PI) (n = 35 patients); an integrase inhibitor (INI), an NRTI, and a PI (n = 7); a nonnucleoside-reverse transcriptase inhibitor and an NRTI (n = 26); an INI and an NRTI (n = 2); an INI and an NNRTI (n = 2); a chemokine receptor type 5 antagonist, an NRTI, and a PI (n = 1); and a chemokine receptor type 5 antagonist, an INI, and a nonnucleoside-reverse transcriptase inhibitor (n = 1). Sixty patients did not receive any therapy. No information was available for 19 patients. Additional information available for patients included HIV-1 viremia and co-infection with hepatitis B virus and hepatitis C virus.

Phylogenetic analysis of the small T antigen gene of KIPyV and WUPyV was performed as described (*6*,*7*). GenBank accession numbers of the sequences used in this analysis are shown in the online Appendix Table (www.cdc.gov/EID/content/16/9/1482-appT.htm).

Total DNA was extracted from 0.2-mL plasma samples by using QIAamp DNA Mini Kit (QIAGEN, Milan, Italy) according to the manufacturer's instructions and stored at –80°C until analysis. Amplification of KIPyV and WUPyV was conducted as described (*8*,*9*). A standard curve was created in a 4-log range by using 1:10 serial dilutions of a virus-specific standard. The dynamic range was determined by using 10-fold dilutions (10^{10}–100 copies/reaction) of each sample. Sensitivity of the 2 methods, which corresponded to the lowest plasma dilution detectable at a frequency of 100%, was evaluated. The dynamic range was 10^2–10^{10} for KIPyV and 10^1–10^{10} for WUPyV.

Statistical analysis was performed by using Epi Info version 3.5.1 software (Centers for Disease Control and Prevention, Atlanta, GA, USA). Odds ratios were determined for associations between infection with HIV and infection with KIPyV and WUPyV and other variables. Statistical significance was assessed by calculating 95% confidence intervals (CIs) and by using standard nonparametric statistics.

Real-time PCR detected KIPyV and WUPyV in 4 (2.6%) of 153 and 7 (4.6%) of 153 HIV-1–infected patients, respectively (Table 1). Of the 130 blood donors examined, 4 and 1 were positive for KIPyV (3.1%) and WUPyV (0.8%), respectively. For KIPyV, no difference was detected in the frequency of infection between

Table 1. Characteristics of 153 HIV+ persons tested for infection with WU and KI polyomaviruses, Italy, 2004–2009*†

Characteristic	WUPyV+, n = 7	WUPyV–, n = 146	KIPyV+, n = 4	KIPyV–, n = 149
CD4+ cells/μL				
≤200	1 (14.3)	45 (30.8)		46 (30.9)
>200	6 (85.7)	101 (69.2)	4 (100)	103 (69.1)
Median (95% CI)	308 (248–523)	282 (153–378)	356 (270–517)	281 (154–378)
Virus load, copies/mL				
≤100,000	7 (100)	99 (67.8)	2 (50)	104 (69.8)
>100,000		47 (32.2)	2 (50)	45 (30.2)
Median (95% CI)	3,210 (108–36,895)	37,460 (5,490–152,000)	60,636 (2,791–246,500)	34,905 (4,980–145,200)
Co-infection				
Yes	2‡ (28.6)	29 (19.9)	2§ (50)	29 (19.5)
No	5 (71.4)	117 (80.1)	2 (50)	120 (80.5)

*Values are no. (%) unless otherwise indicated. WUPvV, WU polyomavirus; KIPyV, KI polyomavirus; CI, confidence interval.
†Of the 153 persons tested, 115 (75.2%) were men, 38 (24.8%) women. A total of 130 blood donors (104 [80%] men, 26 [20%] women) were also tested; 1 (0.8%) was WUPyV+ and 4 (3.1%) were KIPyV+.
‡Co-infection with hepatitis virus.
§Co-infection with hepatitis C virus and hepatitis B virus.

HIV-1–infected patients and blood donors. Patients infected with HIV-1 had a higher risk for infection with WUPyV infection than did blood donors. However, this difference showed borderline statistical significance (odds ratio 6.15, 95% CI 0.93–141; p = 0.054). For WUPyV-positive and KIPyV-positive patients, median CD4+ cell counts were 308 cells/μL (95% CI 248–523 cells/μL) and 356 cells/μL (95% CI 270–517 cells/μL), respectively. No association was observed between CD4+ cell counts and risk for infection with KIPyV or WUPyV.

Median HIV-1 virus load in persons infected with WUPyV or KIPyV was 3,210 copies/mL (95% CI 108–36,895 copies/mL) or 60,636 copies/mL (95% CI 2,791–246,500 copies/mL), respectively. When HIV-1 virus load, CD4+ cell count, and co-infection with hepatitis B and C viruses were analyzed in patients infected with KIPyV or WUPyV, no association was found. Of 11 patients infected with KIPyV or WUPyV, 6 received highly active antiretroviral therapy and 5 did not receive any therapy (Table 2).

Phylogenetic analysis showed that all WUPyVs identified in this study except WUV-IT4 are closely related to the WUV-IT3 strain identified in an HIV-1 patient (*6*) (Figure). The KIPyV strains identified are relatively distant from those identified in another study (*6*), except for strain KIV-RM23, which clusters with KIV-RM21 (*6*) (Figure).

Conclusions

KIPyV and WUPyV have been identified in respiratory secretions of pediatric patients (*3*,*4*). New polyomaviruses have also been detected in immunocompromised patients (*10*–*13*). However, the pathogenic role of these polyomaviruses in immunocompromised patients is unclear. No associations were found between CD4+ cell counts in HIV-1–positive patients and infection with KIPyV or WUPyV. Frequency of KIPyV infection for HIV-1–positive patients was similar to that for blood donors. However, frequency of WUPyV infection was higher for HIV-1–positive patients than for blood donors, although this difference showed borderline significance.

Detection of WUPyV did not show a correlation with virus load for HIV-1 or lower CD4+ cell counts. Seroprevalence of KIPyV and WUPyV in an adult population was 55% and 69%, respectively (*14*). The higher rate of infection for WUPyV may account for the higher rate of detection for WUPyV in our study population. In a previous study (*6*), prevalence of WUPyV in plasma of HIV-1–positive patients was lower than that in our study. This difference may have been caused by the larger sample size in our study.

Phylogenetic analysis did not suggest circulation of specific KIPyV and WUPyV strains in HIV-1–positive patients. The KIPyVs identified in this study cluster with those identified in HIV-1–positive patients, and the WUPyVs identified are closely related to the strain identified previously in an HIV-1–positive patient (*6*). However,

Table 2. Treatment regimens for 11 HIV-1–positive patients co-infected with KI or WU polyomavirus, Italy, 2004–2009*

Patient no.	KIPyV	WUPyV	HAART
1	+	–	None
2	+	–	None
3	+	–	None
4	+	–	NRTI: FTC, TDF; PI: ATV, RTV
5	–	+	NNRTI: EFV; NRTI: 3TC, TDF
6	–	+	NNRTI: NVP; NRTI: 3TC, AZT
7	–	+	NRTI: 3TC, AZT; PI: ATV, RTV
8	–	+	NNRTI: NVP; NRTI: ABC, TDF
9	–	+	None
10	–	+	NNRTI: EFV; NRTI: 3TC, D4T
11	–	+	None

*KIPyV, KI polyomavirus; WUPyV, WU polyomavirus; HAART, highly active antiretroviral therapy; NRTI, nucleoside reverse transcriptase inhibitor; FTC, emtricitabine; TDF, tenofovir; PI, protease inhibitor; ATV, atazanavir; RTV, ritonavir; NNRTI, nonnucleoside reverse transcriptase inhibitor; EFV, Efavirenz; 3TC, lamivudine; NVP, nevirapine; AZT, azidothymidine; ABC, D4T, stavudine.

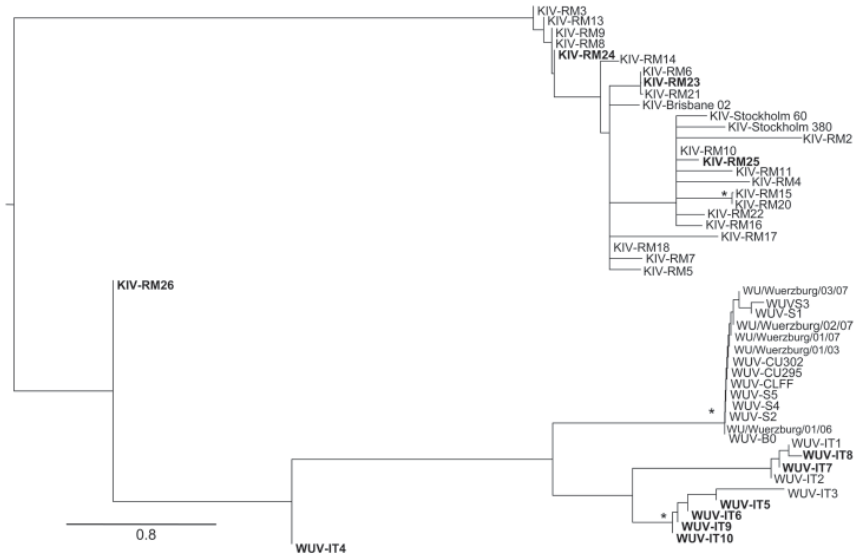

Figure. Maximum likelihood phylogenetic analysis of KI polyomavirus (KIPyV) and WU polyomavirus (WUPyV) small T antigen sequences. Strains identified in this study are in **boldface**. The tree was rooted by using the midpoint rooting method. Branch lengths were estimated by using the best fitting nucleotide substitution (Hasegawa, Kishino, and Yano) model according to a hierarchical likelihood ratio test (*6,7*) and were drawn to scale. Scale bar indicates 0.8 nt substitutions per site. Asterisks along the branches indicate significant statistical support for the clade subtending that branch ($p<0.001$ by the zero-branch-length test and bootstrap support >65%).

these WUPyV strains also cluster with strain WUV-IT1 and 2 strains identified in stool samples (*10*).

Our study design and the complex nature of AIDS-related disease do not enable one to make definitive conclusions on the role of novel polyomaviruses in HIV-1–positive patients. However, our data seem to exclude an active role for KIPyV and WUPyV in HIV-1–positive patients.

We detected WUPyV and KIPyV in healthy persons and immunocompromised persons. BK and JC polyomaviruses persist in peripheral blood mononuclear cells in healthy persons (*15*). However, frequency of detection may vary from 0% to 90% of persons tested. This large variation may reflect recent infection or virus reactivation in a subgroup of persons (*15*). Thus, detection of KIPyV and WUPy in blood cells of immunocompetent persons is needed to identify a possible hematologic reservoir.

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Acute Cervical Lymphadenitis Caused by *Mycobacterium florentinum*

To the Editor: The incidence of nontuberculous mycobacterium (NTM) lymphadenitis appears to be rising, likely because of improved diagnostic techniques. *Mycobacterium avium-intracellulare* is the most common etiologic agent of NTM lymphadenitis in children (*1*,*2*). Newer diagnostic methods, including DNA sequencing, have identified cervical adenitis secondary to other slow-growing species (*M. lentiflavum*, *M. interjectum,* and, most recently *M. florentinum* in 2005) (*3*–*6*). We report a case of acute cervical lymphadenitis caused by *M. florentinum* in a child and briefly describe 4 other patients, both children and adults, with positive culture growth. These results suggest that *M. florentinum* infection is more widespread than previously appreciated.

A previously healthy girl, 3 years of age, came to our outpatient clinic with 2 months of bilateral cervical lymph node enlargement preceded by low-grade fevers for a few days. She had previously received clindamycin for 10 day without improvement. A tuberculin skin test showed 10 mm of induration, and results of a chest radiograph were negative. Both parents were from El Salvador, but the child was born in the United States and had never traveled abroad.

Her examination showed enlarged left (6 × 4 cm) and right (4 × 3 cm) posterior, cervical lymph nodes, which were indurated, erythematous, and fluctuant. Computed tomography scan of the neck showed multiple, bilateral, necrotic lymph nodes in the posterior cervical triangle, more notable on the right, with retropharyngeal abscesses (Figure).

The patient was admitted to the hospital because of concerns about airway obstruction. Laboratory findings included leukocyte count of 9.8 × 10^3 cells/μL (40% neutrophils), increased thrombocytes (523 × 10^3 cells/μL), and a routine, negative blood culture for bacterial growth. She had elevated alanine aminotransferase (822 U/L), aspartate aminotransferase (482 U/L), and lactate dehydrogenase (387 U/L) levels. Computed tomography scan of the abdomen showed no abscesses. She was given intravenous vancomycin.

The following day she underwent neck exploration, excisional left lymph node biopsy, and drainage of her retropharynx. Retropharyngeal cultures grew methicillin-resistant *Staphylococcus epidermidis* and *Streptococcus mitis*. Lymph node histopathologic analysis showed noncaseating granulomas with no malignancy. Results of tests with special stains for acid-fast bacilli and fungi were negative. She was discharged home after receiving oral linezolid for 10 days to complete 14 days of antimicrobial drug therapy. Her liver function test results had improved at the time of discharge.

The excised lymph node and neck abscess grew atypical mycobacteria, which were initially isolated by using an automated broth culture system (MGIT; Becton Dickinson, Sparks, MD, USA) after 4 weeks of incubation. Cultures were negative for *M. avium* complex by DNA hybridization, nonpigmented, and positive for nitrate reductase and inactivation of catalase at 68°C.

At the time of follow-up, the patient's neck swelling had only slightly decreased, which resulted in complete surgical excision of bilaterally infected lymph nodes 3 weeks later with subsequent improvement. Repeat mycobacterial cultures were negative. No relapse occurred within 12 months of observation following surgery.

The isolate was identified as *M. florentinum* at Associated Regional and University Pathologists Labora-

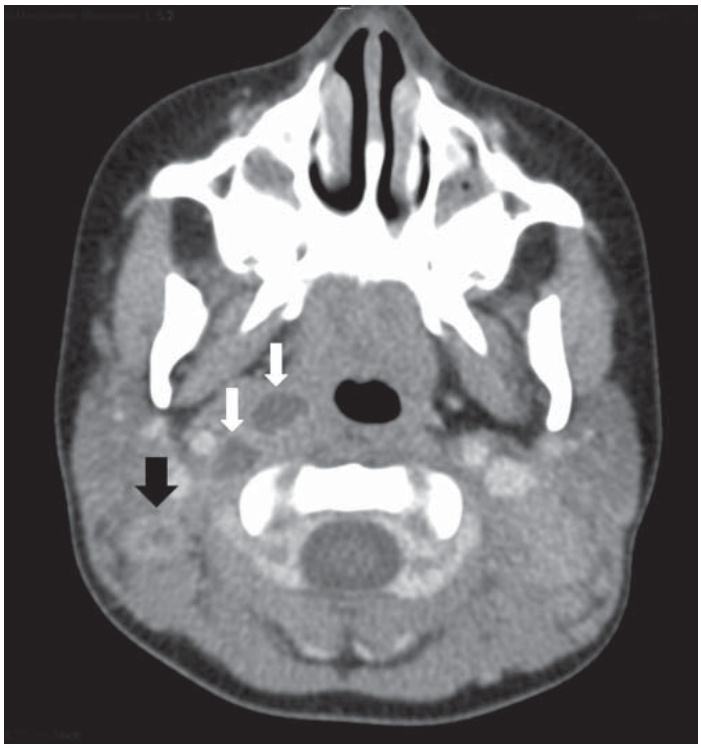

Figure. Computed tomography scan of the neck of a 3-year-old girl, showing right lateral retropharyngeal abscess (white arrows) and enlarged bilateral posterior cervical lymph nodes with low attenuation of a right cervical lymph node (black arrow), consistent with atypical mycobacterium adenitis.

tories (Salt Lake City, UT, USA) on the basis of sequencing of the first 500 bp of the 16S rRNA gene. The patient isolate exhibited 484/484 nt identities with the type strain of *M. florentinum*. The isolate was susceptible to amikacin and clarithromycin with MICs of 0.5 μg/mL but was resistant to ciprofloxacin (MIC 8 μg/mL), according to Clinical and Laboratory Standards Institute guidelines (www.clsi.org). Other drugs tested (with corresponding MICs) included ethambutol (4 μg/mL), gatifloxacin (2 μg/mL), moxifloxacin (1 μg/mL), rifampin (0.12 μg/mL), and streptomycin (0.5 μg/mL). No current Clinical and Laboratory Standards Institute interpretations are available for these agents.

Four additional cases of *M. florentinum* infection have been identified at Associated Regional and University Pathologists Laboratories since 2006 (after Institutional Review Board approval): a 76-year-old man (bronchial aspirate), a 47-year-old man (sputum), a 5-year-old girl (neck lymph node), and a 46-year-old man (unspecified source). Two of these cases were submitted from New York, 1 from Oregon, and 1 from Virginia.

M. florentinum is a recently described, slow-growing, nonpigmented mycobacterium. In 2005, Tortoli et al. described 8 strains obtained over 11 years from the sputum of patients with various pulmonary disorders and, in 1 case, from the lymph node of a 6-year-old girl (*6*). Seven of the 8 patients were hospitalized in Italy.

Atypical mycobacterium lymphadenitis generally shows unilateral lymph node involvement; however, the patient reported here had bilateral lymphadenitis complicated by retropharyngeal abscesses. Although disseminated NTM infection can lead to liver abnormalities, this patient had no risk factors for dissemination such as immunosuppression. Recommended treatment of atypical NTM lymphadenitis in children is complete surgical excision (*5*,*7*), although some studies have suggested antimicrobial drugs (*1*,*7*,*8*) or observation alone (*9*). *M. florentinum* exhibits resistance to several antimycobacterial drugs (*6*); therefore, surgical excision may still be the preferred treatment for this infection. Because the patient reported here had complete surgical excision and no recurrence, she did not require further antimicrobial drugs.

Cervical lymphadenitis in children is thought to result from ingestion of environmental mycobacteria (*10*). Although many NTM species are specific to particular geographic locations, our data suggest that *M. florentinum* is found in diverse locations in the United States. This organism appears to have a predilection for lymph nodes and lung tissue, similar to other NTM species.

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Mobile Messaging as Surveillance Tool during Pandemic (H1N1) 2009, Mexico

To the Editor: Pandemic (H1N1) 2009 highlighted challenges faced by disease surveillance systems. New approaches to complement traditional surveillance are needed, and new technologies provide new opportunities. We evaluated cell phone technology for surveillance of influenza outbreaks during the outbreak of pandemic (H1N1) 2009 in Mexico.

On May 12, 2009, at 2:20 PM, a random sample of 982,708 telephones from an 18 million nationwide network of mostly prepaid cell phones (*1*) received a text message invitation to a Ministry of Health survey. Influenza-like illness (ILI) in April, date of fever onset, severity, number of household members with ILI, age, influenza vaccination, household size, and number of children in each household were assessed (online Technical Appendix Figure 1, www.cdc.gov/EID/content/16/9/1488-Techapp.pdf). ILI was defined as fever and cough or sore throat, and severe ILI was defined as inability to work, study, or maintain family care. Unstructured supplementary service data, an interactive platform available on most cell phones, was used. We obtained daily counts of suspected and confirmed cases of pandemic (H1N1) 2009 from the nationwide clinic-based surveillance system Sistema Nacional de Vigilancia Epidemiológica (SINAVE) (*2*,*3*).

Of 70,856 responses received, 56,551 (78.1%) were unique mobile numbers (5.8% response rate; only the first response was used). Within 3 hours, 53% of responses were received and by 24 hours, 89% were received. Mean (SD) age of respondents was 25.2 (10.4) years (online Technical Appendix Table). A total of 9,333 persons reported ILI and 49.3% had severe symptoms. Mean number of other persons with ILI in the household was 1.6 among respondents reporting severe disease and 0.3 among those with nonsevere disease ($p<0.0001$, by *t* test).

Epidemic curves for disease onset for confirmed and suspected cases of pandemic (H1N1) 2009 from SINAVE and daily proportion of severe cases from the cell phone survey are shown in the Figure. Daily counts of ILI were clustered around multiples of 5, and no distinct pattern was observed (online Technical Appendix Figure 2). Use of the daily proportion of severe cases may partially correct for clustering and artifactual peaks by standardizing by total number of cases. The proportion of severe cases increased throughout the month beginning on April 1 (36.4%) and peaking on April 26 (57.9%). Two distinct decreases in severity of disease coincided with Semana Santa school vacation and school closures on April 24. These decreases are consistent with the decrease in the SINAVE epidemic curve.

The pattern of change in the proportion of severe ILI may be consistent with a decrease in transmission after control measures were implemented. The low response rate (5.8%) made it likely that respondents were not representative of the total population. Therefore, we did not make estimates of disease incidence. We were unable to determine whether a pathogen for which susceptibility was higher was responsible for the difference in number of ILI cases within the household of those reporting severe disease or whether respondents in households with several affected persons were more likely to report severe disease (online Technical Appendix Table). We observed unexpected peaks and a clustering of date of fever onset. However, the peak on April 1 may reflect disease at the end of March, and the decrease in daily proportion of severe cases may indicate lower incidence of ILI after school closures. Comparison of these data with epidemic curves for pandemic (H1N1) 2009 showed less variability than expected; no geographic variation was detected.

Our study was limited by potential selection bias, recall bias, and in-

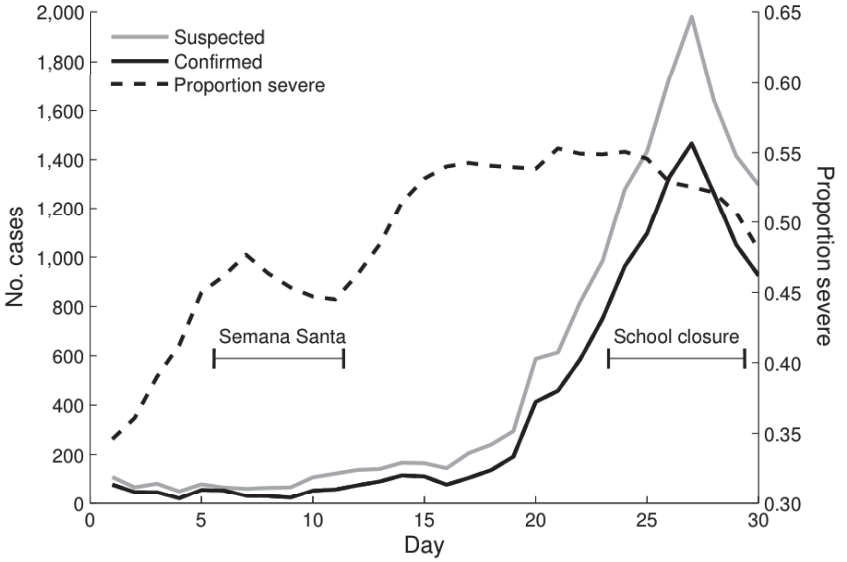

Figure. Proportion of severe cases of influenza-like illness (ILI) in Mexico, April 2009, from unstructured supplementary service data survey and confirmed and suspected cases of pandemic (H1N1) 2009 from Sistema Nacional de Vigilancia Epidemiológica. Suspected cases of pandemic (H1N1) 2009 are ILI cases for which no laboratory confirmation was possible. The daily proportion of reported severe cases and daily counts of confirmed and suspected cases of pandemic (H1N1) 2009 were smoothed by using a 5-day moving average.

clusion of mostly young persons from urban areas. Comparisons between reported cases and noncases are invalid because of the low response rate. However, comparisons within cases may be less prone to bias if they are more likely to respond.

Persons had difficulty remembering the exact date of fever onset. In 2 telephone surveys in New York City outside the influenza season (March and October–November 2003), a total of 20.8% and 19.6% of respondents, respectively, reported ILI within the past month (*4*), which were more than the rate of 12% during the peak of pandemic (H1N1) 2009 in New York (*5*). Use of daily proportion of severe cases may have partially corrected for this recall error. Also, persons may be more likely to report ILI if the date of onset was closer to the date of the survey. Nevertheless, a lower number of cases by the end of the month indicates that more accurate recall for recent dates may not be a serious problem. Generalizability of these results is of concern. However, the age group that was captured was most affected in the early stages of this outbreak (*6*).

Efficient estimation of extent of disease caused by a novel infectious agent may be costly and logistically difficult. When carefully deployed, unstructured supplementary service data surveys may be a practical, low-cost, and timely complement to traditional surveillance. Further refinements of this tool are required to improve its validity. To limit recall errors and increase response rate, repeated surveys at short intervals and specific strategies to improve response rate should be considered.

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Invasive *Klebsiella pneumoniae* Infections, California, USA

To the Editor: A distinctive form of tissue-invasive community-associated *Klebsiella pneumoniae* infection, typified by primary liver abscess and bacteremia, has been well known in Asia for 2 decades (*1*–*4*). Association of these infections with a hypermucoviscous phenotype was discovered in 2004 (*5*). Certain genetic and virulence features were elucidated in that and subsequent reports (*6*).

The phenotype, easily detected at the bench by the string test (*5*), has been associated with a chromosomal gene, the mucoviscosity-associated gene A (*magA*), and a plasmid gene, the regulator of the mucoid phenotype A gene (*rmpA*). Usually serotypes K1 and K2 can be demonstrated. Hypermucoviscous isolates demonstrate increased virulence in mice, are serum insensitive, and resist phagocytosis (*5*).

Reports of such infections from Europe and North America are rare. Recently 2 of us (L.L and B.F.) reported 4 cases in persons seeking care at the Alameda County Medical Center in Oakland, California, USA (*7*). We report 9 more cases, 7 from Alameda County Medical Center and 2 from St. Rose Hospital in Alameda County. The 13 cases are described in aggregate.

One case occurred in 2006, 3 in 2007, 7 in 2008, and 2 in January 2009. Median patient age was 52 years (range 37–70 years), and 9 were men. Ten patients were born in Asia (Philippines, Vietnam, South Korea, Cambodia, and Yemen), but all had emigrated years earlier. Two patients were born in the United States (1 Filipino and 1 African American); the birth site of 1 Filipino was unknown. Five patients had no underlying illness. Seven had diabetes mellitus, 1 had α-thalassemia, 2 had uncontrolled cancer, and 1 had preexisting multiple organ failure. Three patients had gallstones.

Case-patients exhibited diverse clinical features. The site of infection was easily detected by dramatically abnormal results of computed tomographic scan or magnetic resonance imaging (Figure). Seven patients had liver abscesses. One of these patients also had cholecystitis and choledocholithiasis. One other patient with healthcare-associated bacteremia most likely had multiple small liver abscesses that were superinfected cancer metastases.

Two patients had neck abscesses, 1 complicated by extensive descending mediastinitis (Figure). One patient had kidney abscesses complicated by septic and bland pulmonary emboli and numerous brain abscesses detected by magnetic resonance imaging (Figure).

Healthcare-associated bacteremia occurred in 3 patients. One patient had sustained bacteremia without a clear source on hospital day 115. Two patients with uncontrolled cancer also had healthcare-associated bacteremia.

Venous thrombotic complications occurred in 6 patients, most diagnosed at admission. Two patients had bland pulmonary emboli, and 1 patient with uncontrolled cancer had thrombosis of an upper extremity vein and both femoral veins. Two other patients had septic pulmonary emboli suggested by computed tomographic scan. One patient with α-thalassemia had kidney abscesses and renal vein thrombosis, followed by femoral deep vein thrombosis and pulmonary embolus. A patient with a neck abscess had a thrombosed neck vein at surgery.

Four patients died, and 1 was lost to follow-up, for a death rate of at least 31%. None died directly of sepsis.

All isolates were resistant in vitro to ampicillin but susceptible to all other antimicrobial drugs tested.

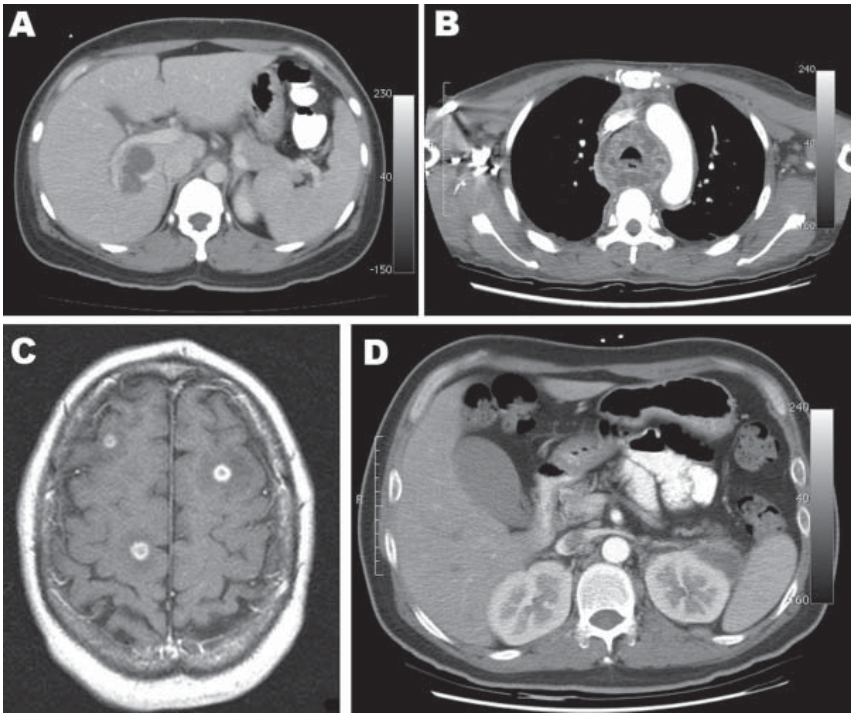

Figure. A) Computed tomography (CT) scan of the abdomen showing a liver abscess adjacent to the portal vein. B) CT scan of the chest at the level of the aortic arch showing mediastinum abscesses surrounding the trachea. C) Brain magnetic resonance imaging (T1 weighted, spin echo, with contrast) showing multiple intracerebral abscesses (smooth ring-enhancing lesions with surrounding vasogenic edema). D) CT scan of the abdomen of patient from panel C, showing a left perinephric abscess and thrombus.

Genotyping was performed on isolates from 4 patients. One isolate contained the *rmpA* gene; 3 contained *rmpA* and *magA* genes. Three of these isolates also underwent capsule serotyping; 2 were type K1 and 1 was K2.

We found 4 additional patients infected with *K. pneumoniae* in 2009 who did not have invasive infections. Briefly, a 21-year-old pregnant recent emigrant from Yemen and a 35-year-old Hispanic pregnant woman each had a urinary tract infection; a 78-year-old Vietnamese man had nosocomial aspiration pneumonia in which *K. pneumoniae* was considered a pathogen; and a 34-year-old African American woman who was receiving mechanical ventilation had sputum transiently colonized with *K. pneumoniae*.

This case series confirms that the clinical syndrome of *K. pneumoniae* bacteremia and primary liver abscess has emerged in Alameda County. Other sites of infection included kidney, brain, lung, pleural space, neck, and mediastinum, as reported in Asia (*1*–*4*). Although *K. pneumoniae* infections are predominantly a community-associated phenomenon, nosocomial infections as we observed have been reported (*8*). Death reflected underlying disease rather than *K. pneumoniae* infection in this study. We present evidence that hypermucoviscous *K. pneumoniae* also can behave as a colonizer or low-virulence pathogen, as manifested in our patients with urinary tract infection, sputum colonization, and aspiration pneumonia.

Our *K. pneumoniae* isolates appear similar to those from Asia (*5*) with respect to in vitro susceptibility, capsule serotypes, and *magA* and *rmpA* genes. Most of our patients were Asian but of widely dispersed origin and without recent travel to Asia. The number of thrombotic complications in this series is striking. Such complications appear not to have been noted in the literature, and this finding requires further investigation. Our data show the emergence of hypermucoviscous *K. pneumoniae* in northern California and suggest that it might be unrecognized elsewhere in North America.

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Family Outbreak of Shiga Toxin–producing *Escherichia coli* O123:H–, France, 2009

To the Editor: Shiga toxin–producing *Escherichia coli* (STEC) is a major cause of foodborne disease in industrialized countries. We present results of the investigation of a family outbreak in France caused by a rare STEC serotype.

Surveillance of STEC infections in France since 1996 has been based on national surveillance of STEC-related pediatric hemolytic uremic syndrome (HUS) (*1*). On February 11, 2009, two cases of diarrhea were reported to a surveillance coordinator: 1 in a child with HUS and the other in that child's sibling.

The 2 siblings, 2 and 6 years of age, had diarrhea beginning on February 4 and 5, 2009. Bloody diarrhea

developed in the younger child, and HUS was diagnosed on February 9. The older child had nonbloody diarrhea for 3 days and abdominal pain. Questioning of the patients' parents identified no recent history of travel, contact with farm animals, or outdoor bathing. A food history indicated that the 2 patients had shared an undercooked ground beef burger 4–5 days before symptom onset. The patients' parents also ate burgers from the same package (box); they did not report any gastrointestinal symptoms.

Fecal specimens of the patients were tested for STEC by direct PCR for STEC genes (*stx*); after which culture and identification of *stx1*, *stx2, eae*, and *ehxA* (*hlyA*) virulence genes; and serotyping with a panel of 22 serum samples were conducted as described (*1*,*2*). Molecular serotyping was subsequently conducted on nonagglutinating strains by using the *rfb*–restriction fragment length polymorphism technique for O antigen (*3*) and sequencing of the *fliC* gene for H antigen (*4*).

A trace-back investigation was conducted for the implicated beef burgers, which were obtained from a box of 10, frozen, 100-g ground beef burgers purchased in late January 2009. The remaining beef burger in the box from which the patients had eaten a beef burger was obtained from the family's freezer for microbiologic testing. Stored production samples from the implicated batch underwent microbiologic testing.

After broth enrichment, ground beef samples were tested by PCR for *stx* and *eae* virulence genes and O antigens of serotypes O157, O26, O145, O103, and O111 (*2*,*5*,*6*). Subsequently, strains isolated from *stx*-positive and *eae*-positive enrichment broths were biochemically tested and underwent serotyping and PCR identification of virulence genes. Genetic relatedness of clinical and ground beef STEC strains was studied by using pulsed-field gel electrophoresis with *Xba*I as described (*7*).

A nonmotile strain of STEC *stx2 eae ehxA*, which was not serotypeable by the panel of 22 serum samples, was identified in fecal samples from patients and in the remaining ground beef. Molecular serotyping of clinical isolates and an isolate from the beef identified a strain of STEC O123:H2. Analysis by pulsed-field gel electrophoresis indicated that the clinical and meat isolates were genetically related (Figure). The level of STEC contamination in the meat was 30–40 CFU/g. All stored meat production samples tested were negative for STEC.

A clinical strain and a ground beef STEC strain were sent to the World Health Organization Collaborating Centre for Reference and Research on *Escherichia* and *Klebsiella* in Copenhagen, Denmark, in December 2009 for analysis. The clinical strain was confirmed as STEC O123:H–, and the meat strain was confirmed as a nonmotile STEC rough type by serum agglutination. Both strains had virulence genes *stx2a*, *eae*, and *ehxA* (F. Scheutz, pers. comm.).

We identified a family outbreak of STEC O123:H– *stx2a*, *eae ehxA* infections associated with ingestion of undercooked ground beef. No similar cases of STEC infection were identified by active case finding. This serotype is rarely described as a cause of human clinical infection. No human

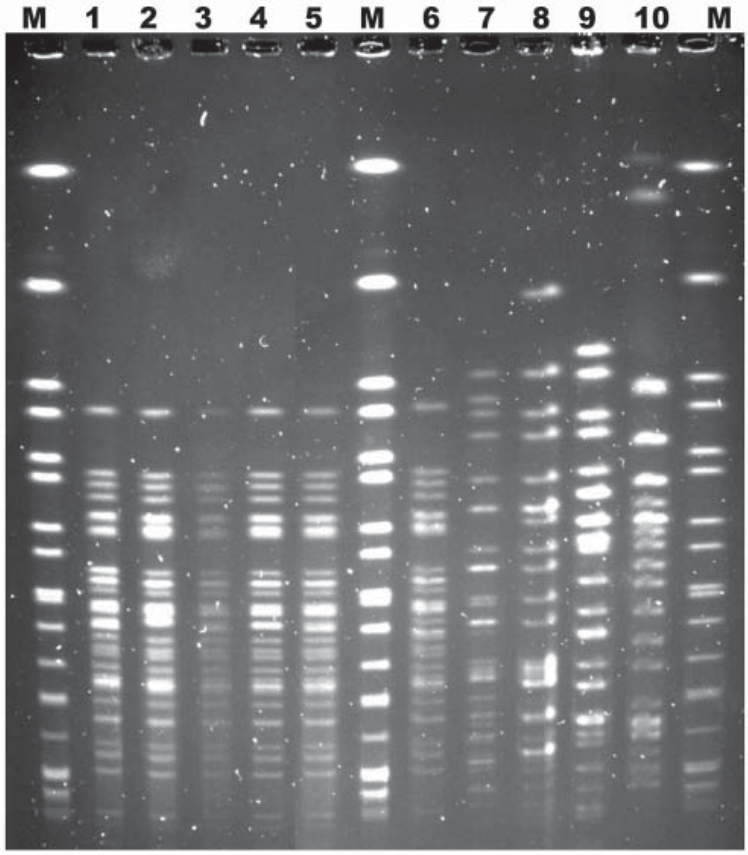

Figure. Representative *Xba*I pulsed-field gel electrophoresis patterns of Shiga toxin–producing *Escherichia coli* (STEC) O123:H– strains isolated from patient fecal samples and strains isolated from ground beef obtained from patients' home, France, 2009. Lanes M, *Xba*I-digested genomic DNA from *Salmonella enterica* serovar Braenderup H9812 used as molecular mass markers; lane 1, Shiga toxin–producing STEC O123:H– isolated from patient with hemolytic uremic syndrome; lane 2, STEC O123:H– isolated from patient with diarrhea; lanes 3–6, STEC O123:H– isolated from ground beef from patients' home; lanes 7 and 8, STEC O123 reference strains; lane 9, STEC 0111 isolate not related to the strains of the patients; lane 10, STEC 0157:H7 isolate not related to the strains of the patients.

isolate of serotype O123:H– is recorded in the database of the World Health Organization Collaborating Centre for Reference and Research on *Escherichia* and *Klebsiella* (F. Scheutz, pers. comm.).

Two strains of STEC O123:H– *stx2d* were isolated from asymptomatic persons in Germany during 1996–2000 (*8*). A study in Australia in 2003 reported using a strain of O123:H– *stx1 stx2 ehxA* from Switzerland that had been isolated from a person with diarrhea (*9*).

We report foodborne transmission of STEC O123:H– that resulted in a cluster of clinical cases of infection. Eating ground beef is a well-established mode of STEC transmission, particularly for serotype O157:H7. STEC serotype O123:H– has been isolated from feces of healthy lambs and sheep in Spain (*10*) and in southwestern Australia (*9*) and is considered to be among the predominant ovine STEC serotypes in these countries.

This family outbreak shows that STEC serotype O123:H–, albeit rarely described as causing human illness, can cause severe human infection. This serotype can also cause clusters of STEC infections and be transmitted by ingestion of undercooked ground beef.

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Austrian Syndrome Associated with Pandemic (H1N1) 2009 in Child

To the Editor: In 1957, an American internist reported the preference of *Streptococcus pneumoniae* for the aortic valve and its frequent association with meningitis and pneumonia (*1*), an association now known as Austrian syndrome. This syndrome mainly occurs in middle-age men who have predisposing factors, such as chronic alcoholism, altered immune state, dural fistula, and ear or sinus infections.

One case of Austrian syndrome has been reported in the pediatric age group, in a 7-year-old girl in whom aortic valve endocarditis developed

after pneumococcal meningitis infection (*2*). In pneumococcal endocarditis, the native aortic valve is the most frequent location of vegetation. Valve replacement must be considered to avoid development of cardiogenic shock, whereas medical treatment alone may be adequate in some cases of mitral endocarditis (*3*,*4*).

We report a previously healthy adolescent with Austrian syndrome associated with pandemic (H1N1) 2009 infection. Cardiac involvement resulted in extensive mitral valve destruction requiring surgical valve replacement.

A 13-year-old boy had cough, nasal congestion, and a fever ≥103°F for 2 weeks before being seen at a hospital. On the day he was admitted to hospital, he became unarousable, and weakness was noted on his left side. His medical history included mild asthma requiring no therapy over the past 3 years. His immunizations were up to date except for seasonal influenza and pandemic (H1N1) 2009 vaccination.

When admitted to hospital, his temperature was 38.7°C, blood pressure 97/47 mm Hg, respiratory rate 77 breaths/min, and heart rate 150 beats/min. Pupils were 3 mm in diameter and reactive to light and accommodation. Weakness and hypertonia of the left upper and lower extremities were noted. He had neck stiffness without Kernig sign or Brudzinski sign. Complete blood count showed a leukocyte count of 26,400 cells/mm^3 and differential of 92.1% neutrophils, 5.3% lymphocytes, 2.6% monocytes, and 0.1% eosinophils; hemoglobin level of 12.9 g/dL; hematocrit 38.6%; and a thrombocyte count of 102,000 cells/mm^3. A plain chest radiograph showed a left lower lobe infiltrate. Computed tomography of the head showed a large infarction involving the right frontal lobe at the distribution of the right middle cerebral artery; small infarcts involved the left frontal lobe and the right parietal lobe.

Lumbar puncture showed a leukocyte count of 100 cells/mm^3, 71% neutrophils, 8% bands, 15% lymphocytes, 5% monocytes, and 1% eosinophils; protein 195 mg/dL, and glucose 6 mg/dL, with a blood glucose level of 140 mg/dL. Gram stain of the cerebrospinal fluid showed gram-positive cocci in pairs. The patient was treated with intravenous ceftriaxone, vancomycin, and dexamethasone. He subsequently became unconscious and hypotensive and required intubation with mechanical ventilation and intravenous dopamine. His nasal wash sample was positive for pandemic (H1N1) 2009 RNA by real-time reverse transcription–PCR; oseltamivir (75 mg) through a nasogastric tube every 12 h for 5 days was administered. Because of heart murmur, a 2-dimensional echocardiography was conducted; it showed a large mitral valve vegetation 1.6 cm × 2.1 cm attached to the posterior mitral leaflet and mild to moderate mitral insufficiency. Because of this finding, he was transferred to Children's Hospital of Michigan for surgical intervention.

Physical examination at Children's Hospital showed a well-nourished adolescent who was intubated and sedated. A grade 3 systolic ejection murmur at the left lower sternal border was noted. Neurologic examination showed sluggish pupils, decreased tone of the left extremities, and bilateral Babinski sign. No meningeal signs were observed. Magnetic resonance imaging of the head showed multiple areas of infarction, with the largest being in the right middle cerebral artery distribution and a smaller one in the left frontal region (Figure). A few small scattered areas of infarction bilaterally were suggestive of cerebral embolism.

Two days after transfer, the patient underwent mitral valve replacement with a St. Jude prosthetic valve (St. Jude Medical, St. Paul, MN, USA). A 2.5-cm vegetation was found on the atrial surface of the inferior aspect of the posterior mitral leaflet involving the inferior commissure. The posterior

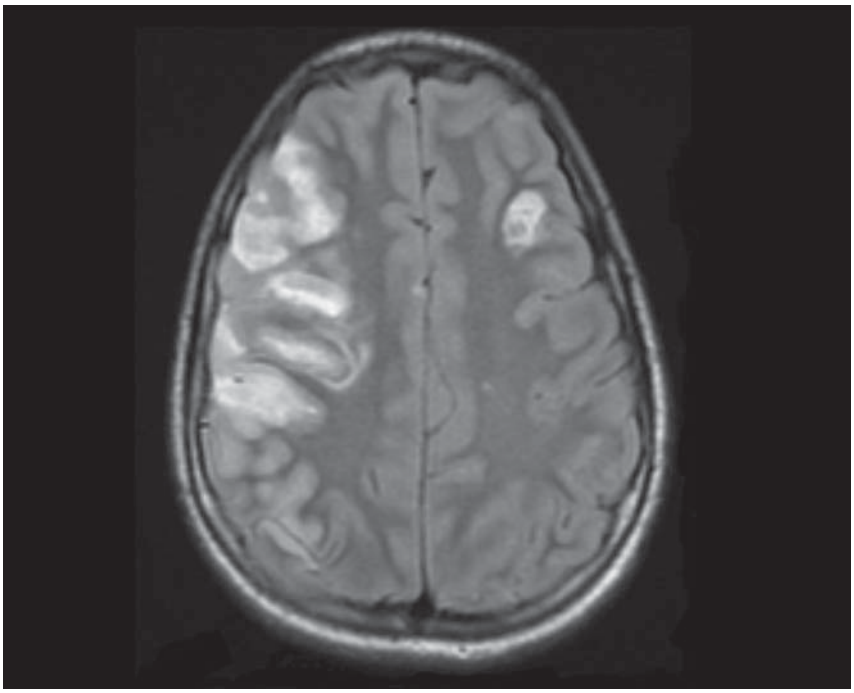

Figure. Magnetic resonance image of the head of the patient, a 13-year-old boy, showing multiple areas of infarction bilaterally with the largest in the right middle cerebral artery distribution and a smaller one in the left frontal region consistent with embolic infarcts.

leaflet associated with the vegetation was destroyed. The vegetation culture, pericardial fluid culture, tissue culture from the resected mitral valve, and 3 blood cultures yielded no bacterial growth.

The patient required ventilator support for 7 days. Follow-up computed tomography on day 8 showed a stable appearance of cerebral infarcts. Coumadin was started to prevent thrombus development at the prosthetic valve. He went home after completing a 4-week treatment course of ceftriaxone. At that time, there was still noticeable left-sided weakness of the extremities. He could only communicate by using eye movements with no verbal response.

During the 20th century influenza pandemics, secondary bacterial pneumonia was a notable cause of death. The current pandemic (H1N1) 2009 outbreak is evolving rapidly, and it is unknown if pandemic (H1N1) 2009 may lead to an increase in rare complications of pneumococcal infection, such as endocarditis. Thus, Austrian syndrome should be considered in any patient with pandemic (H1N1) 2009 complicated by pneumococcal infection and a new heart murmur.

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Rickettsia sibirica mongolitimonae in Traveler from Egypt

To the Editor: Tick-borne rickettsioses are zoonoses caused by spotted fever group (SFG) *Rickettsia* spp. (*1*), which have been reported as a frequent cause of fever in international travelers (*2*). In Egypt, Mediterranean spotted fever caused by *Rickettsia conorii* transmitted by the brown dog tick, *Rhipicephalus sanguineus,* is known to be present, although cases are rarely documented. Moreover, an emerging pathogen, *R. aeschlimannii,* has been detected in *Hyalomma dromedarii* ticks, collected from camels, and in *H. impeltatum* and *H. marginatum rufipes,* collected from cows (*3*). We report a case of *Rickettsia sibirica mongolitimonae* infection in a French traveler who returned from Egypt

In September 2009, a previously healthy 52-year-old man living in France was admitted to the infectious diseases unit of a hospital in Nantes, France, with a 10-day history of fever, asthenia, headache, and arthromyalgia. Three days earlier, he had returned from a 2-week trip to Egypt. He had fever (38°C), painful axillary lymphadenopathies, and an inoculation eschar surrounded by an inflammatory halo on the left scapular area (online Appendix Figure, www.cdc.gov/EID/content/16/9/1495-appF.htm), but he did not have a rash. During his travel, he had been unsuccessfully treated for headache, arthromyalgia, and diarrhea by amoxicillin-clavulanate (3 g/d), nonsteroidal antiinflammatory drugs, and gentamicin cream on the eschar for 3 d. No tick bite was reported by the patient. We suspected an SFG rickettsiosis. The patient received 200 mg doxycycline in a single dose and rapidly improved.

The immunofluorescence assay for antibodies reactive against SFG antigens showed increased levels of immunoglobulin M (titer 16) and G (titer 128). Results of Western blot with cross-adsorption assays supported the hypothesis that the infection was caused by *R. sibirica mongolitimonae* (*1*). To identify the involved rickettsiae, PCR amplifications and sequencing *glt*A, *omp*A, and *omp*B fragment genes of *Rickettsia* spp. and multispacer typing (MST), based on the sequence of variable intergenic spacers, were performed by using DNA samples obtained from an eschar biopsy and a lesion swab (*4*,*5*). A negative control (sterile water and DNA from a sterile biopsy specimen) and a positive control (DNA from *R. montanensis*) were included in each test. Amplicon sequencing confirmed the presence of *R. sibirica mongolitimonae* DNA in patient samples. The sequence homology to *R. sibirica mongolitimonae* DNA was *omp*A, 99.4%; *glt*A, 99.7%; and *omp*B, 100% (GenBank accession nos. DQ097082, DQ097081, and AF123715, respectively). The MST sequences were 100% homologous to the genotype of *R. sibirica mongolitimonae* MST type U (idem HA-91). We injected shell vial cultures with eschar biopsy specimens (*4*). Fifteen days later, positive Gimenez staining and immunofluorescence confirmed

the presence of *Rickettsia* sp. in cell culture, and *R. sibirica mongolitimonae* was identified by PCR and sequencing as described above (online Appendix Figure).

R. sibirica mongolitimonae was first isolated in Beijing in 1991 from *H. asiaticum* ticks (formerly named *R. sibirica* HA-91), and the first human infection was reported in 1996 (*4*). Since that time, *R. sibirica mongolitimonae* infections have been diagnosed in 15 additional patients: 12 from Europe (France, Portugal, Greece, and Spain) and 3 from Africa (Algeria, South Africa, and the present patient who returned from Egypt). The application of genotypic criteria to *R. sibirica mongolitimonae* classified the organism as a subspecies of *R. sibirica* group, in spite of its distinct serotypes and specific epidemiologic features compared to *R. sibirica sibirica*, the causative agent of Siberian tick typhus or North Asian tick typhus (*1*).

R. sibirica mongolitimonae causes lymphangitis-associated rickettsiosis. The available clinical features for the only 16 reported cases (10 men, 6 women) include fever in all patients (range 38˚C–39.5°C), chills (3/16 patients), headache (13/16), myalgia (13/16), arthralgia (3/16), cutaneous rash (11/16), enlarged lymph nodes (10/16), lymphangitis expanding from an inoculation eschar to the draining node (6/16), and retinal vasculitis in a pregnant woman (*6*,*7*). Two patients exhibited 2 eschars. Most eschars were on the legs, but some patients had an eschar on the back, the abdomen, the arm, or the face. The patients' median age was 50 years (range 20–76 years). A tick bite or tick handling was reported for 5 patients, but no tick was collected for further examination. In France, 7 patients probably came in contact with *R. sibirica mongolitimonae*–infected ticks in their gardens, and 2 other patients were probably exposed during a walk in the Camargue National Park, where migratory birds are frequently present (*7*). Infection with *R. sibirica mongolitimonae* occurred primarily between March and September. A single case was reported in December in Greece. *R. sibirica mongolitimonae* has been detected in several *Hyalomma* spp. ticks in Niger, Greece, the People's Republic of China, Senegal, and in *Rh. pusillus* ticks in Portugal (*6*–*8*). Although *Hyalomma* spp. ticks seem to be associated with *R. sibirica mongolitimonae*, more experimental data are needed to determine the tick vectors and reservoirs of this rickettsia.

Clinicians in Egypt and those who may see patients returning from this country should be aware that several species of rickettsiae are found in this region. Thus, they should consider a range of SFG rickettsial diseases in the differential diagnosis of patients with febrile illnesses.

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Increase in *Neisseria meningitidis* Serogroup W135, Niger, 2010

To the Editor: Meningococcal epidemics in the African meningitis belt are generally caused by *Neisseria meningitidis* serogroup A strains, but they also can be caused by serogroup W135 or X strains. The largest reported outbreak caused by serogroup W135 occurred in Burkina Faso in 2002 with ≈13,000 suspected cases (*1*). Sporadic cases of meningitis caused by serogroup W135 have, however, been

detected previously, notably in Niger since the early 1980s (*2*). This serogroup has also been associated with outbreaks in pilgrims to Mecca, Saudi Arabia, in 2000, and several clusters of cases occurred worldwide before 2002 (*3*). After 2003, no major outbreak caused by serogroup W135 was detected in sub-Saharan countries, only sporadic cases. Although Niger borders Burkina Faso, Niger has not experienced a large outbreak of meningitis caused by serogroup W135, with the exception of 7,906 suspected cases and 595 deaths declared in 2001; serogroup W135 represented 12 (38.7%) of the small number (n = 31) of confirmed cases (*4*). In 2010, serogroup W135 may have caused a major outbreak (a large proportion of this serogroup was detected during the first 12 weeks). Niger residents have not been in contact with this serogroup in recent years and have never been immunized with the trivalent polysaccharide vaccine (A/C/W135).

From January 1 through March 28, 2010, the Ministry of Public Health of the Republic of Niger reported 1,188 suspected cases of meningococcal disease, including 103 deaths (case-fatality rate 8.7%). Suspected cases were reported from all 8 provinces but predominantly in the provinces of Maradi (40%) and Tillabéry (24%). At week 12, the districts of Maradi Commune and neighboring Madarounfa crossed the alert, or epidemic, threshold with cumulated attack rates per 100,000 inhabitants of 57.0 and 48.5, respectively. Zinder City district also crossed the alert threshold.

Laboratory confirmation and microbiologic surveillance of meningococcal meningitis is conducted by the Centre de Recherche Médicale et Sanitaire by using culture or PCR (*5*) techniques on cerebrospinal fluid (CSF) or CSF-inoculated trans-isolates. During the study period, the Centre received 816 CSF or trans-isolate specimens (from 69% of the notified cases). Culture (n = 23, 2.8%) and PCR (all specimens) identified *N. meningitidis* as the predominant pathogen (n = 248, 30.4%), followed by *Streptococcus pneumoniae* (n = 35, 4.3%) and *Haemophilus influenzae* (n = 13, 1.6%). Among the 248 cases with confirmed meningococcal etiology, the most frequent serogroup was W135 (n = 121, 48.8%), followed by A (n = 116, 46.8%) and X (n = 2), indicating that serogroup W135 had increased markedly compared with the past 2 years (Figure). Among the 816 CSF specimens, 454 (56%) remained negative when tested for the presence of *N. meningitidis*, *S. pneumoniae*, or *H. influenzae* by PCR. Eighty-four (69.4%) of the serogroup W135 strains originated from the province of Maradi (southern Niger) and, more specifically, 36%.4% (n = 44) and 19.8% (n = 24) originated from the Madarounfa and Maradi districts, respectively. In contrast, serogroup A was mainly present in Tillabéry (western Niger) with 49.1% (n = 57) of the strains and, to a lesser extent, in the provinces of Maradi (16.4%, n = 19) and Dosso (13.8%, n = 16). All meningococcal strains (n = 9 for W135, n = 1 for A) recovered from trans-isolates and analyzed by Etest (AB bioMérieux, Marcy l'Etoile, France) were susceptible to beta-lactams (penicillin, amoxicillin, and ceftriaxone), chloramphenicol, and rifampin. This finding supports the appropriateness of World Health Organization recommendations for antimicrobial drug treatment. The A strain belonged to the sequence type (ST) 7 and the W135 strains to ST 11, the same ST of the strain associated with outbreaks in pilgrims in Saudi Arabia in 2000 (*3*) and the strain that caused the large epidemic in Burkina Faso in 2002 (*1*).

The mean ages of patients with confirmed cases of infection with serogroup W135 and serogroup A were 8.1 (SD 8.5) and 10.9 (SD 7.9) years, respectively. Although no significant difference was found in the mean ages, the age group was 1–4 years of age had more disease caused by serogroup W135, and children 5–14 years of age were most affected by serogroup A. Similarly, the attack rate during the outbreak of meningitis caused by serogroup W135 in Burkina Faso in 2002 was highest in patients <5 years

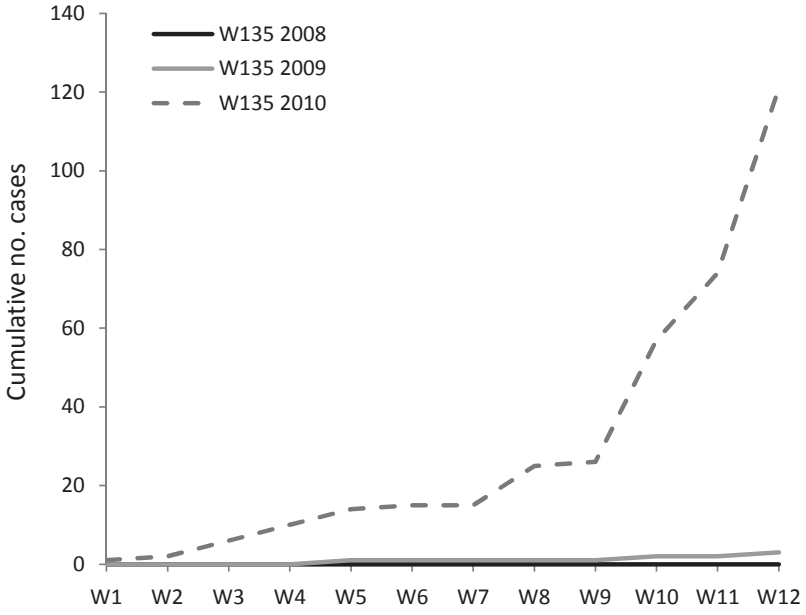

Figure. Epidemic curve of cumulative confirmed cases of *Neisseria meninigitidis* serogroup W135 infections in Niger, 2008, 2009, and 2010 (weeks 1–12). No cases were found in 2008. A color version of this figure is available online (www.cdc.gov/EID/content/16/9/1496-F.htm).

of age, and the attack rate decreased as patients' ages increased (*6*).

Reactive vaccination campaigns in some communes of Madarounfa district that had reached the epidemic threshold were launched by the Ministry of Public Health with a remaining 2009 stockpile (16,527 doses, 35.7% coverage) of the quadrivalent polysaccharide vaccine (A/C/Y/W135) from Médecins sans Frontières. The International Coordinating Group on Vaccine Provision for Epidemic Meningitis Control has also recently approved the release of 381,526 doses of trivalent polysaccharide vaccine (A/C/W135) for vaccination campaigns in Maradi and Zinder districts. Future immunization campaigns will be implemented by Ministry of Public Health with the support of the World Health Organization and partners, including Médecins sans Frontières and The United Nations Children's Fund.

Given the large population at risk, and the low availability and high cost of the trivalent vaccine, a sound vaccination strategy is of particular importance to mitigate the expansion of serogroup W135 in the country. Microbiologic surveillance is critical in the early and accurate detection of meningococcal serogroups for determining the appropriate vaccine.

Acknowledgments

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Toscana Virus Infection in American Traveler Returning from Sicily, 2009

To the Editor: Since the discovery of Toscana virus (TOSV) in 1971 in Tuscany (*1*), sandfly-borne TOSV has become recognized as a leading cause of acute meningitis in central Italy during the summer (*2*). France, Spain, Portugal, Greece, and Cyprus have also reported cases of TOSV infection (*2*). Although TOSV has been detected in sandflies in Sicily (*3*), we are not aware of any historically documented human infection with TOSV in this southernmost region of Italy.

We report TOSV infection of an American male physician, 65 years of age, who traveled to Sicily for 3 weeks and returned to the United States in October 2009. Two days after his return, he awoke with a headache, and hours later he noticed difficulty finding words. His headache progressed, and during the next few hours, he experienced severe expressive dysphasia. At admission to the hospital, he denied having fever, nuchal rigidity, photophobia, nausea, vomiting, or diarrhea.

Other than changing planes in Milan, the patient had remained in Sicily during the entire 3 weeks of his visit. He had sustained both mosquito and what he thought were flea bites while in Sicily. He had no known exposure to bats, rabid animals, or ticks.

Computed tomographic scan and magnetic resonance imaging of the brain showed no mass lesions or abnormality of the cerebral vessels. A sample of cerebrospinal fluid (CSF) obtained at admission showed 14 leukocytes/mm^3 (reference range 0–5 leukocytes/mm^3) with 100% lymphocytes, a protein level of 126 mg/dL (reference range 15–45 mg/dL), and a glucose level of 63 mg/dL (reference range 50–80 mg/dL). A nasopharyn-

geal swab specimen was negative for influenza A and B virus antigens. Other than a decreased thrombocyte count and an elevated serum glucose level, the results of complete blood count, comprehensive chemical panel, and coagulation studies were within normal limits. PCR results for CSF were negative for herpes simplex virus, enterovirus, and parechovirus. Test results for acute-phase and convalescent-phase serum specimens performed at the Washington State Department of Health Laboratory were negative for West Nile virus and St. Louis encephalitis virus immunoglobulin M.

Serum and CSF were sent to the Centers for Disease Control and Prevention in Fort Collins, Colorado. TOSV RNA was detected in a CSF sample collected on day 1 of illness by using reverse transcription–PCR (*4*). Plaque-reduction neutralization assays demonstrated a >4-fold rise in TOSV neutralizing antibodies between paired serum specimens collected on days 1 (titer <1:10) and 21 (titer 1:320) of illness. No similar rise in neutralizing antibodies to serologically related phleboviruses (e.g., sandfly fever Naples virus and sandfly fever Sicilian virus) was detected. The patient received supportive care only. He had a complete neurologic recovery in 10 days and was able to return to work.

Phylogenetic analyses indicate that 2 geographically distinct genotypes, the Italian and Spanish lineages of TOSV, circulate throughout the Mediterranean region (*5*). To determine the lineage of the infecting strain, we performed advanced molecular analyses of TOSV RNA isolated from the infected traveler's CSF. These analyses used published consensus primers that target the small (S) segment (*4*) as well as primers newly designed to target the medium (M) segment: M 851F, 5′-ACCAAATACAACCATAGCCCC-3′ (forward) and M 1327c, 5′-ATA CAATTCCCACAGTCGTTAG-3′ (reverse) of the multisegment TOSV genome. Reverse transcription–PCR amplification and nucleotide sequencing generated 2 nt sequences of 332 (S segment) and 424 (M segment) nucleotides in length. Phylogenetic analyses of the newly determined sequences and sequences previously determined for Mediterranean TOSV isolates of diverse origin were carried out by using MEGA version 4 (*6*). According to phylogenetic inference, the TOSV RNA identified in the returning traveler is of the Italian lineage (Figure). Of interest, the TOSV M segment sequence generated from this patient aggregates with extreme bootstrap support along with that generated previously from a strain of TOSV that was isolated from sand flies in Palermo, Sicily, in 1993 (Figure), indicating that the infecting strain is likely representative of strains that have circulated in Sicily for years.

This case represents the third report of meningitis or meningoencephalitis caused by TOSV infection in a US traveler to the Mediterranean (all acquired in Italy) (*7*,*8*). As is shown by this and other recent reports of TOSV infections in the Mediterranean islands surrounding Italy (*9*), the geographic range of TOSV human infections is larger than previously known. Reports of TOSV infection among European travelers returning from disease-endemic regions have provided additional evidence of the emergence of TOSV-related illness on a global scale (*10*).

Although the clinical course varies from asymptomatic infection to severe meningoencephalitis, TOSV should be included in the differential list of viral pathogens among patients who seek treatment with symptoms consistent with meningitis or encephalitis if the patients have recently traveled to Mediterranean areas, including Sicily. Because neither a vaccine nor specific antiviral drug treatment is available to prevent or treat TOSV infection, travelers to TOSV-endemic areas should be advised to take all precautions to prevent insect bites.

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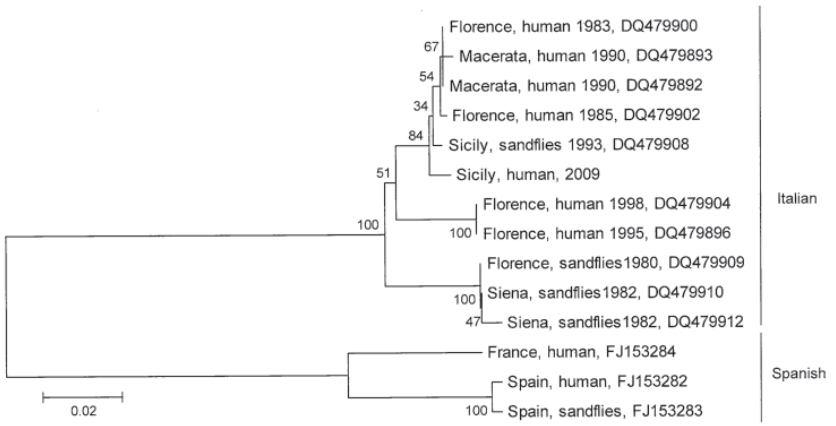

Figure. Phylogeny of Toscana viruses (TOSVs) of diverse origin. Partial small (S) and medium (M) segment sequences of interest were aligned by using ClustalW (www.ebi.ac.uk/Tools/clustalw2/index.html), and neighbor-joining and maximum-parsimony trees were generated by using 2,000 bootstrap replicates with MEGA version 4 (*6*). Highly similar topologies and confidence values were derived by all methods, and a neighbor-joining tree generated from a comparison of 424 nt of the M segment polyprotein gene open reading frame is displayed here. GenBank accession numbers appear after the location and source of isolation for each taxon. Scale bar represents the number of nucleotide substitutions per site. Of interest, the 2009 Sicilian TOSV described in this study (Sicily, human, 2009) aggregates with extreme support along with other Italian viruses, including an isolate that was derived from sandflies in Palermo, Sicily, in 1993.

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Hospital Discharge Data for Guillain-Barré Syndrome and Influenza A (H1N1) Vaccine Adverse Events

To the Editor: As part of the public health response to the current pandemic (H1N1) 2009, surveillance for adverse events following vaccination for influenza A (H1N1) is a high priority (*1*). Surveillance for Guillain-Barré syndrome (GBS) has been of particular interest, because the syndrome was associated with the 1976–1977 swine influenza vaccine (*1*,*2*). To study this association, reliable ascertainment of recent incident cases of GBS is necessary.

GBS is an acute, immune-mediated paralytic disorder of the peripheral nervous system (*3*–*5*) with an estimated annual incidence of 0.8–1.9/100,000 (*6*). Most cases are associated with an antecedent infection (*6*). Several surveillance systems are in place to monitor rates of postvaccination GBS (*1*–*3*), most of which include a component of electronic administrative record review for case detection. Analysis of computerized medical databases is a well-established method of monitoring for vaccine adverse events (*7*). Although the validity of such data varies, depending on the diagnosis and region, few studies have evaluated the use of hospital discharge data for GBS specifically (*8*,*9*).

We reviewed the Tennessee Department of Health Uniform Hospital Discharge Dataset for all hospital discharge diagnoses in 4 major metropolitan regions of Tennessee in 2002–2003 with codes from the International Classification of Diseases, 9th Revision, Clinical Modification (ICD-9-CM), that might indicate acute GBS. Records with ICD-9-CM code 357.0 (acute infective polyneuritis) or other combinations suggestive of GBS within the top 10 diagnoses were requested. These data were compared with information on cases identified by directly requesting lists of patients with discharge diagnoses of GBS from hospital medical record departments. Charts of all reported cases were validated by chart review. Patients were classified as having acute GBS if they met Brighton Criteria Levels 1, 2, or 3 (*10*).

A total of 344 records of possible cases of acute GBS were identified. Of these cases, 215 (63%) were identified through the state hospital discharge database, 315 (92%) were reported directly by hospitals, and 186 (54%) were identified by both systems. Among all suspected cases identified, only 103 (30%) met criteria for acute GBS (annual rate 2.1/100,000 population), 14 (4%) were in out-of-state residents, 114 (33%) were nonacute cases that occurred before the study period and patients were readmitted for other reasons, 90 (26%) had no documentation of GBS in the medical record, 17 (5%) were duplicate reports, and 6 (2%) had insufficient information for further investigation. The predictive-value positive of a

GBS diagnostic code from the statewide hospital discharge database representing acute GBS was only 30%. Of the 103 confirmed cases, 26 (25%) would have been missed if only the state hospital discharge database was used to identify potential cases.

Of 103 cases, all were identified with ICD-9-CM diagnosis code 357.0; in 91 (88%) cases, this was the primary diagnosis. Other combinations of codes did not identify additional cases. Of cases of acute GBS identified, 32 (30%) met only clinical criteria (Brighton Level 1), 40 (39%) had either laboratory or electrophysiologic evidence (Brighton Level 2), and 32 (31%) had both (Brighton Level 3).

Because the 2 surveillance systems we compared both relied on medical record discharge diagnoses, they were not independent, and we could not perform a capture/recapture analysis. Because GBS is a diagnosis for which the great majority of patients are hospitalized, and our overall incidence rate is within the range identified in other studies, it is likely that the combination of these methods is reasonably sensitive. The administrative hospital discharge database could not be relied on to confirm that all coded GBS cases were acute. Even if the 114 nonacute cases could easily have been identified and excluded from the initial list of 344 records, only 103 (45%) of the remaining 230 reports were identified as confirmed acute cases.

Although the use of large hospital discharge databases may be useful as an adjunct for identification of GBS cases as part of public health surveillance, they lack sufficient sensitivity or specificity to be relied upon exclusively. The poor specificity of the system is particularly problematic for public health surveillance. A large investment of time and resources was necessary to perform manual chart reviews to confirm possible cases, two-thirds of which were ultimately found not to be cases at all. Statewide administrative hospital discharge diagnosis databases should not be solely relied on for GBS surveillance. Additional methods of reliable and efficient ascertainment and verification of cases are crucial to ensure valid data. Obtaining reliable methods is particularly important for urgent situations such as current surveillance for adverse events after pandemic (H1N1) 2009 virus vaccination, in which the detection of problems will have immediate public health effects.

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Contact Lens Solution–associated *Acanthamoeba* and *Fusarium* Keratitis

To the Editor: Verani et al. (*1*) detailed the 2004–2007 outbreak of *Acanthamoeba* keratitis (AK) in persons wearing soft contact lenses who used Complete MoisturePlus (CMP) multipurpose contact lens solution (Advanced Medical Optics, Santa Ana, CA, USA). They noted similarities between the AK outbreak and the *Fusarium* keratitis (FK) outbreak of 2004–2006, including the concomitant time frame and association with a particular solution, ReNu with MoistureLoc (Bausch & Lomb, Rochester, NY, USA). Both solutions were new products introduced within 1 year before the respective outbreaks.

In neither outbreak was the solution contaminated; in both outbreaks,

implicated bottles were from multiple lots, suggesting that each outbreak resulted from insufficient antimicrobial activity. However, in the FK outbreak, all reported cases involved bottles produced at 1 (Greenville, SC, USA) of 4 multinational Bausch & Lomb manufacturing plants (*2*). After a Food and Drug Administration inspection of the Greenville facility, Bausch & Lomb was cited for inadequacies in temperature control during production, storage, and transport of its products in and beyond the plant (*3*).

We experimentally demonstrated that, when exposed to prolonged temperature elevation, ReNu with MoistureLoc loses more in vitro fungistatic activity than do other contact lens solutions. We concluded that improper temperature control of ReNu with MoistureLoc may have contributed to the FK outbreak (*4*). We are aware of no other theory that adequately explains why only ReNu with MoistureLoc from only 1 plant was implicated.

CMP was manufactured and used internationally; AK has a much higher incidence in Europe and Hong Kong than in the United States (*5*), and CMP–associated AK has been reported internationally (*6*). Therefore, it would seem critical to know, and we would like the authors to comment on, whether the geographic pattern of the AK coincided with distribution of CMP solution from ≥1 Advanced Medical Optics manufacturing plants and, if so, the relevance of that information.

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In Response: We thank Bullock and Warwar for offering their theory of potential consequences of manufacturing inadequacies in temperature control during production of ReNu with MoistureLoc (Bausch & Lomb, Rochester, NY, USA) associated with the *Fusarium* keratitis (FK) outbreak during 2004–2006 (*1*). They note the substantial similarities between the FK outbreak and the *Ancanthamoeba* keratitis (AK) outbreak that we reported (*2*). They inquire whether the geographic pattern of AK outbreak–associated cases coincides with distribution of ≥1 manufacturing plants for the associated product, Complete MoisturePlus (CMP) multipurpose contact lens solution (Advanced Medical Optics [AMO], Santa Ana, CA, USA).

We obtained lot numbers for 22 bottles of CMP that AK case-patients used before symptom onset. Because no lot number was repeated, intrinsic contamination was unlikely as the source of the AK outbreak; the geographic and temporal distribution of cases further argued against a point-source outbreak. All 17 lot numbers for which AMO plant of origin was determined were manufactured in Spain (Food and Drug Administration, pers. comm.). According to a press release from AMO in November 2006, the "vast majority of AMO's contact lens solution products distributed in the U.S." were manufactured in the company's production facility in Spain, 1 of its 2 international manufacturing plants (*3*).

CMP was produced and used internationally at the time of the US multistate outbreak (*4*). Por and colleagues (*5*) reported an increase in the number of AK cases among contact lens users in Singapore that temporally coincided with the US outbreak. However, their retrospective case series did not include a control group; therefore, measuring associations between particular contact lens products and AK was not possible for those case-

patients. The authors reported that a case–control study was underway, and we look forward to seeing the results of that investigation to better understand the magnitude of AK cases associated with CMP use.

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New Infectious Diseases and Industrial Food Animal Production

To the Editor: Cutler et al. bring welcome attention to the importance of new and reemerging zoonotic diseases in the industrialized world (*1*). However, they make no mention of industrialized systems of food animal production, major sources of antimicrobial drug–resistant bacterial pathogens (*2*) that are among the most globally prevalent and emerging infectious diseases (*3*). These systems have practices characterized by crowded and unsanitary confinement of animals and routine use of antimicrobial agents in animal feeds (*2*). For example, in the same issue, Dutil et al. (*3*) reported on increases in ceftiofur resistance in *Salmonella enterica* isolates from food, which they associate with use of this drug in broiler poultry production.

Recognition of the role of industrial food animal production in driving vancomycin resistance in enterococci prompted restrictions on agricultural antimicrobial drug use in the European Union; unfortunately, few measures have been implemented in the rest of the world (including the United States) (*4*). Industrialized food animal production is now assumed to contribute to the emergence of new strains of community-associated methicillin-resistant *Staphylococcus aureus* with varying potential for infecting humans (*5*). Because the industrial model of food animal production is rapidly expanding globally (*2*), this source must be included in surveillance, research, and tracking programs for effective prevention of emerging zoonotic disease.

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In Response: Silbergeld et al. highlight pertinent points about how stochastic events can lead organisms to acquire adaptive advantages through lateral gene transfer (*1*). Word constraints of our earlier article precluded detailed debate of many such topics, and we welcome the opportunity to discuss this further. The role of industrial food animal production in driving the development of antimicrobial drug–resistant pathogens is indeed a topic of great concern.

Commonly, reemergence of infections is caused by changes in the environment or the host, genetic changes of pathogens, or alteration in the dynamic interactions that unite them. Our need for intensive protein production can have explosive consequences, as seen with the recent outbreak of Q fever among humans residing near goat farming areas in the Netherlands (*2*) and the emergence of antimicrobial drug–resistant organism variants with selective advantages, such as methicillin-resistant *Staphylococcus aureus* (*3*). The bombardment of livestock with antimicrobial drugs for therapy and prophylaxis and as growth-enhancing agents (in Europe before 2006) has provided selective pressure for acquisition of resistance, which occurs globally (*4*). Even exposure to various biocides has been linked with acquisition of resistance to therapeutic antimicrobial agents (*5*), although such resistance has not yet been demonstrated in natural populations. Risk prevention within and management of intensified food production systems is a continuing challenge. Similarly problematic are pathogens that increase in general, such as RNA viruses that under the recent selective pressure have rapidly acquired resistance to oseltamivir (*6*). A common feature of all these facts is that such traits and clones of increased fitness can disseminate rapidly around the globe. For these reasons, we need robust surveillance mechanisms; ability to predict spread; cohesive intervention strategies; and lastly, but by no means least, strong collaborative links between previously segregated human and veterinary fields that extend to producers and policy makers.

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Antimicrobial Resistance: Beyond the Breakpoint

J. Todd Weber, editor

S. Karger AG, Basel, Switzerland

IBSN: 3805593236
Pages: 174; Price: US $189.00

The Fifth Decennial International Conference on Healthcare-Associated Infections, held March 2010 in Atlanta, Georgia, USA, brought the issue of antimicrobial drug resistance to the forefront. Results of a survey that polled members of the Society for Healthcare Epidemiology of America regarding critical knowledge gaps and research priorities were presented. Respondents reported that 3 of the 5 most important issues they face are directly associated with antimicrobial drug resistance: multidrug–resistant gram-negative bacteria, antimicrobial stewardship, and methicillin-resistant *Staphylococcus aureus* (MRSA).

The publication of this collection of authoritative reviews of these issues is therefore timely. This concise volume draws on the knowledge of 23 authors, many of whom are either current or past staff members of the Centers for Disease Control and Prevention. In 10 chapters and 174 pages, these authors address contemporary issues in bacterial, fungal, parasitic, and viral (HIV) resistance, as well as some aspects of the effects of antimicrobial drug resistance on healthcare facilities. The main emphasis of the book is on the epidemiology and mechanisms and public health implications of resistant pathogens, not on details of treatment. Chapters on MRSA, extended-spectrum β-lactamase–producing gram-negative bacteria, and fluoroquinolone resistance cover the epidemiology and mechanisms of resistance, laboratory detection, and treatment considerations. A historical review of the contribution of bacterial pneumonia to the death rate of previous influenza pandemics clearly discusses the likelihood and consequences of resistance in strains of *Streptococcus pneumoniae* and *S. aureus*. Questions raised here about how these antimicrobial drug–resistant bacteria affect a modern pandemic, such as the impact of antiviral drugs and diagnostic testing on antibiotic use, can now be investigated in light of the subsequent pandemic (H1N1) 2009.

Three chapters address resistance in healthcare settings and the promotion of appropriate antimicrobial drug use. The first describes interventions to reduce the inappropriate use of antimicrobial agents for respiratory conditions and quality initiatives that can improve prescribing. The chapter that reviewed effective strategies for controlling resistant pathogens in hospitals is the only chapter that was not well referenced on some of the more controversial issues, such as silver coating of devices. However, the chapter on estimating costs attributable to infections caused by antimicrobial drug–resistant bacteria is comprehensive.

Three final chapters expand the scope of this volume into issues of parasitic, fungal, and viral resistance. Resistance in helminths is presented in the context of mass treatment during eradication programs and the need for enhanced surveillance programs. The chapter on antifungal resistance reviews available drugs and laboratory detection of resistance. The final chapter on HIV drug resistance in the developing world reviews initial concerns and current encouraging data on antiretroviral drug–resistance in sub-Saharan Africa and southern Asia. Current public health strategies for detecting and controlling drug–resistant HIV are given, along with a clear account of the biological and pharmacologic factors that affect HIV resistance and a review of areas needing continued attention and resources.

The strength of this book is the wide scope of its coverage of antimicrobial drug resistance. Most chapters are well written in a succinct style and format easily accessible to the general reader. The text has supportive references from primary sources, and contains a good index. This book is a valuable resource for those beginning their careers or who are looking for a research focus, or for anyone already an expert in an aspect of antimicrobial resistance who is seeking a broader perspective.

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Tuberculosis (Biographies of Disease)

Carol A. Dyer

Greenwood Press, Santa Barbara, California, USA, 2010

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Pages: 146; Price: US $45.00

A great story has drama, mystery, conflict, interesting characters, intrigue, and excitement. The story of tuberculosis (TB) has all of these. It is a story of epic proportions. Carol Dyer's Tuberculosis (Biographies of Disease) is not a textbook or a history book; it tells us the story of this fascinating but deadly disease.

Dyer, a science writer, takes us from the mummies of ancient Egypt, with their visible signs of TB, to the

skepticism that met Robert Koch's discovery of the tubercle bacillus, to today's global TB public health emergency. Thought by many to be a disease of the past, the final chapters of the book jolt us into today's reality.

As the drama unfolds, Dyer describes how TB ravaged Europe's working class during the industrial revolution. More personal accounts from the Romantic Age are especially interesting. She tells us how 6 siblings of the famed literary Bronte family died of TB. She describes the tragic death from TB of the poet John Keats at age 26. And the personal anecdotes continue; from the scientists who advanced our knowledge of TB to the artists whose lives and art were affected. By describing its influence on literature and the arts, Dyer brings to life the profound effects of TB on humanity.

Her discussion of the victories and setbacks in the fight against TB provide a context for what she considers to be the story's main plot: how is it possible that TB remains a leading cause of death from infectious disease globally? Her description of the complex biology of the organism and the societal characteristics of the disease help us understand why, despite the discovery of effective chemotherapy, TB continues to devastate.

The final chapters provide a sobering picture of the current state of TB. Dyer describes the serious effect of the HIV epidemic on TB and warns of the alarming rates of more dangerous forms of drug-resistant TB. However, the story she tells ultimately becomes a hopeful one. She discusses how the world community has come together, leading to new funding initiatives and prevention and control strategies.

This book is a quick and easy read (120 pages). However, the organization of its 8 chapters is peculiar. The reader might be tempted to skip to the third chapter on the history of TB or even stop reading, as he or she get bogged down in some of the medical details in the first 2 chapters. Occasional overuse of technical detail gets in the way of the story. Sidebars with anecdotes and scientific summaries, scattered throughout, are a nice addition to the format of the book. The timeline at the end is also helpful.

The book falls short in describing the epidemiology of TB. More quantitative information would provide the reader with a better understanding of the magnitude of the problem. Despite this issue and several minor technical inaccuracies, the book is informative and at times exciting. It captures all the elements of this great story. Overall, this book is a great read for public health professionals and the general public. For the reader engaged in global public health efforts, the book should be a call to action.

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Thomas Hart Benton (1889–1975), *Interior of a Farm House* (1936) Tempera on board (45.7 cm × 76.2 cm), Courtesy of The John and Mable Ringling Museum of Art, The State Art Museum of Florida, a division of Florida State University, USA

The Soot That Falls from Chimneys

Polyxeni Potter

"The best damned painter in America," is how Harry S. Truman described Thomas Hart Benton, his choice to create a mural for the Truman Library in Independence, Missouri. The President's fellow Missourian lived in Kansas City at the time, his artistic career in a slump, though his reputation as a fine muralist still intact. "I picked him," Mr. Truman told the crowd at the mural's dedication, "because he was the best, and this is the finest work by the best."

During work on the Library mural, the President noted that he got along with the painter, even though, he joked, "That's hard for anyone to do." A gifted musician, writer, and prolific lithographer, Benton was also direct, impatient, radical, and often tactless. He hated museums, professing that art works belonged in clubs and barrooms, "Anywhere anybody had time to look at 'em." In his autobiography, he mused, "A few people have, at times, expressed a belief that I was not the most desirable kind of fellow to have around. But, all in all, my differences with the home folks, when looked at in perspective, have not amounted to much."

Born in a fiercely political family, the artist was named after his uncle Thomas Hart Benton, the first and longest serving U.S. Senator of Missouri. His father, a populist, was a member of the U.S. House of Representatives. Benton bucked family tradition and entered the Art Institute of Chicago, in 1907, aspiring to become a cartoonist. His longtime love of painting affirmed in the fine arts environment, he traveled to Paris, where he attended the Académie Julien and Académie Colarossi. He met Mexican muralist Diego Rivera and studied the masters at the Louvre, among them El Greco, whose exaggerated forms found their way into his mature style.

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Back in New York for the lack of funds, he explored the local scene and such prevailing styles as impressionism and synchromism, especially in the work of Stanton MacDonald-Wright, who became his friend. His career took a different turn when he joined the Navy. "The most important thing, so far, I had ever done for myself as an artist." This work, which involved extensive documentary drafts and drawing, had an enduring effect on his style. "When I came out of the Navy after the First World War, I made up my mind that I wasn't going to be just a studio painter, a pattern maker in the fashion then dominating the art world—as it still does. I began to think of returning to the painting of subjects, subjects with meanings, which people in general might be interested in."

Though by this time well-versed in modernism, Benton turned against it unable to embrace its "colored cubes and classic attenuations," rejecting it as "divorced from the common ways of the day." He moved toward naturalistic and representational work focused on the American scene. He taught at the Art Students League, instructing many who went on to become famous in their own right, not the least of them abstract expressionism icon Jackson Pollock. "Even after I had castigated his innovations and he had replied by saying I had been of value to him only as someone to react against, he kept in personal touch with me …."

Benton's style, a blend of modern and academic elements, came to be known as regionalism. Others in this movement were Grant Wood, most famous for his *American Gothic*, and John Steuart Curry, who painted life in his native Kansas. Many criticized their choice of the local over the cosmopolitan, but the regionalists, particularly Benton, struggled with the notion of an authentic American voice long before New York became an art center. "You just can't think of art in terms of progress," Benton explained in an interview, "It is not progressive. It is just different from age

to age. One age gets used to a certain kind of art form and thinks that is better, but the next age will deny that thought and go back to some older form. So I wouldn't compare the animal paintings of the cavemen with those of our times or any other times …. We were as good, as artists, when we began our history as we are now—sometimes better."

The 1930s, a decade of unprecedented economic hardship in the United States, witnessed renewed interest in history reflected in all aspects of culture. Murals, among them *The Social History of Missouri* in Jefferson City, which Benton considered his masterpiece, were part of this resurgence, recording as they were milestones in the country's development. Benton captured his era's transformation from rural and agrarian to urban and industrial. He painted the growth of business and technology, and the consequent changes in the lives of the common people, in paintings of steamboats and trains, factories, logging and mining operations, offices and farmhouses. "History was not a scholarly study for me but a drama." He was innovative, bold, outspoken, and unafraid of controversy, allowing myth to blend with observation, casting ordinary people as heroes and pioneers. "I wasn't so much interested in famous characters as I was in Missouri and the ordinary run of Missourians that I'd known in my life."

Benton made a habit of gallivanting around the countryside, meeting people and sketching them and their surroundings. Later he would lay out his designs from these pencil sketches, using pen and ink to define and preserve them. These and three-dimensional clay models he created served as prototypes for oil and tempera studies and for larger compositions. While many critics objected to his subjects, bold colors, manipulated forms, or muscular style, few found fault with his compositional and architectural skills. He had a talent for incorporating multiple themes in limited space and still maintaining cohesiveness.

Interior of a Farmhouse on this month's cover offers a glimpse of the brilliant color, energy, and movement that characterize Benton's art and the complexity and richness of his murals. The title understates this intricate composition. The farmhouse at center stage anchors a community of scenes connected by a fence here, a doorway there, an angle, a partial wall, and contains his favorite people: workers doing what they do in the kitchen, the barn, the fields, at rest. On the periphery, steamboat navigation and the wheels of industry are rolling, their ubiquitous smokestacks belching above the Missouri River. Court is in progress; a worker reads the daily news; another washes up; animals wander in and outdoors. The painter reviews American industry in the 1930s, which pulsates, as if it were a live, breathing organism itself.

The values of honest living and hard labor, at the heart of Benton's work, went hand in hand with the belief that harmony between humans and nature resided on the farm, the interior of which in this painting is not altogether filled with agrarian bliss. Despite the energy emanating from the vibrant community, there are tensions, political and ecologic undertones, part and parcel of industrialization. Benton the social historian sensed the dark side of factories and increased transportation, which he noted in palpable terms, a cloud so menacing against the pristine horizon it unfolded half way across the painting.

"The yellow smoke that rubs its muzzle on the window-panes/…Let fall upon its back the soot that falls from chimneys/," Benton's fellow Missourian T.S. Eliot wrote prophetically in "The Love Song of J. Alfred Prufrock." As they settle, the dark plumes from smokestacks, a fixture in the artist's work signaling the machine's intrusion, cause havoc in the farmhouse. "The harmony man had with his environment has broken down," he wrote. "Now men build and operate machines they don't understand and whose inner workings they can't even see."

Choked by industrial and other pollution, we have come to resemble Benton's farmhouse, an organism under stress, because "man doesn't escape his environment." The human lung, at the center of the body's complex internal operations is also affected by external factors in the environment: pollution, infectious agents, allergens. These factors, along with causing many other local and global adverse effects, complicate and aggravate a host of respiratory problems from rhinovirus infection, influenza, and pneumonia, to pneumococcal disease, tuberculosis, and legionellosis, now found to spread around the community from the water tank of a paving machine. Once again, the farmhouse is threatened by what's lurking in "The yellow fog that rubs its back upon the window-panes."

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EMERGING INFECTIOUS DISEASES

Upcoming Issue

Efficacy of Personal Protective Equipment and Oseltamivir during Influenza A (H7N7) Epidemic, the Netherlands

Monitoring for Slow Response to Tuberculosis Therapy in a State Control Program, Virginia, USA

Highly Pathogenic Avian Influenza (H5N1) Risk in Southern Asia and Satellite Tracking of Wild Waterfowl

Oral Fluid Testing during 10 Years of Rubella Elimination, England and Wales

Human Monkeypox Outbreak Caused by Novel Virus belonging to Congo Basin Clade, Sudan

Bloodstream Infection with *Mycobacterium tuberculosis* and Other Pathogens among HIV-positive Outpatients, Southeast Asia

Mobile Phone–based Surveillance System, Sri Lanka

Pandemic (H1N1) 2009 Virus Seroconversion in Hospital Staff, Singapore

Influenza (H5N1) Viruses from Pigs, Indonesia

Changing Epidemiology of Pulmonary Nontuberculous Mycobacterial Disease

Erythema Migrans-like Illness among Caribbean Island Residents

Type 2 Diabetes and Increased Risk for Malarial Infection

Imported Lassa Fever, Pennsylvania, USA, 2010

Genetic Characterization of Nipah Virus from Naturally Infected Bats, Malaysia

Predicting Hospitalization for Patients with Pandemic (H1N1) 2009

Patterns of Death for Hemorrhagic Fever with Renal Syndrome Caused by Puumala Virus

Malaria in Traveler Returning from Africa and Misuse of Artesunate

Klassevirus Infection in Children, South Korea

Complete list of articles in the October issue at
http://www.cdc.gov/eid/upcoming.htm

Upcoming Infectious Disease Activities

August 28–September 1, 2010
Infectious Disease 2010 Board Review Course – 15th Annual Comprehensive Review for Board Preparation
Hyatt Regency Crystal City
Arlington, VA, USA
http://www.IDBoardReview.com

November 6–10, 2010
APHA 138th Annual Meeting and Expo
Denver, CO, USA
http://www.apha.org/meetings

November 11–13, 2010
European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE)
Lisbon, Portugal
http://www.escaide.eu

Announcements

To submit an announcement, send an email message to EIDEditor (eideditor@cdc.gov). In 50–150 words, describe timely events of interest to our readers. Include the date of the event, the location, the sponsoring organization(s), and a website that readers may visit or a telephone number or email address that readers may contact for more information.

Announcements may be posted on the journal Web page only, depending on the event date.

Announcements are no longer submitted through Manuscript Central.

Earning CME Credit

To obtain credit, you should first read the journal article. After reading the article, you should be able to answer the following, related, multiple-choice questions. To complete the questions and earn continuing medical education (CME) credit, please go to **http://cme.medscape.com/viewpublication/30063**. Credit cannot be obtained for tests completed on paper, although you may use the worksheet below to keep a record of your answers. You must be a registered user on Medscape.com. If you are not registered on Medscape.com, please click on the New Users: Free Registration link on the left hand side of the website to register. Only one answer is correct for each question. Once you successfully answer all post-test questions you will be able to view and/or print your certificate. For questions regarding the content of this activity, contact the accredited provider, CME@medscape.net. For technical assistance, contact CME@webmd.net. American Medical Association's Physician's Recognition Award (AMA PRA) credits are accepted in the US as evidence of participation in CME activities. For further information on this award, please refer to http://www.ama-assn.org/ama/pub/category/2922.html. The AMA has determined that physicians not licensed in the US who participate in this CME activity are eligible for *AMA PRA Category 1 Credits*™. Through agreements that the AMA has made with agencies in some countries, AMA PRA credit is acceptable as evidence of participation in CME activities. If you are not licensed in the US and want to obtain an AMA PRA CME credit, please complete the questions online, print the certificate and present it to your national medical association.

Article Title

Recurrent *Granulibacter bethesdensis* Infections and Chronic Granulomatous Disease

CME Questions

1. . A 35-year-old man presents with a history of X-linked chronic granulomatous disease (CGD) in the hospital. He was admitted after 3 weeks of fever. Physical examination reveals obvious lymphadenopathy. Laboratory evaluation reveals an elevated erythrocyte sedimentation rate, but a Gram stain for organisms on 1 of his lymph nodes is negative.

This patient is most likely infected with which of the following organisms?

A. Group B *Streptococcus* species
B. *Mycoplasma pneumoniae*
C. *Aspergillus* species
D. *Campylobacter jejuni*

2. Which of the following statements about a possible infection with *Granulibacter bethesdensis* in this patient is most accurate?

A. This infection is unlikely, given the patient's duration of fever
B. Lymphadenopathy was present in all cases of infection with *G. bethesdensis*
C. Infection with *G. bethesdensis* does not promote an elevation in the erythrocyte sedimentation rate
D. *G. bethesdensis* was evident on bacterial staining in all cases

3. The patient has a battery of tests and procedures to establish a causative organism for his infection. Which of the following statements about the diagnosis of G. bethesdensis infection is most accurate?

A. *G. bethesdensis* cultures grew readily, but only on Middlebrook media
B. PCR amplification testing of excised tissue could identify *G. bethesdensis*
C. All infections with *G. bethesdensis* could be traced to the same strain
D. There was no evidence of reactivation of latent infection

4. A bacterial culture from your patient is now growing *G. bethesdensis*. Which of the following statements about his treatment and prognosis is most accurate?

A. Ceftriaxone is a good choice of treatment
B. Macrolide antibiotics are particularly effective for treating *G. bethesdensis*
C. Disseminated *G. bethesdensis* infection was uniformly fatal
D. *G. bethesdensis* responds to only 1 or 2 antibiotics

Activity Evaluation

1. The activity supported the learning objectives.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
2. The material was organized clearly for learning to occur.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
3. The content learned from this activity will impact my practice.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
4. The activity was presented objectively and free of commercial bias.				
Strongly Disagree				Strongly Agree
1	2	3	4	5

Earning CME Credit

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Article Title

Illicit Drug Use and Risk for USA300 Methicillin-Resistant *Staphylococcus aureus* Infections with Bacteremia

CME Questions

1. Who is most likely to acquire soft-tissue community-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) USA300 strain in an outbreak in the United States?

A. A 21-year-old male wrestler
B. A 40-year-old female prisoner
C. A 29-year-old female prostitute
D. A 32-year-old female illicit drug user

2. A 54-year-old, female veteran is admitted to a Veterans Affairs hospital with a suspected diagnosis of sepsis. Blood cultures are positive for MRSA USA300 strain. On the basis of this study, what are the implications of this information for directing this patient's care?

A. Illicit drug users are more likely to have an infection with an S aureus strain other than USA300, making a history of drug use unlikely in this patient
B. Although illicit drug use is a risk factor for acquisition of infection, the percentage of infection occurring in persons without a history of drug use has increased, and this individual should not be assumed to have a history of drug use
C. The percentage of infection occurring in illicit drug users increased over 4 years, and it is very likely that this individual acquired her infection through this mechanism
D. Although the risk for community-acquired MRSA increased in drug-using men over the period from 2004 to 2008, no similar increase occurred in women and a history of illicit drug use is less likely in female patients

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

EMERGING INFECTIOUS DISEASES www.cdc.gov/eid

JOURNAL BACKGROUND AND GOALS

What are "emerging" infectious diseases?

Infectious diseases whose incidence in humans has increased in the past 2 decades or threatens to increase in the near future have been defined as "emerging." These diseases, which respect no national boundaries, include

- New infections resulting from changes or evolution of existing organisms.
- Known infections spreading to new geographic areas or populations.
- Previously unrecognized infections appearing in areas undergoing ecologic transformation.
- Old infections reemerging as a result of antimicrobial resistance in known agents or breakdowns in public health measures.

Why an "Emerging" Infectious Diseases journal?

The Centers for Disease Control and Prevention (CDC), the agency of the U.S. Public Health Service charged with disease prevention and health promotion, leads efforts against emerging infections, from AIDS, hantavirus pulmonary syndrome, and avian flu, to tuberculosis and West Nile virus infection. CDC's efforts encompass improvements in disease surveillance, the public health infrastructure, and epidemiologic and laboratory training.

Emerging Infectious Diseases represents the scientific communications component of CDC's efforts against the threat of emerging infections. However, even as it addresses CDC's interest in the elusive, continuous, evolving, and global nature of these infections, the journal relies on a broad international authorship base and is rigorously peer-reviewed by independent reviewers from all over the world.

What are the goals of Emerging Infectious Diseases?

1) Recognition of new and reemerging infections and understanding of factors involved in disease emergence, prevention, and elimination. Toward this end, the journal

   - Investigates factors known to influence emergence: 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.
   - Reports laboratory and epidemiologic findings within a broader public health perspective.
   - Provides swift updates of infectious disease trends and research: new methods of detecting, characterizing, or subtyping pathogens; developments in antimicrobial drugs, vaccines, and prevention or elimination programs; case reports.

2) Fast and broad dissemination of reliable information on emerging infectious diseases. Toward this end, the journal

   - Publishes reports of interest to researchers in infectious diseases and related sciences, as well as to public health generalists learning the scientific basis for prevention programs.
   - Encourages insightful analysis and commentary, stimulating global interest in and discussion of emerging infectious disease issues.
   - Harnesses electronic technology to expedite and enhance global dissemination of emerging infectious disease information.

EMERGING INFECTIOUS DISEASES®

August 2010

Influenza

Courtesy of Kröller-Müller Museum, Otterlo, the Netherlands

Emerging Infectious Diseases is a peer-reviewed journal established expressly to promote the recognition of new and reemerging infectious diseases around the world and improve the understanding of factors involved in disease emergence, prevention, and elimination.

The journal is intended for professionals in infectious diseases and related sciences. We welcome contributions from infectious disease specialists in academia, industry, clinical practice, and public health, as well as from specialists in economics, social sciences, and other disciplines. Manuscripts in all categories should explain the contents in public health terms. For information on manuscript categories and suitability of proposed articles see below and visit www.cdc.gov/eid/ncidod/ EID/instruct.htm.

Emerging Infectious Diseases is published in English. To expedite publication, we post articles online ahead of print. Partial translations of the journal are available in Japanese (print only), Chinese, French, and Spanish (www.cdc. gov/ncidod/EID/trans.htm).

Instructions to Authors

Manuscript Preparation. For word processing, use MS Word. List the following information in this order: title page, article summary line, keywords, abstract, text, acknowledgments, biographical sketch, references, tables, figure legends, appendixes, and figures. Each figure should be in a separate file.

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. Include up to 10 keywords; use terms listed in Medical Subject Headings Index Medicus.

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. Provide figures as separate files, not embedded in MS Word. Use Arial font for text content. Place keys within figure area. Provide footnotes and other information (e.g., source/copyright data, explanation of boldface) in figure legend. Submit figures with text content in native, editable, PC file formats (e.g., MS Excel/PowerPoint). Submit image files (e.g., electromicrographs) without text content as high-resolution (300 dpi/ppi minimum) TIFF or JPG files. Submit separate files for multiple figure panels (e.g., A, B, C). EPS files are admissible but should be saved with fonts embedded (not converted to lines). No PNG or BMP files are admissible. For additional guidance, contact fue7@cdc.gov or 404-639-1250.

Manuscript Submission. Include a cover letter indicating the proposed category of the article (e.g., Research, Dispatch) and verifying that the final manuscript has been seen and approved by all authors. Complete provided Authors Checklist. To submit a manuscript, access Manuscript Central from the Emerging Infectious Diseases web page (www.cdc.gov/eid).

Types of Articles

Perspectives. Articles should be under 3,500 words and should include references, not to exceed 40. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), a one-sentence summary of the conclusions, and a brief biographical sketch. Articles in this section should provide insightful analysis and commentary about new and reemerging infectious diseases and related issues. Perspectives may also 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. If detailed methods are included, a separate section on experimental procedures should immediately follow the body of the text.

Synopses. Articles should be under 3,500 words and should include references, not to exceed 40. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), a one-sentence summary of the conclusions, and a brief 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. If detailed methods are included, a separate section on experimental procedures should immediately follow the body of the text.

Research Studies. Articles should be under 3,500 words and should include references, not to exceed 40. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), a one-sentence summary, and a brief 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 be under 3,500 words and should include references, not to exceed 40. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), a one-sentence summary of the conclusions, and a brief 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 a brief biographical sketch. Dispatches are updates on infectious disease trends and research. The articles 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.

Commentaries. Thoughtful discussions (500–1,000 words) of current topics. Commentaries may contain references but no figures or tables.

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.

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.

Books, Other Media. Reviews (250–500 words) of new books or other media on emerging disease issues are welcome. Name, publisher, number of pages, other pertinent details should be included.

Announcements. We welcome brief announcements (50–150 words) of timely events of interest to our readers. (Announcements may be posted online only, depending on the event date.)

Conference Summaries. Summaries of emerging infectious disease conference activities are published online only. Summaries, which should contain 500–1,000 words, should focus on content rather than process and may provide illustrations, references, and links to full reports of conference activities.