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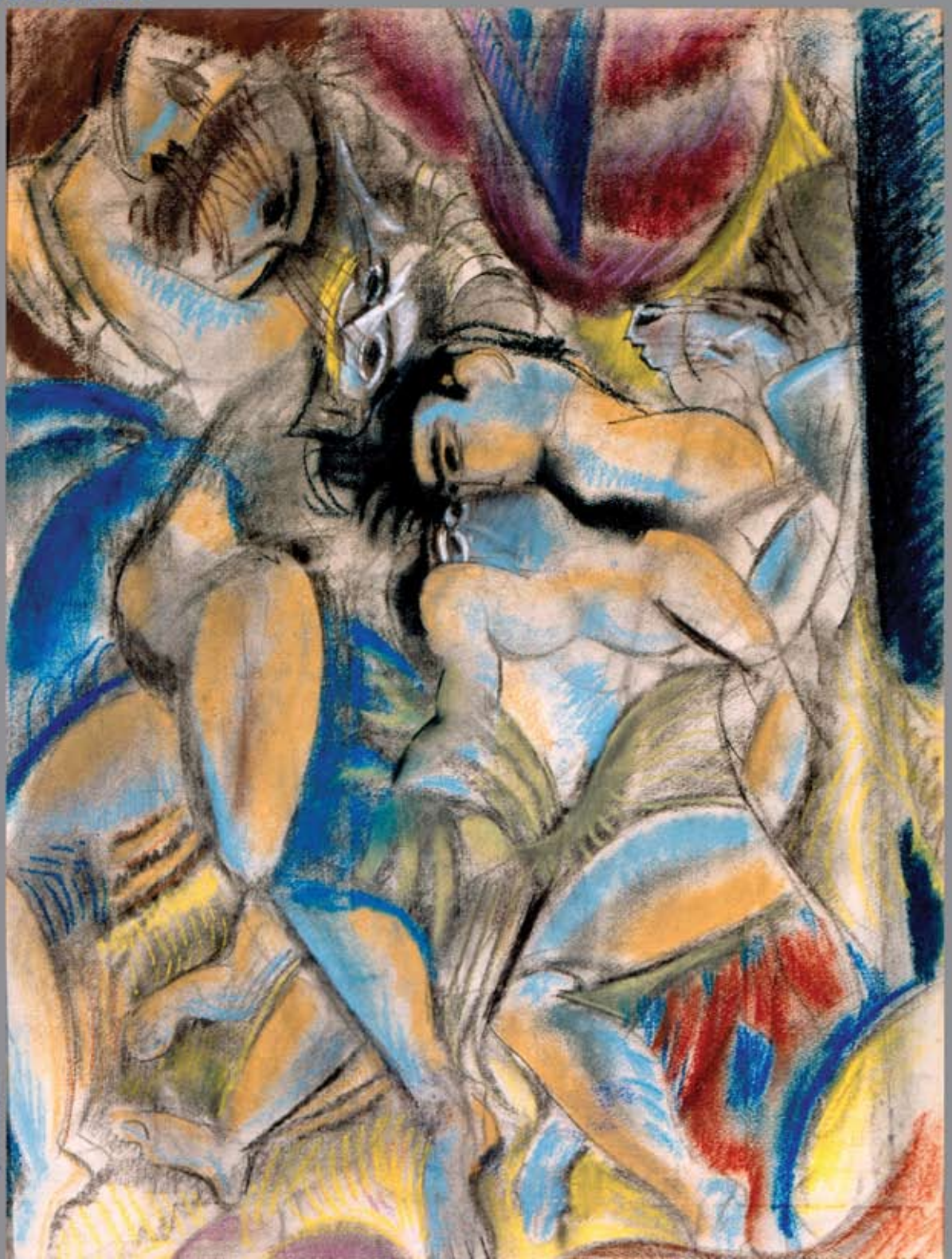
June 2011

30 Years of AIDS



EMERGING INFECTIOUS DISEASES®

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EMERGING INFECTIOUS DISEASES

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On the Cover

Max Weber (1881–1961)
Figures (c. 1914)
Pastel on paper (61 cm × 45.7 cm)
High Museum of Art, Atlanta
Gift in memory of Louis Regenstein by his wife Helen and sons Lewis and Kent

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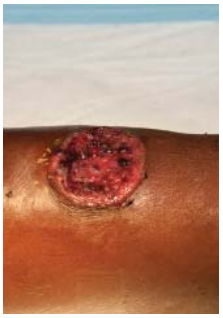

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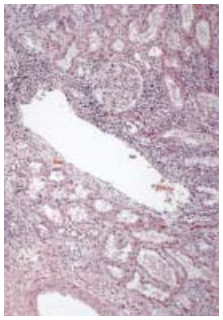

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Reality Check of Laboratory Service Effectiveness during Pandemic (H1N1) 2009, Victoria, Australia

Michael Catton, Julian Druce, Georgina Papadakis, Thomas Tran, and Christopher Birch

No campaign plan survives first contact with the enemy.—Helmuth Graf von Moltke

In Australia, the outbreak of pandemic (H1N1) 2009 began in Melbourne, Victoria; in the first 17 days, the Victorian Infectious Diseases Reference Laboratory detected 977 cases. Although the laboratory had a pandemic plan in place, a retrospective evaluation found 3 major variations from plan assumptions: 1) higher peak demand not limited by a case definition, 2) prolonged peak demand because containment attempts continued despite widespread influenza, and 3) unexpected influence of negative test results on public health actions. Although implementation of the plan was generally successful, the greatest challenges were limited availability of skilled staff and test reagents. Despite peak demand of 1,401 tests per day, results were provided within the usual 24 hours of specimen receipt; however, turnaround time seemed slower because of slow transport times (≥3 days for 45% of specimens). Hence, effective laboratory capability might be enhanced by speeding transport of specimens and improving transmission of clinical data.

The pandemic (H1N1) 2009 outbreak in Australia was detected in Victoria on May 18, 2009, and during the following weeks spread to other states. Pandemic planning guidelines for Australia consist of 4 phases (*1*): delay (identify and test persons who meet a clinical case definition), contain (home quarantine laboratory-confirmed case-patients and give antiviral prophylaxis to their contacts), sustain (restrict laboratory testing to persons with clinically defined cases who are at increased risk for severe outcomes), and protect (identify and manage those at risk for severe illness and those in vulnerable settings such as aged-care facilities). The pandemic plan envisaged all Australian states moving together through the pandemic phases. In practice, however, Victoria implemented the sustain phase, referred to as modified-sustain, sooner than other states.

The first 3 case-patients were siblings who had recently returned from the United States (Figure 1). When the outbreak began, Victorian health authorities implemented the contain phase (*3*), and laboratory confirmation of cases was conducted by the Victorian Infectious Diseases Reference Laboratory (VIDRL). Attempted containment ceased on June 3 when confirmed cases totaled 977, at which time laboratory testing was restricted to that appropriate under a modified-sustain phase. By June 23, when the modified-sustain phase ended, 1,406 cases had been laboratory confirmed and 1 patient had died. Testing efforts subsequently moved to those required under the protect phase. By September 27, a total of 6,895 cases in Victoria had been reported, 24 of them fatal (*3*), although the true number of cases is probably greater.

We describe VIDRL provision of laboratory support for the pandemic (H1N1) 2009 outbreak response in Victoria. We critically appraise the effectiveness of this laboratory's pandemic planning from 3 perspectives: 1) how the reality of the pandemic matched planning assumptions, 2) how successfully this planning facilitated workflow in practice, and 3) how successfully the laboratory delivered the required testing.

Pandemic Planning

Our planned algorithm for influenza A virus testing involved extraction of RNA from clinical specimens by using QIAxtractor or BioRobot Universal System

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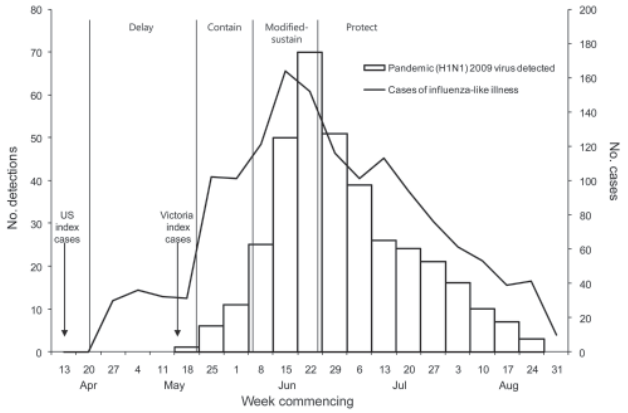

Figure 1. Number of patients with influenza-like illness and numbers of laboratory detections of pandemic (H1N1) 2009 derived from primary care physician influenza surveillance together with the phases of the outbreak in Victoria (VIC). The phases are as follows: delay (conduct active surveillance and border control measures), contain (restrict establishment of the pandemic), modified-sustain (minimize transmission and maintain health services), and protect (focus on those at risk for severe outcomes). Modified from (*1,2*),

extraction robots (each from QIAGEN, Valencia, CA, USA), followed by reverse transcription with random hexamers. cDNA was amplified in parallel assays by using an Applied Biosystems 7500 Fast Real-Time PCR System (Foster City, CA, USA) and incorporating primers and probes selective for the matrix gene of influenza A viruses, including that of the pandemic (H1N1) 2009 virus, and for the hemagglutinin (HA) gene of that virus. (Sequences of all primers and probes used in these assays are available upon request to M.C.).

Our model of anticipated pandemic influenza testing comprised 2 phases. First, an initial peak of intense testing needed to identify early cases would result in ≥500 additional PCRs being conducted each day for 2 weeks. Second, a step-down in demand with a focus on severe or atypical cases that needed testing for clinical management would result in ≈200 tests being conducted each day for several months. Implicit in the latter phase was that a clinical case definition would suffice for most uncomplicated influenza cases and that dominant circulation of the pandemic strain would enable a test result of "influenza A detected" from many laboratories to be a de facto diagnosis of pandemic (H1N1) 2009 infection. Some laboratory capacity would be reserved for outbreak monitoring by sentinel surveillance and detailed strain characterization. All routine diagnostic laboratory activity (≈1,000 tests/day) for diseases other than influenza would proceed routinely, but elective activities such as research would be delayed as needed.

To realize this pandemic plan, certain measures were undertaken at VIDRL. They were 1) assembly of enough nucleic acid extraction robotics and real-time PCR analyzers for >500 daily PCRs, 2) recruitment and training of 2 additional scientists who could work in the testing laboratory during a major outbreak, 3) planning for the temporary reassignment of scientific staff with appropriate skills from other laboratory areas during an outbreak, 4) cross-training of secretarial and clerical staff to enter patient and specimen data into the laboratory information system, 5) manning of the laboratory telephone switchboard by clerical staff, and 6) creation of a small stockpile of essential laboratory reagents.

Effectiveness of Testing

During the initial contain phase, the number of tests run was high. On June 1, the day of peak testing, 1,401 PCRs for influenza were performed, this being the sum of the matrix gene PCRs performed on each referred specimen and HA gene PCRs performed on matrix gene PCR-positive

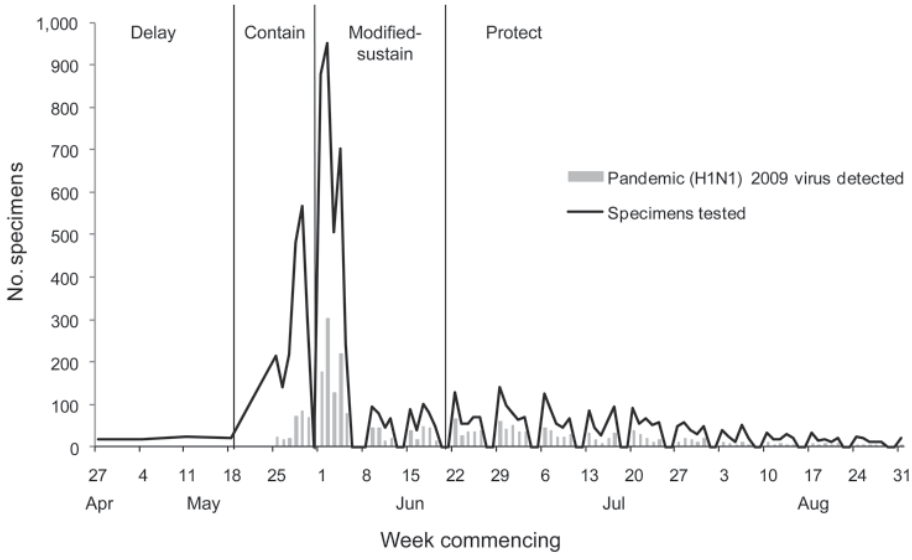

Figure 2. Number of diagnostic specimens received at the Victorian Infectious Diseases Reference Laboratory and laboratory detections of pandemic (H1N1) 2009 virus, Victoria, Australia, 2009.

samples (Figure 2). In contrast, a typical daily peak number in winter would be ≈100. However, the laboratory was able to sustain peak levels of influenza testing and provision of results within typical turnaround times. The times from specimen data entry into the laboratory information system to result reporting were calculated by extracting data from the Laboratory Information System (Medipath, LRS Health; Melbourne, Victoria, Australia) with an integral analytic software module. Because the actual time of specimen arrival is not searchable on our system, the representativeness of this electronic data as a proxy for total test turnaround time was verified by a manual audit of 200 Medipath files. This procedure compared the manually stamped arrival time and date on scanned digital images of specimen request forms received on June 1, the busiest day of the outbreak, with the corresponding time and date recorded electronically for result reporting. This manual audit gave a faster estimate for turnaround time than the electronic search, probably because the latter includes data from weekends (data not shown).

The mean turnaround time from specimen data acquisition to result reporting for the 4 peak months of the 2009 outbreak was ≤24 hours (Figure 3). For all except a 2-week period in June, this turnaround time was faster than the equivalent turnaround time for the winter of 2008. The main contributors to this outcome were longer than usual working hours for scientific and support staff, coupled with high levels of automation.

Specimens were transported by courier to VIDRL from Melbourne hospitals, other laboratories, and general practitioners on behalf of Victorian health authorities. The duration of time from specimen collection to arrival at VIDRL varied. Transport times for all pandemic (H1N1) 2009–positive samples were calculated by comparing the interval between the laboratory receipt time and date stamp and the recorded collection time and date on digital images of specimen request cards. Positive samples were chosen for analysis because of the relative ease with which this dataset could be collated from the laboratory information system. The positive samples were representative of the total sample group from which they came; ≈15% of positive specimens arrived on the day of collection, 40% arrived the next day, and ≈30% arrived over the next 2 days (Figure 4). Despite maintenance of typical test turnaround times, these transport times contributed to clinicians' perception of slow turnaround times (*4*), for which VIDRL received numerous complaints. During the pandemic, it was common to receive telephone inquiries for results for specimens that had arrived only hours earlier or had yet to arrive.

Our pandemic planning had focused primarily on resources and processes under our control within the laboratory. However, for optimal functioning of the whole testing cycle, the movement of specimens and accompanying

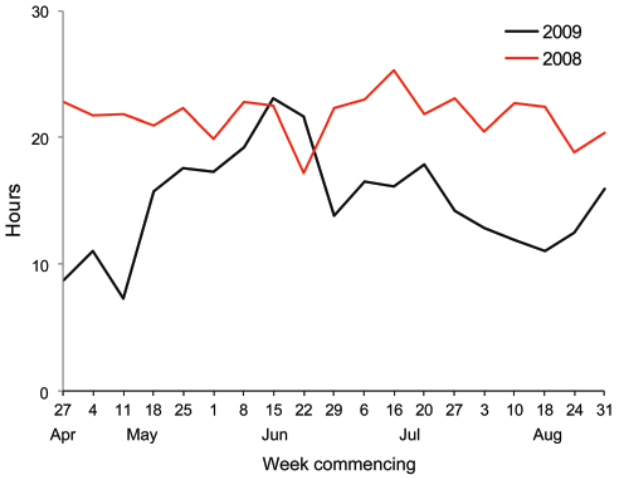

Figure 3. Mean turnaround times for Victorian Infectious Diseases Reference Laboratory detection of influenza, Victoria, Australia, 2008 and 2009.

data from patient to testing site and provision of results back to the patients' caregivers must also be optimal. To do so required a systemwide planning approach that was less than complete at the onset of the pandemic. More planning will be needed for optimal functioning under the pressures imposed by a future large outbreak (Table).

Effectiveness of Pandemic Planning

During the pandemic, 3 key elements differed substantially from our planning assumptions: 1) we did not predict the expectation that all community respiratory disease would be tested, 2) we did not plan for testing to continue long after widespread community spread of influenza was evident, and 3) we had not considered that negative test results would be so influential to the public health response. This outbreak was the first influenza pandemic during which provision of real-time diagnostic

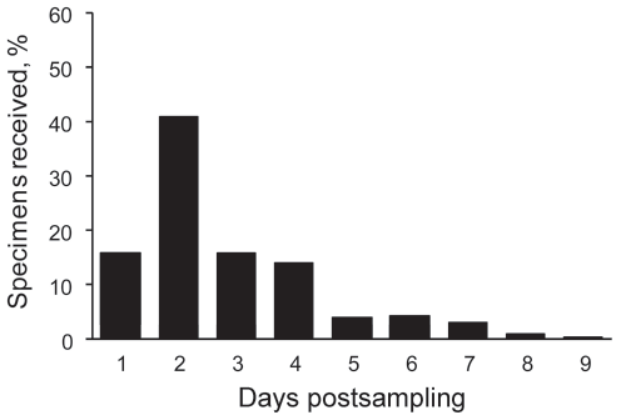

Figure 4. Timing of receipt of pandemic (H1N1) 2009 virus–positive specimens by the Victorian Infectious Diseases Reference Laboratory, Australia, 2009.

Table. Summary of laboratory effectiveness during pandemic (H1N1) 2009, Victoria, Australia, 2009

Challenge	Potential solution
Data management	
Pressure on specimen data entry into laboratory information system	Direct electronic communications of specimen data from referring source to laboratory
Missing telephone, fax, address details on request forms	Direct electronic communication of results from laboratory to referring source
Volume of negative results precluding telephone contact with referring source	Direct electronic communication of results from laboratory to referring source
Specimen transport	
Slow	Multi-institution planning of efficient emergency specimen transport
Poor interfacing with test start times in laboratory	Multi-institution planning of efficient emergency specimen transport
Staff	
Finite laboratory staff resources	Further minimization of manual steps for specimen processing and additional staff cross-training
Telephone inquiries	
Difficulty manning switchboard over extended laboratory hours	Planning for additional agency staff during emergencies
High call volume to laboratory taking scientific staff away from testing	Minimization of inquiries through improved specimen transport and data management
Reagents	
Shortages threatening test capacity	Expansion of reagent stockpile and use of validated test protocols using reduced reagent volumes
Communication	
Misunderstandings regarding scope and objectives of laboratory testing	Strengthened lines of communication between laboratories, clinicians, and health authorities
Pandemic planning	
Lack of flexibility to accommodate verging levels of influenza activity at state jurisdiction level	Adapted pandemic plan

virologic testing on large numbers of specimens had been a practical possibility. This testing capability created high expectations among users of our service. Our pandemic planning had sought to provide a realistic volume of testing capacity for anticipated public health and clinical needs. However, the initial expectation from the community and many clinicians during the contain phases was that all cases of respiratory disease in the community would be tested. This expectation is not unusual in highly publicized infectious disease outbreaks, but because the at-risk population was effectively unlimited in this outbreak, the demand was extreme. Most samples received were from persons who were relatively healthy, as evidenced by telephone conversations between our medical staff and patients, clinical details when provided on request forms, and by the dramatic drop in demand later during the sustain phase when testing was focused on those truly at risk for serious illness (Figure 2).

Our planning model of a 2-week initial surge followed by a step-down to clinically focused testing proved correct. However, the contain phase of high-demand testing continued well beyond the point at which it was first evident that community transmission was widespread. Only 9 of the first 978 case-patients had a history of overseas travel (*3*), and pandemic (H1N1) 2009 began to be detected from our sentinel general practitioner influenza surveillance network within the first week of the outbreak (*3*). Unlimited testing as influenza spread rapidly in the community drove testing demand to extremely high levels. The reasons for continuation of the contain phase are complex but were in part a consequence of the pandemic plan's treatment of the country as a homogeneous whole, although in reality the Victoria outbreak occurred several weeks sooner than outbreaks in other Australian states (*5*). In contrast to the higher than expected peak, testing levels during the subsequent step-down phase were lower than provided for in our plan (Figure 2). This finding is consistent with the relative clinical mildness of the pandemic (H1N1) 2009 virus strain; in Victoria, only 0.3% of infected patients were hospitalized in the first 10 weeks of the outbreak (*6*).

In past outbreaks, we focused on urgent and accurate communication of positive laboratory results that identified cases, and we communicated negative results en masse by routine systems, including electronic links to major health care institutions. However, during pandemic (H1N1) 2009, major public health actions were triggered by negative results, including cessation of quarantine restrictions and decisions about antiviral prophylaxis. While communication of large numbers of positive results to clinicians and public health authorities challenged resources, urgent and personalized transmission of a much larger number of negative results was not possible. This

limitation was further compounded by the frequency with which telephone or fax numbers of primary care physicians were missing on request forms; hence, laboratory reporting depended on postal addresses, which were also frequently incomplete or missing. Spot checks of request forms performed several times during the outbreak found this problem on up to 10% of request forms.

Implementation of Planning

Many aspects of our laboratory pandemic planning worked well in practice; outbreak testing facilities and equipment platforms provided the required test capacity (as many as 1,400 extra PCRs in 1 day). Employment of additional scientists before the outbreak also provided considerable benefits. In other areas, a great deal of commitment and hard work from staff compensated for planning shortcomings. Notably, preparations for surge capacity in several support areas, including patient data entry and dealing with telephone inquiries, could not match demand and required additional effort to resolve bottlenecks. Because our system of data entry requires specific skills, we could not use temporary agency staff for data entry. In practice, cross-trained secretarial staff and volunteers proved too slow for the demand, and their needs for support impeded the work of skilled staff. Particularly after hours, laboratory test results were often available before complete data entry had been performed, delaying release of hard-copy laboratory reports. A technical solution involving electronic upload of test requests from clinicians seems the best future approach to this problem.

Scientists in our organization who were not involved in influenza testing, envisaged as providing a pool of supplementary staff with PCR or virology skills, were rarely able to perform this function during the outbreak. The capacity of support staff who were performing functions such as specimen reception was almost entirely consumed by the demands of receiving influenza specimens. Staff in other laboratory areas helped absorb demand by taking over these functions for their own specimens but then could not reasonably release scientific staff to supplement influenza testing. As a result, those involved in influenza testing worked long hours, supported by scientists from other laboratory areas who were also working overtime. Although this approach was sustainable for weeks, it could not have continued through the outbreak.

Lastly, the small stockpile of PCR reagents proved insufficient. The high demand for testing during the contain phase required a commensurate amount of reagents. Suppliers in Australia were initially unable to keep up with our rapidly escalated demand. This limitation was successfully managed by using reduced reaction volumes (because of a shortage of random hexamers, the volume of reverse-transcribed cDNA was halved); changing aspects of our testing algorithm (from an initial test algorithm involving influenza A matrix gene PCR primers and H1 HA gene primers run in parallel to an algorithm involving the matrix gene alone with subsequent HA subtyping of positive samples on the same day); and, immediately after introduction of the modified-sustain phase, adhering rigidly to the criteria for test eligibility circulated by health authorities. Adhering to these criteria included storing, but not testing, samples from persons determined to not be at substantial clinical risk. This practice caused unhappiness among some clinical colleagues but preserved sufficient capacity to guarantee testing for patients in clinical need.

Outbreak Monitoring

As described elsewhere (*2*,*3*), a network of 80 general practitioners in metropolitan Melbourne and rural Victoria conducted influenza surveillance, coordinated by VIDRL, from May through October 2009. Laboratory testing for influenza was conducted for a subset of these cases, and test results were made available online (*7*). This testing activity was maintained during the time of heavy laboratory demand because of the perceived need to collect unbiased data on influenza activity comparable to data collected during the previous 10 years of influenza surveillance.

The number of laboratory-confirmed cases of pandemic influenza (*3*) was heavily influenced by community testing behavior and by guidelines for testing promulgated by health authorities. This influence is shown clearly in the abrupt reductions in testing and detections of influenza in Victoria after June 3, when the pandemic response phase changed from contain to modified-sustain (Figure 2). Hence, the number and timing of laboratory-confirmed cases were unrepresentative of the wider outbreak. In contrast, laboratory-supported influenza surveillance undertaken in parallel with diagnostic testing provided monitoring of the course of the outbreak relatively free of these effects (Figure 1) and, as described elsewhere, enabled direct comparison of the outbreak with >10 years of seasonal influenza (*3*,*7*,*8*).

Conclusions

Operationally, the pandemic (H1N1) 2009 outbreak tested our laboratory preparedness in ways that no exercise could; yet some of the potential pressures were limited by the relatively low clinical severity of the virus. The numbers, speed, and accuracy of tests conducted, along with real-time tracking of the outbreak through laboratory-supported influenza surveillance, were unimaginable less than a decade ago. Facilities, equipment, and PCR-based testing performed extremely well. Limits to the available pool of skilled staff and the threat of reagent shortages provided challenges where contingency plans had only been partly successful. Staff performed admirably in the

face of these challenges, but in the future, more effective solutions will be required. The greatest improvements in overall performance of the laboratory testing cycle will be achieved through increasing the speed of specimen transport and improving transmission of clinical data to and from the laboratory.

Acknowledgment

We thank Kristina Grant for reproduction of influenza-like illness data shown in Figure 1.

Dr Catton is director and head of virology at VIDRL in Melbourne, Australia. His professional interests are molecular viral diagnostics and emerging viruses.

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Multiple Introductions of Multidrug-Resistant Tuberculosis into Households, Lima, Peru

Ted Cohen, Megan Murray, Ibrahim Abubakar, Zibiao Zhang, Alexander Sloutsky, Fernando Arteaga, Katiuska Chalco, Molly F. Franke, and Mercedes C. Becerra

Two cases of multidrug-resistant tuberculosis (MDR TB) in a household are assumed to reflect within-household transmission. However, in high-incidence areas of MDR TB, secondary cases may arise through exposure to MDR TB in the community. To estimate the frequency of multiple introductions of MDR TB into households, we used spoligotyping and 24-loci mycobacterial interspersed repetitive unit–variable number tandem repeats to classify isolates from 101 households in Lima, Peru, in which >1 MDR TB patient received treatment during 1996–2004. We found different MDR TB strains in ≥10% of households. Alternate approaches for classifying matching strains produced estimates of multiple introductions in ≤38% of households. At least 4% of MDR TB patients were reinfected by a second strain of MDR *Mycobacterium tuberculosis*. These findings suggest that community exposure to MDR TB in Lima occurs frequently. Rapid drug sensitivity testing of strains from household contacts of known MDR TB patients is needed to identify optimal treatment regimens.

The discovery and use of discriminating genetic markers such as IS*6110* restriction fragment length polymorphisms (RFLPs), spacer oligonucleotides (spoligotyping), and mycobacterial interspersed repetitive unit–variable number tandem repeats (MIRU-VNTRs) (*1*) have improved our understanding of the transmission dynamics of tuberculosis (TB) (*2*,*3*). Genotyping studies, in which strains with matching sets of markers are considered potential members of a single transmission chain, have demonstrated that recent transmission plays a major role, even in low-incidence settings (*4*,*5*); that persons with recurrent episodes of TB may be having reinfection rather than relapse (*6*–*8*); that persons may be infected by >1 isolate of *Mycobacterium tuberculosis* at the same time (*9*–*11*); and that transmission may occur in casual social settings (*12*).

Molecular epidemiologic studies have also demonstrated that secondary cases among close associates of known case-patients are not always members of the same chain of transmission, i.e., that infection may have been acquired from independent sources (*13*). Molecular investigations of households of multiple TB patients showed that cohabitating TB patients may be infected with distinct isolates of *M. tuberculosis* (*14*–*16*). For example, in 2 suburbs of Cape Town, South Africa, which have TB notification rates of ≈320 cases per 100,000 population, researchers found that less than half (46%) of secondary TB cases within households had a TB isolate that matched an isolate from another case within the household by RFLP (*16*). Overall, <1 (19%) in 5 new TB cases occurring in these communities was the result of within-household transmission.

Although studies have shown that household contacts with TB are likely to have acquired infection independently in high-incidence settings, there are no published estimates of the probability that 2 household members with multidrug-resistant TB (MDR TB: resistance to at least isoniazid and rifampin) share a similar genotype and are members of the same transmission chain. Molecular epidemiologic data from households with >1 MDR TB case can help shed

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light on the transmissibility of highly drug-resistant disease and also help guide public health policy. For example, international guidelines for the management of known contacts of MDR TB patients recommend an empirical drug regimen based either on the drug-resistance profile of an isolate from the suspected index MDR TB case-patient or on the most common drug-resistance pattern in the community while drug sensitivity tests are pending (*17*–*19*). A better understanding of the relative importance of intrahousehold or community transmission may help to inform the choice of empirical regimen.

Despite a decreasing overall incidence of TB in Peru of ≈3.7% per year since 1996, the incidence of MDR TB has increased by ≈4.5% over the same period (*20*). The increasing incidence of MDR TB in densely occupied urban communities of Lima, Peru, poses obvious challenges for TB control. We report a molecular epidemiologic study within households in Lima in which >1 person received a diagnosis of MDR TB. We used spoligotyping and 24-loci MIRU-VNTR typing (*21*,*22*) to identify households that have had >1 introduction of MDR TB, and we explored the association of household factors with these multiple introduction events.

Materials and Methods

Study Setting, Participants, and Data

The estimated incidence of TB in Lima, Peru, is >130 cases/100,000 persons; this estimate masks substantial heterogeneity in the actual distribution of TB within this large metropolitan area where poor areas often experience several-fold higher local incidence of disease than higher-income areas (*23*). For example, in 2000 in northern metropolitan Lima (population 3,186,199), the incidence of active TB was 232 cases/100,000 persons (*24*). A nationwide survey in 2006 reported that 5.3% of all new cases and 23.6% of retreatment cases were MDR TB (*25*). Since 1996, Partners in Health and Socios en Salud Sucursal Peru have worked with the Peru Ministry of Health to implement a program to treat patients with active MDR TB by using supervised, individualized, antimicrobial drug regimens delivered on an ambulatory basis (*26*–*28*).

We previously reported the TB incidence in a cohort of household contacts of the patients treated for MDR TB (*29*). A household was eligible for inclusion in the study if ≥2 members had been treated for MDR TB by this program during 1996–2004, and if ≥1 MDR *M. tuberculosis* isolate obtained from each person was available for analysis. All available (pretreatment and ongoing treatment) MDR isolates from patients in eligible households were included in this analysis. Demographic data, drug-susceptibility test results, and information about the physical condition of the household structure were abstracted from the electronic records of the MDR TB program. This study was reviewed and approved by the Committee on Human Studies of the Office of Research Subject Protection of Harvard Medical School.

Laboratory Methods and Drug-Susceptibility Testing

Drug-susceptibility testing and genotyping by using MIRU-VNTR and spoligotyping were performed by the Supranational Reference Laboratory at the University of Massachusetts Medical School. A standard agar plate proportion method was used for drug-susceptibility testing of *M. tuberculosis* isolates. The first-line and second-line drugs tested were isoniazid (0.2 mg/L, 1.0 mg/L, and 5.0 mg/L), rifampin (1.0 mg/L), streptomycin (2.0 mg/L and 10.0 mg/L), ethambutol (5.0 mg/L), kanamycin (5.0 mg/L), ethionamide (10.0 mg/L), capreomycin (10.0 mg/L), ofloxacin (2.0 mg/L), and *p*-amino salicylic acid (8.0 mg/L). Susceptibility to pyrazinamide (100 mg/L) was determined by using the BACTEC 460 Liquid Medium System (Becton Dickinson, Sparks, MD, USA). We only included drugs to which resistance had been tested for ≥70% of isolates in the study.

MIRU-VNTR Genotyping

DNA for PCR analysis was prepared by using a simple thermolysis procedure. PCR amplification of the 24 MIRU-VNTR loci was conducted as described (*22*,*30*) with minor modifications. The PCR mixture contained 2 μL of thermolysate, 1× PCR buffer, 1 mol/L betaine, 0.5 U Taq DNA polymerase (Takara Bio, Madison, WI, USA), 200 μmol/L of each dNTP, and 0.3 μmol/L of each flanking primer.

An ABI Thermal Cycler 2720 (Applied Biosystems, Foster City, CA, USA) was used for PCRs. Initial denaturation at 94°C for 5 min was followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 62°C for 30 s, and elongation at 70°C for 45 s; and a final extension step at 72°C for 10 min. *M. tuberculosis* H37RV DNA and sterile distilled water were included in each test run as positive and negative controls, respectively.

PCR products were analyzed in 2 ways. First, DNA fragments from amplification with primers specific for loci ETRA, ETRB, ETRC, ETRD, MIRU2, MIRU20, MIRU23, MIRU24, MIRU26, Mtub21, Mtub29, Mtub30, Mtub34, and Qub11b were separated by using standard 2% agarose gel electrophoresis. Second, DNA fragments from amplification with primers specific for loci ETRE, MIRU10, MIRU16, MIRU27, MIRU39, MIRU40, Mtub04, Mtub39, Qub26, and Qub4156 were analyzed by electrophoresis with the QIAxcel System and the QIAxcel DNA Screening Kit (both from QIAGEN, Valencia, CA, USA).

Spoligotyping

Mycobacterial DNA was prepared by using the same thermolysis protocol as for MIRU-VNTR typing. For DNA amplification, 0.15 μL *Tth* polymerase (5 U/μL; Roche, Pleasanton, CA, USA) was added to 50 μL of PCR mixture, and the following amplification profile was used: 3 min at 96°C; 35 cycles for 1 min at 96°C, 1 min at 55°C, and 30 s at 72°C; and 5 min at 72°C.

Spacer oligonucleotide typing was performed by using the Multianalyte Profiling System (Luminex Inc., Austin, TX, USA). The procedure was conducted according to the protocol reported by Cowan et al. (*31*) with adaptations for a 96-well format. Fluorescence signals indicating hybridization strength were analyzed by using Bio-Plex Suspension Array System Instrument Luminex 100xMAP Technology (Luminex Molecular Diagnostics Inc., Toronto, Ontario, Canada) and the Bio-Rad BioPlex Manager Program version 4.1.1 (Bio-Rad Laboratories, Hercules, CA, USA). Lineage and the shared type for each isolate were assigned based on matching the spoligotype patterns with those listed in the SpolDB4 database (*32*).

Identification of Multiple Introductions of *M. tuberculosis* into a Household

Households were classified as having evidence of repeated introduction of TB from the community if isolates from ≥2 patients with MDR TB within 1 household had different molecular genotypes. Supply et al. proposed a standard approach for characterizing the relatedness of *M. tuberculosis* isolates by spoligotyping and 24-loci MIRU-VNTR. They found that the combination of these methods (which requires including ≥15 of the most diverse loci for MIRU-VNTR analysis) has comparable discriminatory power to IS*6110* RFLP typing (*22*). We present minimum and maximum estimates of the proportion of households judged to have evidence of multiple TB introductions on the basis of spoligotyping and MIRU-VNTR genotyping data.

We also examined a classification approach recently used by Narayanan et al. (*7*). Nonmatching strains are defined as those strains with >1 spoligotype spacer or >1 MIRU-VNTR locus difference. Enabling different degrees of stringency in calling 2 (or more) strains a match reflects our underlying uncertainty about how rapidly spoligotypes and MIRU-VNTR genotypes change because of mutations at marker loci during the natural history of disease and through chains of transmission that may span decades.

Identification of Reinfection Events

We genotyped all available MDR isolates of patients within study households. Among participants from whom ≥2 isolates were available, we identified episodes of reinfection on the basis of differences in genotypes. We used a similar approach for comparing genotypes for identifying episodes of reinfection and repeated household introduction.

Statistical Analysis

SAS version 9.2 (SAS, Cary, NC, USA) was used for statistical analysis. We performed standard nonparametric tests for assessing univariate associations between household-level factors and the probability of repeated introduction.

Results

We identified 105 households in which ≥1 MDR *M. tuberculosis* isolate was available from each of ≥2 different household members. In total, 391 MDR isolates from 236 persons were available for molecular typing. Spoligotyping and MIRU-VNTR analyses were successfully completed on samples from ≥2 participants from 101 (96%) of these households. These analyses resulted in a set of 384 (98%) isolates from 232 (98%) persons. Characteristics of persons and households included in the study are shown in Table 1. There were an additional 142 households for which we knew of ≥2 patients with MDR TB, but for whom *M. tuberculosis* specimens were no longer available for genetic analysis. No statistically significant differences in size, density, or age distribution of members were found between the households that were included and those not included in this study.

Of 384 isolates, 228 (59%) were tested for susceptibility to a sufficient number of second-line drugs to identify extensively drug-resistant *M. tuberculosis* strains (MDR plus additional resistance to a fluoroquinolone and a second-line, injectable antimicrobial drug [either kanamycin, amikacin, or capreomycin]). Thirty-one (14%) of these 228 isolates were confirmed as extensively drug resistant and were obtained from 15 patients, none of whom were living in the same household.

Multiple Introductions of MDR *M. tuberculosis* into Households

Using a permissive definition of matching in which we included strains that differed by 1 spoligotype spacer to be matched, we estimated that 10 (10%) of households had

Table 1. Characteristics of 101 households with MDR TB, Lima, Peru, 1996–2004*

Characteristic	Median (IQR)
Persons per household	8 (6–10)
Persons per bedroom	2.5 (1.75–4.33)
Participants per household	2 (2–2)
Participants, n = 232	
Age, y	23.8 (19.2–30.5)
Male sex, %	57.2

*MDR TB, multidrug-resistant tuberculosis; IQR, interquartile range.

distinct MDR isolates and showed evidence of repeated introduction. The strictest definition of matching, which required exact matches in spoligotype and at all 24-loci of the MIRU-VNTR analysis, showed that 38 (38%) of households had evidence of repeat introduction of MDR TB from the community (Figure). Using the approach of Narayanan et al. (*7*) for identifying nonmatching strains (pairs with >1 spoligotype spacer or 1 MIRU-VNTR locus difference), we classified 16 (16%) households as settings with multiple introductions of MDR TB.

The 16 households in which ≥2 persons had an MDR *M. tuberculosis* isolate that was different from that obtained from another person in the household, according to the definition of Narayanan et al. (*7*), are shown in online Appendix Table 1 (www.cdc.gov/EID/content/17/6/969-appT1.htm). Seven of these households also had evidence of within-household transmission of MDR TB. Closer inspection of spoligotypes isolated from these households indicated that 6 of the 16 households, although failing to meet the proposed criterion for matching, had similar isolates (households 112, 192, 557, 960, 263, and 645). If these 6 households are classified as having evidence of within-household transmission, our best estimate of the number of households with evidence of multiple introductions of MDR strains is reduced to 10 (10%). Under these criteria, the percentage of households with only evidence of probable within-household transmission is 90%.

We used the 10 households as our most conservative set of households with evidence of multiple introductions of MDR stains and searched for household factors that were associated with multiple introduction events. We did not find any significant associations; specifically, the size and density of households, the quality of the household structure, and time span over which isolates were accrued from households all appeared to be unrelated to multiple introductions (Table 2). In addition, no significant difference was found in the number of drugs to which the isolate from the first patient was resistant between households that had repeated introduction (mean 5.1 drugs) and households that had evidence of probable within-household transmission (mean 5.3 drugs; p = 0.75).

Evidence of MDR Reinfection

Ninety persons had >1 MDR TB isolate available for analysis. Using the definition of matching strains of Narayanan et al. (*7*), we found that 5 (6%) of these persons had 2 distinct strains of MDR *M. tuberculosis* during the period of follow-up and the remaining 85 (94%) showed repeated isolation of the same MDR strain (online Appendix Table 2, www.cdc.gov/EID/content/17/6/969-appT2.htm). Closer inspection of the isolates available from these 5 persons showed that 1 person (a 20-year-old man) from household 977 may not have been reinfected. Three isolates were available from this person. The first isolate had a slightly different spoligotype than the 2 isolates subsequently obtained, but the MIRU-VNTR pattern was the same for all 3 isolates.

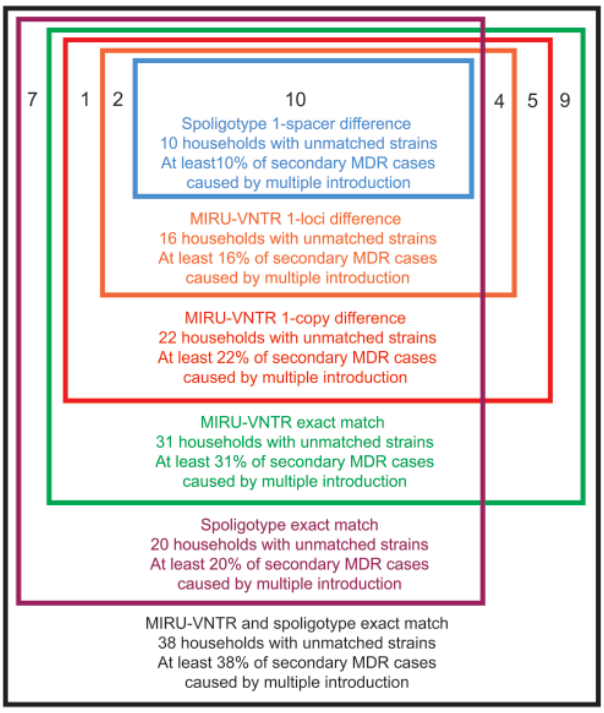

Figure. Numbers of households classified as having multiple multidrug-resistant (MDR) tuberculosis introductions by 6 definitions of matching genotypes, Lima, Peru, 1996–2004. MIRU-VNTR, mycobacterial interspersed repetitive unit–variable number tandem repeat.

Discussion

In the absence of molecular epidemiologic data, secondary cases of MDR TB within a household are generally assumed to be the result of within-household transmission. In an area with increasing incidence of MDR TB (*20*), we found that 90% of household contacts of MDR TB index cases with active disease and drug-susceptibility test results had MDR TB (*29*). Our present study, in a subset of that cohort, used genotyping on the basis of spoligotyping and 24-loci MIRU-VNTR, which has been shown in other settings to have comparable discriminatory power to IS*6110* RFLP (*21*). Our study shows that there was at least a 10% risk that a subsequent case of MDR TB occurring within the home of a known MDR TB patient was the result of transmission in the community rather than transmission in the household. This estimate represents a lower boundary of the contribution of community transmission to the appearance of secondary MDR cases

Table 2. Association between household factors and repeated introduction of MDR TB, Lima, Peru, 1996–2004*

Factor	Introduction, n = 10†	No introduction, n = 91	p value
No. persons	7.5 (6–8)	8 (7–11)	0.18
Persons per bedroom	2.6 (1.7–2.7)	2.4 (1.75–5)	0.43
Homes of substandard quality‡	1/9 (11)	23/64 (36)	0.44
Mean age of household members, y	28 (23–32)	26 (21–30)	0.39
Duration between first and last isolate obtained from household, d	389 (167–724)	345 (204–599)	0.92

*Values are median (interquartile range) or no. positive/no. tested (%). MDR TB, multidrug-resistant tuberculosis.
†Households classified as having repeated MDR TB introductions for these analyses are indicated in online Appendix Table 1 (www.cdc.gov/EID/content/17/6/969-appT1.htm).
‡Substandard housing was defined as a dwelling with a dirt floor; walls made of straw matting, plastic, or plywood; a roof made of straw matting, plastic, or plywood; or no access to water in the home (data were not available for all households).

within a home because matching strains within a household (which we would categorize as within-home transmission) may be caused by transmission from other sources in the community. Because circulating MDR strains were heterogeneous (Table 3), the magnitude of this bias may not be substantial.

We did not find any easily measured household factors associated with risk for repeated introductions compared with within-home transmission. We had hypothesized that a high household density (persons/bedroom) or low quality of household structure may be associated with a higher probability of within-home transmission, conditional upon observing multiple cases within a home, but this hypothesis was not supported by these data. This finding may reflect an absence of this association between household characteristics and risk for within-home transmission or, alternatively, it may reflect the relatively small number of repeated introduction events that we observed and our limited power to test such associations. Accordingly, although our observations provide convincing evidence that repeated introduction of MDR TB into households occurs in these settings, further studies are needed to determine whether household factors, number of persons within these households, or strains present within these households are associated with an increased risk for within-home transmission or repeated exposure in the community.

Genetic (*33*) or acquired susceptibility (*34*) to infection and disease may play a role in the accumulation of multiple TB cases within households. Because household members are likely to share genetic or environmental risk factors, or both, persons living with TB case-patients may be particularly likely to be infected and acquire disease whether they are infected by their household contact or in the community.

Our findings provide evidence to support international guidelines for management of active TB among contacts of known MDR TB cases (*17–19*) because they confirm that among strains from persons for which genotyping test results are available, ≤90% of household contacts with MDR TB were infected with the same strain as the index patient. Our findings also highlight limitations associated with such policies. Because subsequent cases of MDR TB in a household may be caused by community transmission, policies that specify that apparent secondary case-patients receive therapy on the basis of the drug-susceptibility profile of an isolate from the initial MDR TB patient may result either in effective drugs being needlessly withheld or in administration of drugs to which the strain is already resistant. This policy may result in acquisition of additional resistance to second-line drugs and prolonged opportunity for transmission of highly drug-resistant strains within homes and in the community (*35*,*36*).

These findings support the use of rapid drug-resistance tests to determine drug susceptibility profiles in known contacts of MDR TB patients. Molecular tests for resistance, such as line probe assays and cartridge-based PCRs (i.e., GeneXpert; Cepheid, Sunnyvale, CA, USA), are promising and have been endorsed by the World Health Organization for determining resistance to first-line drugs (*37*). However, although new diagnostic tests in development also detect resistance to second-line drugs (*38*,*39*), these tests have not yet been optimized for use in guiding clinical care. New rapid phenotypic tests for resistance, such as the microscopic-observation drug-susceptibility assay, have also not yet been adequately tested under field conditions for their capacity to be used in selection of tailored regimens for MDR TB (*40*). Known contacts of MDR TB patients should be a high-priority,

Table 3. Strain lineages of *Mycobacterium tuberculosis* detected in the study population, Lima, Peru, 1996–2004

Lineage	No. (%)
Beijing	19 (4.9)
H1	22 (5.7)
H3	22 (5.7)
LAM1	25 (6.5)
LAM3	12 (3.1)
LAM4	6 (1.6)
LAM5	47 (12.2)
LAM9	38 (9.9)
T1	85 (22.1)
T2	19 (4.9)
T5_MAD2	2 (0.5)
U	1 (0.3)
X3	17 (4.4)
No match	69 (18.0)

high-yield study population for assessing the immediate utility of these new tools.

A limitation of our study is that we cannot definitively distinguish the 2 mechanisms by which distinct MDR isolates may appear within households. First, household members may have been infected by different drug-susceptible strains in the community and acquired drug resistance through deficient drug treatment. Second, household members may have been directly infected by different MDR strains in the community. Distinguishing between these 2 possibilities is essential because each would cause a distinct public health response. The first mechanism suggests that detailed investigation of individual-level or household-level risk factors for acquisition of MDR TB was needed and would indicate a need for greater treatment support and supervision for patients with drug-susceptible disease. The second mechanism indicates a need to improve infection control in the community or to facilitate diagnosis and effective treatment for persons with MDR TB to reduce the duration of infectiousness. In most circumstances, we expect acquisition and transmission to contribute to the appearance of multiple cases of MDR TB within homes, and efforts to reduce the incidence of drug-resistant disease will need to address these factors.

Although we have insufficient data for previous TB episodes and treatment for persons in our study to exclude possible independent acquisition of MDR TB among household members because of inadequate treatment, our finding that ≥4 persons showed evidence of reinfection by a second (i.e., different) MDR TB strain provides evidence that there is a high risk for MDR TB exposure in this community. HIV status was known for only ≈50% of the persons in the study. Among those tested, only 3 (3%) of 102 were HIV infected and none of the 3 HIV-infected persons were among persons in households in which multiple introductions of MDR TB were detected. If co-infection with HIV was common, it would be expected to increase the probability of rapid progression to disease and lead to higher risks of multiple cases of unlinked disease within households. Because HIV co-infection was so rare, it is unlikely that this explains the study results.

Our results extend findings from previous studies showing that a substantial fraction of cohabiting persons have independently acquired TB in the community (*13–16*). In contrast to earlier studies that compared relative contributions of within-home and community transmission, all persons in our study had MDR TB. We found that although 90% of households had evidence of intrahousehold transmission, 10% had ≥2 independent introductions of MDR *M. tuberculosis* strains from the community. This finding suggests that the risk for community or extrahousehold transmission of MDR TB in Lima is high. Furthermore, it indicates that known MDR TB contacts initiating empirical treatment for MDR TB treatment require access to drug susceptibility testing to ensure that they receive the drugs to which their isolate is susceptible. National TB programs should be wary of applying empirical regimens on the basis of population-level drug susceptibility data without better understanding of the relative role of intrahousehold and community transmission of MDR TB.

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Binary Toxin and Death after *Clostridium difficile* Infection

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We compared 30-day case-fatality rates for patients infected with *Clostridium difficile* possessing genes for toxins A and B without binary toxin (n = 212) with rates for patients infected with *C. difficile* possessing genes for A, B, and binary toxin. The latter group comprised patients infected with strains of PCR ribotype 027 (CD027, n = 193) or non-027 (CD non-027, n = 72). Patients with binary toxin had higher case-fatality rates than patients without binary toxin, in univariate analysis (relative risk [RR] 1.8, 95% confidence interval [CI] 1.2–2.7) and multivariate analysis after adjustment for age, sex, and geographic region (RR 1.6, 95% CI 1.0–2.4). Similar case-fatality rates (27.8%, 28.0%) were observed for patients infected with CD027 or CD non-027. Binary toxin either is a marker for more virulent *C. difficile* strains or contributes directly to strain virulence. Efforts to control *C. difficile* infection should target all virulent strains irrespective of PCR ribotype.

Clostridium difficile infection (CDI) is a common cause of health care–associated diarrhea in industrialized countries (*1*), and the leading cause of intestinal infection related to antimicrobial drug consumption (*2*). Clinical manifestations range from mild to severe diarrhea, pseudomembranous colitis, toxic megacolon, sepsis, and ultimately death. Risk factors for CDI include duration of hospital stay, underlying illness, age (*3*), and previous use of virtually any antimicrobial drug, most frequently cephalosporins and fluoroquinolones (*4–10*).

The hypervirulent fluoroquinolone-resistant *C. difficile* PCR ribotype 027 North American pulsed-field type 1 (NAP1) (REA type BI, toxinotype III) has received attention as the cause of increasingly severe outbreaks and higher death rates, longer hospital stays, and frequent relapses (*8*,*9*,*11*,*12*). However, whether it really causes increased severity is questionable. Characteristics observed by previous studies may be due to selection bias or to the procedures used for diagnostic testing and reporting of cases; disease severity was similar in 2 groups of patients (PCR ribotype 027 and non-027) when recruitment to the study was done without reference to clinical signs and symptoms or PCR ribotype (*13*).

The pathogenicity of *C. difficile* is based on the action of at least 1 of the 2 main cytotoxins (A and B) acting as glycosyltransferases that modify guanose triphosphatases within the intestinal epithelial cells and lead to the disruption of the actin cytoskeleton. A recent study, which used a gene knock-out system, reinforced the fact that toxins A and B are comparable in terms of virulence, as shown by in vitro cytotoxicity and virulence in vivo (*14*). A binary toxin *C. difficile* transferase is found in some strains and belongs to the actin-modifying adenide dinucleotide protein–ribosyltransferases, which also impair the structure of actin cytoskeleton in epithelial cells (*15*,*16*). The pathologic significance of binary toxin is not yet clear. However, a recent study reports that binary toxin not only affects the actin cytoskeleton but also induces the formation of microtubule-based protrusions on the surface of epithelial cells, leading to increased adherence of bacteria (*17*).

Cultures positive for *C. difficile* are notifiable by the diagnostic laboratories in Denmark as part of the surveillance for gastrointestinal infections; in addition, isolates are selected under certain criteria and submitted to the National Reference Laboratory at Statens Serum Institut for further typing. The aim of the present study was to determine the case-fatality rate after diagnosis with *C. difficile*, according to toxin profile and PCR ribotype.

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Methods

Surveillance System and Registries

All entries to 3 national registries in Denmark (the Danish Civil Registration System, the national Registry of Enteric Pathogens, and the *C. difficile* Microbiological Database) use a unique person registration number. These identifiers were used for the study. The study was retrospective, and we used a cohort design in which 4 groups of case-patients with *C. difficile* infection (Figure 1) were monitored from the date of diagnosis until the date of death or date of extraction from the registry. The study was conducted during week 1 of 2008 through week 22 of 2009. The unique patient identifier was used to link the registries. None of the registries contain clinical data.

The Danish Civil Registration System contains demographic information on all residents of Denmark and was used to retrieve the date of death. This registry does not contain information on the cause of death. The national Registry of Enteric Pathogens includes weekly case-based notifications of cultures positive for *C. difficile* from all departments of clinical microbiology of regional hospitals in the country. A second case-based database, the *C. difficile* Microbiological Database, which is separate from the Registry of Enteric Pathogens, contains information on isolates that undergo genotypic toxin detection and PCR ribotyping at the National Reference Laboratory at Statens Serum Institut, Copenhagen. Isolates are forwarded by departments of clinical microbiology if they are resistant to moxifloxacin, if severe clinical course is observed, or if an outbreak is suspected. These criteria were established in 2007, when sporadic cases of *C. difficile* PCR ribotype 027 were found for the first time in Denmark (*7*). They were reinforced in 2009, when the country experienced the first large *C. difficile* PCR ribotype 027 outbreak, which involved different hospitals of the Copenhagen Capital Region (*18*). Information on which specific criteria were used for submission of the individual isolates for subtyping was not available. No laboratory standard for primary diagnostics of CDI has been developed at the national level, and clinical microbiology departments use different methods, including environmental impact assessment, culture, PCR, or standard cytotoxin assays.

All isolates referred to Statens Serum Institut are genotyped to detect genes for the 3 toxins (A and B, and binary toxin). PCR ribotyping is subsequently performed on isolates possessing the genes for all 3 toxins (Figure 1). The methods used for genotyping of toxins and PCR ribotyping have been described in detail elsewhere (*19*,*20*). This study was approved by the Danish Data Protection Board.

Definitions

Patients were assigned to 4 groups, depending on the characteristics of the isolates (Figure 1). Infected patients with an isolate possessing genes for toxins A and B and binary toxin were categorized either as *C. difficile* PCR ribotype 027 (CD027) or *C. difficile* PCR ribotype non-027 (CD non-027). A third group included patients infected with a strain possessing genes encoding for toxins A and toxin B, but not the binary toxin genes (CD A and B). A fourth group was created by subtracting the other 3 groups from patients with *C. difficile* infection that were notified to the surveillance laboratory system. Therefore, such patients were infected with isolates not referred for typing, presumably because the criteria for submission were not fulfilled. We refer to this group as unselected *C. difficile* unselected (CD-unselected).

Only the first episode of infection of the patient was considered. The first episode of CD027 overruled the first episode of CD non-027; the first episode of CD non-027 overruled the first episode of CD A and B; and the first episode of CD A and B overruled the first episode of unselected *C. difficile* infection. Therefore, the final dataset included only 1 observation per patient.

Statistical Methods

Kaplan Meier survival curves were created to determine the effect of time after diagnosis on the risk for death. Differences between curves were compared by using the log-rank test. Multivariate Poisson regression was used to estimate the risk ratio of death within 30 days after diagnosis. For survival analysis, patients were categorized into 2 groups, according to the presence or absence of binary toxin. Analysis was performed with STATA version 10 (StataCorp, College Station, TX, USA). Case-patients for whom 30 days of follow-up after infection could not be completed were excluded from the analysis (163 cases).

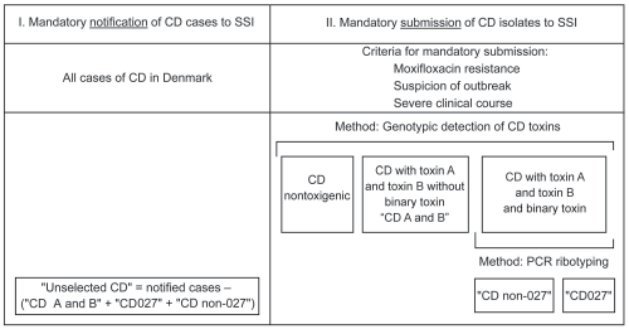

Figure 1. Description of *Clostridium difficile* (CD) infections surveillance in Denmark, with the 4 groups of *C. difficile*–infected patients included in the study, week 1, 2008–week 22, 2009. SSI, Statens Serum Institut; R, resistance.

Results

After the 2 microbiological datasets were merged, 2,299 case-patients with a first episode of infection were identified for the 17-month study. Of the 2,299 case-patients, isolates from 477 were referred to the national laboratory and were genotyped for toxins; of these 265 had genes for toxin A, toxin B, and binary toxin and were further ribotyped by PCR. Therefore, the 4 groups of patients with *C. difficile* infection used for the study consisted of 1,822 CD unselected, 212 CD A and B, 193 CD027, and 72 CD non-027. None of the isolates were positive for genes encoding only toxin A or B. The group of 72 CD non-027 consisted of 24 *C. difficile* PCR ribotype 078 (33%), 26 *C. difficile* PCR ribotype 066 (36%), and 22 *C. difficile* PCR ribotype 023, together with 9 other PCR ribotypes (31%).

Gender was equally distributed among the 4 groups of patients with CD unselected, CD 027, CD non-027, and CD A and B. The proportion of case-patients <50 years of age was much higher in the group with CD unselected (27.1%), compared with that of groups CD027 (4.6%) and CD non-027 (9.7%), which had more case-patients ≥80 years of age (Table 1). Most of the CD unselected, CD A and B, and CD non-027 were submitted by local clinical microbiology laboratories from areas not including the Capital region; most CD027 occurred in the Capital region, where the outbreaks of CD027 occurred in 2008–2009 (Table 1).

The case-fatality rate 30 days after diagnosis was independent of PCR ribotype in patients infected with strains that were positive for the binary toxin. More specifically, 54/193 case-patients with CD027 (28.0%, 95% confidence interval [CI] 21.8–34.9), and 20/72 case-patients with CD non-027 (27.8%, 95% CI 17.9–39.6) died within 30 days after infection. Case-fatality rate was 17.0% (36/212) for the group infected with CD A and B (95% CI 12.2–22.7) that did not possess genes for binary toxin, and lower (13.6%) for the 247/1,822 case-patients infected with CD unselected (95% CI 12.0–15.2). Among patients with CD non-027, seven deaths (29.2%) in CD078 were reported, 8 deaths (30.8%) in CD066, and 5 deaths in the group of other PCR ribotypes. No statistically significant difference was found between these case-fatality rates.

Kaplan Meier curves were created for 1 year after diagnosis. A steep increase was seen in the case-fatality rates within 30 days after the diagnosis for all groups of patients, but especially evident for the 2 groups possessing the binary toxin genes (Figure 2). The shape of the curve for case-patients with binary toxin genes (CD027 and CD non-027) almost overlapped in the first 30 days; curves for the other 2 groups had a different shape (log-rank test, $p<0.001$). The curve of the group of patients infected with CD A and B showed an intermediate case-fatality rate as compared with the 2 groups with binary toxin and CD unselected. The cumulative risk of death (Kaplan Meier function) after 60 days was 18.4% in case-patients with CD unselected (336/1,822), 24.5% in those with CD A and B infection (52/212), 37.1 % with CD027 (71/193), and 30.5% with CD non-027 (22/72). After 90 days, the cumulative risk of death rose to 20.9% for CD unselected (381/1,822), 26.8% for CD A and B (57/212), 38.9% for CD027 (75/193), and 36.1% for CD non-027 (26/72). Kaplan Meier curves were also created after excluding all case-patients <50 years of age for all 4 groups (550 case-patients) because of the higher proportion of patients <50 years of age in the group of CD unselected strains. The curves showed a similar shape as compared when using the full dataset (figure not shown, log rank test, $p<0.001$). The cumulative case-fatality rate at 30 days also remained comparable: 18.1% for case-patients with CD unselected strains (239/1,319), 19.8% for CD A and B (36/182), 29.0% for CD027 (53/183), and 29.2% for CD non-027 (19/65).

On the basis of these observations, which showed a similar case-fatality pattern for the groups that possessed the genes for the binary toxin, in the regression analysis, we combined these 2 groups with the genes for the binary toxin (CD027 and CD non-027) into 1 group, and compared it with the group not possessing the binary toxin (CD A and B). Therefore, in the regression analysis, the group of CD

Table 1. Characteristics of case-patients according to group of *Clostridium difficile* infection, week 1, 2008–week 22, 2009, Denmark

		No binary toxin	Presence of binary toxin	
Characteristic	No. (%) CD unselected, n = 1,822	No. (%) CD A and B, n = 212	No. (%) CD 027, n = 193	No. (%) CD non-027, n = 72*
Male sex	796 (43.7)	100 (47.2)	87 (45.1)	31 (43.1)
Age group, y				
<50	494 (27.1)	30 (14.2)	9 (4.6)	7 (9.7)
50–59	166 (9.1)	14 (6.6)	8 (4.1)	8 (11.1)
60–69	280 (15.4)	33 (15.6)	25 (13.0)	14 (19.4)
70–79	367 (20.1)	70 (33.0)	52 (26.9)	16 (22.2)
≥80	514 (28.2)	65 (30.7)	99 (51.3)	27 (37.5)
Region of local microbiology laboratory				
Capital region	263 (14.4)	46 (21.7)	164 (85.0)	22 (30.5)
Other parts of Denmark	1,502 (83.4)	158 (74.4)	29 (15.0)	50 (69.4)

*Consisting of *C. difficile* (CD) PCR ribotype 078 (n = 24), PCR ribotype 066 (n = 26), and PCR ribotype 023 and others (n = 22).

unselected isolates was excluded because these isolates were not submitted for characterization.

Univariate analysis showed that the relative risk (RR) for death within 30 days after diagnosis was 1.8 (95% CI 1.2–2.7) for case-patients infected with *C. difficile* that possesses the genes for binary toxin in addition to toxin A and B, as compared with those infected with strains possessing only genes for toxin A and B, which provided the reference level (Table 2). Multivariate analysis, after adjustment for age, sex, and region, showed that the RR became 1.6 (95% CI 1.0–2.4) for case-patients infected with the strains encoding the genes for the binary toxin when compared with the reference group of patients infected with strains without the genes for binary toxin (Table 2).

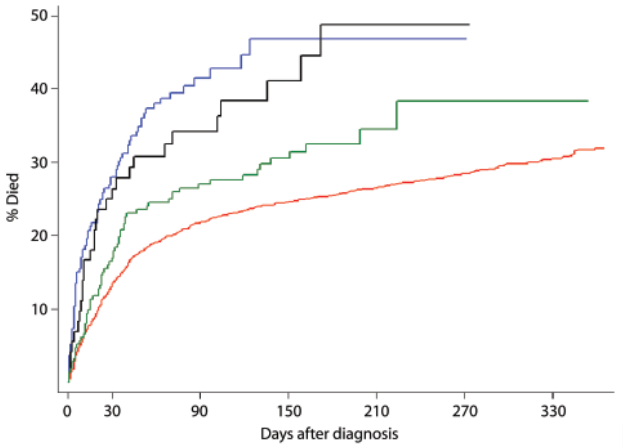

Figure 2. Kaplan Meier curves showing the probability of patient survival after diagnosis of *Clostridium difficile* infection according to the 4 different infection groups (log-rank test, p<0.001). Blue line, *C. difficile* PCR ribotype 027; black line, *C. difficile* PCR ribotype non-027; green line, *C. difficile* with toxins A and B without binary toxin; red line, *C. difficile* unselected strains not referred for typing.

Discussion

We used surveillance data to describe the case-fatality rate after a diagnosis of *C. difficile* infection. We found that the case-fatality rate is highest after infection with strains that possess genes for the binary toxin in addition to toxins A and B, irrespective of the PCR ribotype. Strains encoding genes for toxins A and B, but not binary toxin, showed a lower case-fatality risk.

A number of studies have addressed the issue of risk for death and severity of disease after infection with *C. difficile*. Overall, *C. difficile* PCR ribotype 027 has been associated with more severe disease and increased death rates. Nevertheless, many studies did not have a strict sampling frame or appropriate epidemiologic design, and their findings have been questioned by recent evidence (*13*,*21*). Our results are consistent with the initial findings that *C. difficile* PCR ribotype 027 is associated with elevated risk of death, but we elaborate further on the molecular characterization according to toxin profile. We suggest that the previously observed high case-fatality rate observed in *C. difficile* infection cannot be solely ascribed to excess risk for death after infection with PCR ribotype 027; other markers of virulence may be more appropriate than the PCR ribotype itself. The inclusion of case-patients on the basis of clinical findings only (*1*,*8*,*9*,*12*), the different criteria used to select strains for PCR ribotyping (*13*,*21*–*23*), or the lack of differentiation in separate groups according to toxin profiles (*24*) might have accounted for variation of estimates across the studies, as well as an overestimation of the risk for death associated with *C. difficile* PCR ribotype 027.

We observed a 28% case-fatality rate at 30 days for the 2 groups possessing the binary toxin: estimates from previous studies in Canada indicated a risk for death of 23% for patients with *C. difficile*–associated disease (CDAD), in a hospital in which *C. difficile* PCR ribotype 027 strain made up two-thirds of the isolates (*12*); or of 25% in another study involving 12 hospitals in which case-patients with CDAD were compared with controls without CDAD. In the latter study, 129/157 strains examined had

Table 2. Relative risk for death within 30 days after diagnosis of *Clostridium difficile* infection, univariate and multivariate analysis, week 1, 2008–week 22, 2009, Denmark*

Variable	No. deaths	Crude risk ratio (95% CI)	Adjusted risk ratio (95% CI)
CD A and B	36	Reference	Reference
CD 027 + CD non-027	74	1.8 (1.2–2.7)	1.6 (1.01–2.4)
Male sex	47	0.9 (0.6–1.2)	1.0 (0.7–1.4)
Age group, y			
<50	1	Reference	Reference
50–59	3	4.9 (0.5–47.2)	4.5 (0.5–43.9)
60–69	9	6.2 (0.8–48.8)	6.0 (0.8–47.3)
70–79	37	14.5 (2.0–105.8)	13.8 (1.9–100.9)
≥80	60	17.4 (2.4–125.3)	15.5 (2.1–112.6)
Region			
Capital	63	1.4 (1.0–2.1)	0.9 (0.6–1.2)
Other parts of Denmark	47	Reference	Reference

*CI, confidence interval; CD, *Clostridium difficile*.

pulsed-field gel electrophoresis patterns identical to NAP1 (*8*). In the Netherlands, 12.9% lethality was reported for *C. difficile* PCR ribotype 027 as compared with 7.0% in other *C. difficile* PCR ribotypes non-027 (*21*).

A few clinical studies indicate that the production of binary toxin correlates with the severity of CDI, rendering the strains with binary toxin more virulent. A case–control study conducted in 2005 included 26 patients infected with strains producing binary toxin in addition to toxins A and B and 42 controls infected with strains producing toxins A and B only. Diarrhea in case-patients was more frequently associated with abdominal pain (61.5% vs. 26.2%; p = 0.003) and with liquid stools (76.9% vs. 59.5%; p = 0.14) (*25*). Another case–case study from 2007 confirmed this tendency, showing that binary toxin–positive strains were significantly associated with more severe CDI (RR 3.38, 95% CI 1.29–8.85) and with higher case-fatality rates (RR 2.55, 95% CI 1.25–5.21) (*26*). Binary toxin–positive strains that produced neither toxins A and B were investigated in the rabbit ileal loop model to elucidate the contribution of binary toxin in the pathogenesis of CDI (*27*). This study showed that binary toxin contributed to marked nonhemorrhagic fluid responses when responses of nontoxigenic strains were compared. However, strains that produced toxins A and B gave rise to hemorrhagic fluid responses in this assay. In the same study, challenge with clindamycin-treated hamsters resulted in colonization of the binary toxin–positive strains but not diarrhea and death as seen for the strains that produced toxins A and B. Therefore, binary toxin may play an adjunctive role in the pathogenesis of disease caused by strains positive for toxins A and B (*27*).

Historically, *C. difficile* infection was not considered a severe disease, and studies performed 15 years ago reported case fatality rates of 3.0%–3.5% (*28*,*29*). Due to the current laboratory surveillance system, we were able to quantify 30-day case-fatality rate of a reference group (CD unselected isolates not referred for typing) at 14%, which provides an updated estimate of such baseline category. In a registry-based study in Finland, performed before *C. difficile* PCR ribotype 027 was identified in the country for the first time, a 14.2% 30-day death rate was reported among those discharged with a CDAD-related diagnosis (*30*). In Quebec, 13.8% of deaths reported 30 days after CDAD diagnosis were observed at the beginning of the *C. difficile* PCR ribotype 027 epidemic in 2003 (*9*).

Many studies have reported that a consistent fraction of the deaths occurring after *C. difficile* infection will be attributable to the bacterium (*1*,*8*,*12*,*21*–*23*,*31*) and that attributable death increases linearly with age (*8*,*31*). In our study, we could not differentiate between death after infection and attributable death because the registries did not contain information on the cause of death nor underlying illness. An excess proportion of deaths caused by CD027 and other strains with binary toxin corroborates recent evidence from Canada, which showed an increased risk for death in patients infected with the NAP1 strain (*24*)

Due to the availability of the national registries, we were able to investigate the case- fatality rate for a large cohort of patients and to get statistically significant results when investigating groups with different toxin profiles of the same infection. In addition, we performed multivariate analysis adjusting for age, sex, and region. Multivariate analysis indicated that the risk of death was increased by 60% (RR 1.6) for the strains possessing the binary toxin, irrespective of age, sex, and region of the laboratory submitting the isolates. Use of the registries made it possible to design the study on an individual patient basis, not only on isolates, and made it unlikely that deaths were missed.

The main limitations of the study were that we were not able to collect data on underlying illness from the registries and that the toxin gene profile of the unselected isolates not referred for further typing was not characterized. We accounted for the latter possible bias by excluding this group in the regression analysis, and by using the group toxin profiled without genes for binary toxin (CD A and B) as the reference level. The lack of availability of data on underlying illness means that the long-term case fatality explored with the Kaplan Meier survival function must be interpreted with caution. However, our estimates at 3 months after infection were comparable to those of a previous study in which confounding caused by underlying illness was addressed (*12*). Therefore, *C. difficile* could play a role in risk for death in the longer term. An increase in long-term deaths after bacterial gastrointestinal infections has been observed (*32*,*33*). Complications of operations performed after toxic megacolon, disruption of the colonic flora and intestinal cells, subsequent malabsorbtion, and, most importantly, the recurrence of infection, could be some of the mechanisms involved in long-term deaths after infection with *C. difficile*. About 19%–20% of first episodes of infection with *C. difficile* will be followed by a recurrence (*34*), either due to a relapse or reinfection with another strain.

In conclusion, our registry-based study demonstrates that patients infected with *C. difficile* strains possessing the binary toxin genes and genes encoding toxins A and B have a higher 30-day case-fatality rate, irrespective of PCR ribotype, when compared with strains that have toxins A and B only. Early recognition of the toxin profile might be beneficial in terms of clinical management of the disease. Future studies should address whether the binary toxin or an unknown co-expressed factor might be responsible for increased case-fatality rates. *C. difficile* PCR ribotype 027 can no longer be considered the only PCR ribotype

associated with severe disease, and efforts to control CDI should target all virulent strains of *C. difficile*, not only *C. difficile* PCR ribotype 027.

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Invasive Group A Streptococcal Infection and Vaccine Implications, Auckland, New Zealand

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We aimed to assess the effect of invasive group A streptococcal (GAS) infection and the potential effects of a multivalent GAS vaccine in New Zealand. During January 2005–December 2006, we conducted prospective population-based laboratory surveillance of Auckland residents admitted to all public hospitals with isolation of GAS from normally sterile sites. Using *emm* typing, we identified 225 persons with confirmed invasive GAS infection (median 53 years of age; range 0–97 years). Overall incidence was 8.1 cases per 100,00 persons per year (20.4/100,000/year for Maori and Pacific Islanders; 24.4/100,000/year for persons ≥65 years of age; 33/100,000/year for infants <1 year of age). Nearly half (49%) of all cases occurred in Auckland's lowest socioeconomic quintile. Twenty-two persons died, for an overall case-fatality rate of 10% (63% for toxic shock syndrome). Seventy-four percent of patients who died had an underlying condition. To the population in our study, the proposed 26-valent vaccine would provide limited benefit.

During the 2 decades since recognition of streptococcal toxic shock syndrome (STSS), there have been many publications on invasive group A streptococcal (GAS) infections, some population-based (*1*–*4*). The spectrum of infection caused by *Streptococcus pyogenes* varies widely from invasive disease, such as bacteremia, sepsis, necrotizing fasciitis (NF), and STSS, to noninvasive infection, most commonly pharyngitis with suppurative complications, such as otitis media, and nonsuppurative complications, such as acute rheumatic fever (ARF) and acute glomerulonephritis (APSGN).

GAS infection causes a substantial number of illnesses and deaths, especially in the developing world, with ≈500,000 deaths worldwide annually, attributable mostly to ARF and its sequelae, rheumatic heart disease, and invasive infection (*5*). GAS disease and its sequelae, including GAS pharyngitis, have been well documented in New Zealand (*6*–*12*; http://dnmeds.otago.ac.nz/departments/womens/paediatrics/research/nzpsu/pdf/2008_report.pdf).

With renewed interest in GAS vaccines (*13*), understanding the complete spectrum of disease, including invasive GAS disease, in diverse populations is essential. The vaccine most completely studied is a 26-valent vaccine based on *emm* types and M subtypes collected across GAS diseases from the United States (*14*). We previously published a population-based approach of laboratory surveillance for invasive bacterial diseases in Auckland's public hospitals where all persons with acute disease would be admitted (*8*,*15*–*18*). Using this approach, we demonstrate the effects of invasive GAS on the Auckland population to complement our knowledge of other GAS-associated diseases by using prospectively collected incidence data, clinical characteristics, associated underlying conditions, and the associated *emm* types. This study also provided an opportunity to establish the direction of further investigations and to focus interventions in New Zealand.

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Methods

Surveillance

We enrolled patients during January 1, 2005–December 31, 2006. Patients were included if they resided in metropolitan Auckland and had a GAS isolate cultured from a previously sterile body cavity. Patients with STSS were included in accordance with the consensus definition (*19*); STSS also was the diagnosis for patients who were dead on arrival or who died within 48 hours after illness onset and for whom laboratory data were insufficient in accordance with the methodology of Davies et al. (*1*). NF was defined as tissue necrosis diagnosed by histopathologic examination or by the treating surgeon during surgical debridement. Patients could have had >1 diagnosis, with the exception of bacteremia without a source. Clinical syndromes, such as skin or soft tissue infection, had to be accompanied by recovery of an isolate from a normally sterile site or specimen, such as blood, to meet the case definition. Nosocomial infection was defined as GAS infection in patients who had been hospitalized for >72 hours. Invasive GAS infection was defined as postpartum if it occurred in a woman who was pregnant or ≤30 days after delivery or who had clinician-defined puerperal fever, chorioamnionitis, or a septic abortion. Women from whom GAS was isolated from amniotic fluid or placenta alone were excluded (*20*). Our study was approved by the regional ethics committee and each hospital's research committee and Maori research committee.

Data were collected from the microbiology laboratories serving all 3 Auckland regional District Health Board (DHB) hospitals, i.e., Auckland City Hospital and Starship Children's Hospital (Auckland DHB); Middlemore Hospital, which includes Kidz First Children's Hospital (Counties Manukau DHB); and North Shore Hospital and Waitakere Hospital (Waitemata DHB). All Auckland residents with serious medical illness would attend 1 of these hospitals.

Auckland (2006 population: 1,387,780), New Zealand's largest city, comprises one third of the country's population and is the country's most ethnically diverse city. In 2006, 19.0% of residents self-identified as Asian, 14.4% as Pacific Islander, 11.1% as indigenous Maori, and 56.5% as European. The climate is temperate, with summer occurring during December through March. We used New Zealand birth data for infants <1 year of age and customized New Zealand census charts for DHBs as denominators.

We obtained demographic and clinical features by reviewing medical charts and electronic documents. To ensure complete surveillance, we requested International Classification of Diseases, 10th Revision, diagnoses from DHB data managers. We contacted the regional coroner and forensic pathologist to seek out records of deaths (including deaths in the community) caused by GAS infection and scrutinized intensive care unit (ICU) data for diagnoses of shock from GAS, STSS, or NF. Disease severity was determined by length of stay, ICU admission, and use of surgical and medical procedures.

We assigned each invasive GAS infection in the Auckland region a deprivation score by using the New Zealand Deprivation Index 2006 (www.moh.govt.nz). This index measures socioeconomic status (SES) in small areas according to 9 variables (income, income assistance, education, access to a car and phone, household crowding, employment, single-parent family, housing rented or owned).

Laboratory Techniques

β-hemolytic colonies on blood agar were typed as Lancefield group A by using commercially available latex agglutination kits (Pro-Lab Diagnostics, Austin, TX, USA). Group A isolates were sent to Environmental Science and Research Laboratory (Wellington, New Zealand) for *emm* typing by using established procedures (*21*). Concordance between *emm* types and M serotypes has been established (*21*). Antimicrobial drug sensitivities were determined by routine methods (*22*).

Estimates of Vaccine Benefit

We used *emm* typing to estimate the proportion of cases and deaths caused by *emm* types in the proposed 26-valent vaccine. These *emm* GAS types are 1, 2, 3, 5, 6, 11, 12, 13, 14, 18, 19, 22, 24, 28, 29, 33, 43, 59, 75, 76, 77, 89, 92, 94, 101, and 114 (*14*). We then calculated potential vaccine efficacy in the most at-risk Auckland populations: persons <5 years of age and ≥65 years of age.

Results

Epidemiology

During the 24-month study period, we identified 333 patients who potentially had invasive GAS infections. Of these, we excluded 118 who did not fulfill the inclusion criteria. The most common reasons for exclusion were isolation from a nonsterile site or residence outside metropolitan Auckland at the time of diagnosis. Using the electronic discharge summaries based on International Classification of Diseases, 10th Revision, coding, we identified and included 10 (4%) additional cases that fit the case definition.

The 225 patients were from all ethnic groups: European (77 [34%] patients), Maori (69 [31%]), Pacific Islanders (70 [31%]), and other ethnicities (7 [3%]). For 2 patients, no information was available about ethnicity. For the 225 patients, median age was 53 years (range 0–97 years), and 119 (53%) patients were male. Ethnic disparities, although

notable in the extremes of life, did not differ significantly by age (Figure 1; Table 1). The 198 patients with invasive GAS infection for whom SES information was available were more likely to originate in areas designated by the New Zealand Deprivation Index 2006 as lower SES areas than in higher SES areas (Table 2). Forty-nine percent of case-patients were from the lowest SES quintile.

Case-Fatality Rate

Twenty-two patients died, for an overall case-fatality rate (CFR) of 10% (Figure 1, Table 3). Fourteen of these patients died within 72 hours after hospital admission. Three infants (one 2 months of age and two 4 months of age) who died in the community had STSS. One death previously had been attributed to sudden infant death syndrome.

The median age of patients who died was 62 years (range 2 months–86 years). Eighteen adults who died had multiple concurrent illnesses. The highest CFR (31%) was for infants (a total of 4 deaths in three 4-month-old infants and one 2-month-old infant); these were the only deaths among children <15 years of age.

All infants who died had GAS-positive blood cultures. One who died in the community also had GAS-positive cerebrospinal fluid. Three of the 4 deaths occurred in the community and are attributed to STSS. The illness of the fourth (hospitalized) infant also met the criteria for STSS. Bronchopneumonia was found at post-mortem examination in 2 infants (1 hospitalized, 1 in the community). Two of the infants who died in the community had additional pathogens isolated from postmortem blood cultures (*Staphylococcus aureus* in both cases and *Streptococcus pneumoniae* and viridans streptococci in 1 each) but no gram-negative organisms.

Clinical Features

The most common clinical feature was skin and soft tissue infection (97/225; 43%) (Table 3). Of the 30 patients with STSS, 26 (87%) had an underlying condition before the onset of acute GAS disease. Median age at STSS occurrence was 57 years (range 2 months–86 years). Six cases occurred in children <5 years of age. Empyema (4 cases; p<0.0001) and brain abscess (2 cases; p = 0.0011) occurred more frequently in children ≤14 years of age than in adults. The incidence of bacteremia with no focus of infection was 1.4 cases per 100,000 persons per year overall, but 3.7 per 100,000 for children <5 years of age (n = 7).

Seven cases of GAS postpartum infection were recorded for women 15–49 years of age, for a rate of 0.16 cases per 1,000 live-born infants (Maori and Pacific Islander, 0.21 cases/1,000 live-born infants). No deaths occurred in this group. We also identified 3 premature neonates with invasive GAS disease unrelated to cases in adults; 1 infection was nosocomially acquired.

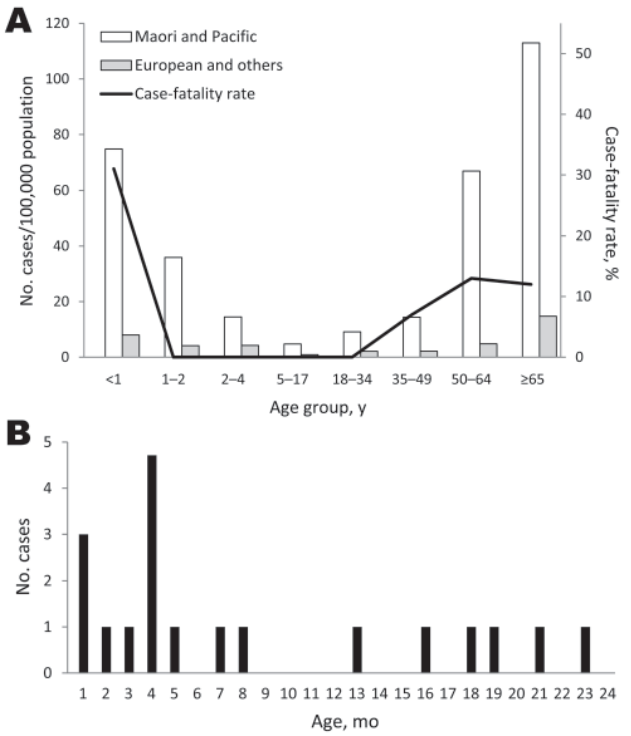

Figure 1. A) Annual incidence rates for invasive group A streptococcal (GAS) disease, Auckland, New Zealand, 2005–2006. The black line indicates age-specific case-fatality rates for combined ethnicities. B) Number of invasive GAS cases among infants ≤24 months of age.

Risk Factors

Of the 223 patients for whom data were available, 58 (26%) had no underlying condition or other risk factor, 114 (51%) had 1 or 2 risk factors, and 64 (28%) had ≥3 risk factors. In the ≥15-years age group, 67 (35%) had heart disease, 60 (32% [23 Maori, 25 Pacific Islanders]) had diabetes, and 21% had either renal disease (39 persons) or lung disease (40 persons). Cigarette smoking was the most common nondisease-related risk factor (56 [30%] of 189 persons ≥15 years of age).

Microbiological Analysis and Potential Vaccine-Preventable Disease

GAS was most frequently isolated from peripheral blood cultures (184 [82%]). Other sources were surgical specimen (37 isolates), tissue specimen (18), joint aspirate (16), pus aspirate (12), catheter blood culture (6), peritoneal aspirate (2), cerebrospinal fluid (2), and postmortem blood (3).

Of the 225 cases, 205 (91%) GAS isolates were available for *emm* typing (Figure 2). Seventy (34%) of 205 cases had an *emm* type that was contained in the 26-valent vaccine. The proposed 26-valent vaccine could prevent

Table 1. Population-based incidence of invasive group A streptococcal disease, by age, Auckland, New Zealand, 2005–2006*

	Age group, y									
	<1		<15		≤50		≥65		All ages	
Population	No.	Rate	No.	Rate	No.	Rate	No.	Rate	No.	Rate
Maori and Pacific Islander	11	20.3	27	13.0	74	80.1	30	113.0	139	20.4
Maori	8	40.9	14	14.1	33	82.5	15	146.8	69	21.6
Pacific Islander	3	16.4	13	12.0	41	78.2	15	91.8	70	19.3
Other	2	4.1	9	2.4	53	8.9	36	15.0	84	5.3
Total	13	33.0	36	6.1	127	18.4	66	24.4	225	8.1

*Rate/100,000 population. Table includes only populations at risk. Use of a Poisson regression model indicated no evidence of a difference in the effect of ethnicity on risk in different age groups. The incidence rate ratios for all ages of Maori compared with others was 7.60 (95% confidence interval [CI] 5.10–11.32) and for Pacific Islanders compared with others was 8.84 (95% CI 6.17–12.65). For male vs. female, the incidence rate ratio was 1.29 (95% CI 0.96–1.74).

30% of GAS invasive cases in children <5 years of age and 15% of cases in persons ≥65 years of age (Table 4).

Of the 225 isolates, 1 (0.4%) was resistant to erythromycin and 1 (0.4%) had intermediate sensitivity to erythromycin. Three (1.3%) were resistant to clindamycin.

Disease Severity

Hospitalization was required for 222 patients (3 deaths occurred in the community). Length of stay was >10 days for 105 (47%); mean length of stay was 15.9 days (range 1–153 days). Thirty-eight (17%) required ICU admission (mean length of stay 4.5 days; range 1–9 days); maximum length of stay was 19 days. Nosocomial infection was responsible for 12 (5%) of the 225 cases. Seventy-five (33%) patients required at least 1 surgical procedure, predominantly drainage, débridement, or washouts. One patient (2 years of age) with STSS required intravenous immunoglobulin.

Discussion

Our New Zealand study is population based and prospective. The overall annual incidence rate for greater Auckland of 8.1 cases per 100,000 persons per year is more than double or triple the rate of earlier reports elsewhere in the industrialized world. Annualized rates reported from other industrialized countries were 3.5 per 100,000 in 2007 in the United States (*2*); 1.5 in Ontario, Canada; and 3.1 in the Netherlands (*1*,*23*).

Table 2. Invasive GAS infection and relation with socioeconomic status, Auckland, New Zealand, 2005–2006*

New Zealand Deprivation Index 2006†	No. (%) confirmed invasive GAS infections, n = 198
10	63 (32)
9	33 (17)
8	23 (12)
7	23 (12)
6	10 (5)
5	10 (5)
4	6 (3)
3	10 (5)
2	15 (8)
1	5 (3)

*Based on the 198 case-patients for whom socioeconomic status information was available. GAS, group A streptococcal.
†New Zealand Ministry of Health, www.moh.govt.nz. 10, most deprived area; 1, least deprived area.

This study was conducted in metropolitan Auckland, where studies are ongoing to assess GAS disease, including endemic ARF (*11*,*12*,*24*), APSGN (http://dnmeds.otago.ac.nz/departments/womens/paediatrics/research/nzpsu/pdf/2008_report.pdf), and streptococcal pharyngitis (*25*) with associated *emm* typing. Our study was conducted in close association with ongoing active surveillance for ARF and its associated *emm* types (*26*) and APSGN surveillance. Our laboratory-based surveillance was supported by discharge data evaluation, chart review, and coroner surveillance, which minimized underestimation of STSS and NF. Approximately 50–70 new ARF cases (90% in persons <20 years) occur each year in this population (*12*,*24*) and a similar number of APSGN. The incidence of streptococcal pharyngitis has been carefully determined in a randomized controlled trial for ARF control at ≈60 cases per 100 child-years during a 4-year period in a population of ≈12,000 persons 5–19 years of age (*11*). This rate is considerably higher than that documented recently from Fiji (14.7/100 child-years) (*27*). Serotypes in ARF cases in our study were diverse (*emm* 58, 74, 75, 76, 92, 99, and 53), mirroring an earlier report (*emm* 53 and 58 associated with ARF) (*28*).

The annualized rate for Maori and Pacific Islanders <1 year of age (75/100,000) was similar to rates reported from Kenya (*29*) and greater than the rate more recently reported from Fiji (44.9/100,000) (*27*) from prospective studies. Nearly 50% of cases occurred in the lowest SES quintile of Auckland. Indigenous Maori and Pacific Islanders are overrepresented in lower SES areas of Auckland. Ethnically disparate rates for invasive GAS parallel these findings, with overrepresentation of these groups. The New Zealand Deprivation Index uses multiple parameters, including housing, income, and education. In addition, access to health care may be deficient (*30*,*31*) and perhaps health knowledge as well. The role of crowded housing in the population in our study has been recently documented for epidemic meningococcal disease (*32*) and may have a

Table 3. Clinical syndromes and CFRs for 225 patients with invasive GAS disease, Auckland, New Zealand, 2005–2006*

	Age group, y					
	0–14, n = 36		≥15, n = 189		All ages, no. (%),‡	
Diagnosis†	No.	CFR	No.	CFR	N = 225	p value§
Skin and soft tissue infection¶	11	0	86	8	97 (43)	0.14
Bacteremia only	7	38	31	19	38 (16)	0.63
STSS#**	6	67††	24	54	30 (13)	0.59
Bone infection	6	0	20	0	26 (12)	0.39
Pneumonia and other respiratory infection	12	13	12	0	24 (11)	0.0001
Necrotizing fasciitis**	1	0	19	15	20 (9)	0.21
Pelvic infection/peripartum‡‡	0	0	12	0	12 (5)	

*CFR, case-fatality rate; GAS, group A streptococcal; STSS, streptococcal toxic shock syndrome.
†Patients may have had >1 diagnosis, with the exception of bacteremia without a source. Other conditions (not shown) included 4 upper airway infections 6 ear/nose/throat infections, 5 central nervous system infections, 4 cases of peritonitis, 3 urinary tract infections, and 2 hemodialysis vascular access infections. No deaths occurred in this group.
‡Overall CFR 10% (22/225).
§p value calculated by using Fisher exact test, a test of difference between age groups.
¶Includes cellulitis (n = 79), cutaneous abscess, boil, lymphadenitis, myositis, bursitis, infected burn, infected scabies, and infected ulcer with evidence of documented bacteremia.
#STSS confirmed and probable (n = 3).
**Five patients had STSS and NF; 1/5 died (20% CFR).
††Three of 6 were community deaths in infants <1 y of age.
‡‡Includes pregnancy-related (n = 6) endometritis and infected products, urinary tract infection/chorioamnionitis, and wound problems.

more substantial role for GAS disease, which is considered to be even more contagious (*33*). The high likelihood of an associated risk factor in the adult population, such as a chronic disease or another association, has been reported many times (*1*,*2*,*27*,*34*).

Skin infections have been documented as a major cause of illness in Auckland (*35*). More recently, New Zealand surveillance data (*24*) reported highly discrepant hospitalization rates for serious skin disease: Maori and Pacific Islanders <15 years of age are more likely to be hospitalized (unadjusted rate ratios 2.77 [95% CI 2.66–2.88] and 4.47 [95% CI 4.27–4.68], respectively) than are New Zealand European children 0–14 years of age. These data also reflected more hospitalizations for persons living in the most deprived quintile (*24*), which most likely is related to poor access to primary care and perhaps health knowledge. High population-based rates of invasive disease caused by methicillin-sensitive *S. aureus*, mostly bone and joint disease, also have been documented (*36*).

The overall CFR from our study (10%), with a high CFR for STSS (63%), mirrors other studies in the industrialized world (*2*). This CFR suggests good access to hospital care and efficiently delivered secondary and tertiary care, including ICU admission. A recently reported CFR (28%) from Fiji suggests otherwise from the developing world (*27*).

We included in our study all 3 infants who died in the community and from whom GAS was cultured (*37*). GAS is a rare finding from postmortem specimens (J. Zucollo, pers. comm.). In all 3 cases, only gram-positive organisms were isolated (1 solely group A streptococcus from blood and cerebrospinal fluid). Studies in which careful precautions have been taken to reduce contamination show that approximately two thirds of blood cultures yield negative results, 2 in 9 yield 1 isolate, and 1 in 9 show mixed growth. GAS infection as the sole cause of death was less certain in 2 cases in our study in which >1 potentially disease-causing species was cultured. We characterized the 3 infant deaths as STSS according to Davies et al. (*1*), a definition

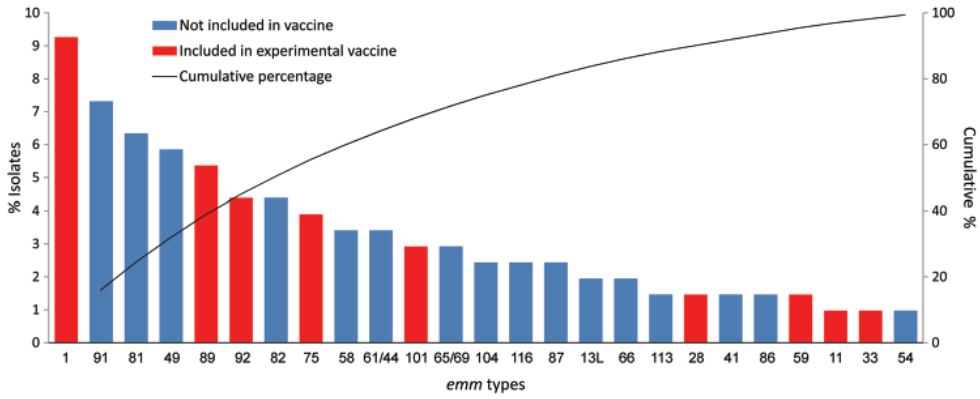

Figure 2. The 25 most common *emm* types as a proportion of all isolates. The remaining *emm* types were as follows: 100, 107, 25, 53, 56, 22, 18, 103, 105, 106, 108, 112, 123, 4, 51, 55, 70, 73, 77/27L, DRX4, ST6030, STN5554, 109, 110, 12, 52, 77, 88, 97, ST4119, ST4547, and 76.

Table 4. Invasive GAS disease and fatalities potentially prevented by vaccination of infants and elderly persons with a proposed 26-valent vaccine, Auckland, New Zealand, 2005–2006*

Age group, y (no. *emm* typed)	Assumed vaccine efficacy, %	Assumed vaccine coverage, %	No. (%) persons with GAS disease from *emm* types in the 26-valent vaccine†	GAS-related deaths from emm type in the 26-valent vaccine†, %	Potential GAS disease prevented %‡	Potential GAS-related deaths prevented, %§
<5 (25)	≥84	80¶	11 (44)	1#	29.6	0.67
≥65 (59)	84	60**	18 (30.5)	14 (1/7)	15	7.1

*GAS, group A streptococcal.
†Among patients with typed isolates.
‡Percentage of assumed vaccine efficacy × percentage of assumed vaccine coverage × persons with GAS disease from 26-valent *emm* types (based on O'Loughlin et al. [*2*]).
§Percentage of assumed vaccine efficacy × percentage of assumed vaccine coverage × persons with GAS disease from 26-valent *emm* types × percentage of GAS-related deaths associated with a 26-valent *emm* type.
¶Craig et al. (*24*).
#Of the 4 children <5 years of age, 2 had an *emm* typed isolate. Neither of these types is in the proposed vaccine. These are very small numbers.
**New Zealand Ministry of Health Immunisation Handbook (www.moh.govt.nz).

that produces higher rates of STSS and a higher CFR in children than in other reports. We look forward to further investigations in this area.

Current health strategies for preventing illness and death from invasive GAS infections are limited. The rate of nosocomial infection in our study was low. The high rates in postpartum women and in infants require further investigation. We were unaware of any links between cases in our series. In New Zealand, index cases of invasive GAS disease are not investigated by public health authorities (*38*). Primordial strategies, such as of the provision of less-crowded housing (*32*) and hand-washing education, need further consideration (*39*).

The currently available vaccine most advanced in clinical trials (*14*) comprises 26 *emm* types representing population-based, practice-based, and historical assessments from the United States (*14*). Its applicability to the population in our study might be less than ideal. Thirty-four percent of disease was caused by *emm* types in the proposed 26-valent vaccine. Data are accruing from other sites (79% *emm* coverage with the 26-valent vaccine in the United States, 69% in Europe, and 40% in Fiji) (*4*,*14*,*27*). Our data can contribute to a recent global estimate suggesting the current formulation of an experimental multivalent GAS vaccine may not be ideal in areas of most need (*40*). The effectiveness estimate in our study (Table 4) suggests that fewer than one third of invasive GAS cases in children <5 years of age and perhaps 15% of cases in persons ≥65 years of age could be prevented. This finding is of particular concern in a New Zealand population where other GAS-associated diseases cause a substantial amount of illness and death.

The rates in our study, driven largely by high rates in indigenous Maori and Pacific Islanders, are higher than those previously reported from industrialized countries and similar to reports from Fiji and Kenya. The rates suggest a need for more investigation and planned interventions in populations at highest risk. Our study also supports the role of GAS as a pathogen for invasive disease, particularly because of its effect on all age groups.

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Dr Safar is an advanced trainee in emergency medicine at Auckland City Hospital, Auckland, New Zealand. His primary research interests include severe sepsis and critically ill patients, trauma, and toxicology.

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Use of Antiviral Drugs to Reduce Household Transmission of Pandemic (H1N1) 2009, United Kingdom[1]

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The United Kingdom implemented a containment strategy for pandemic (H1N1) 2009 through administering antiviral agents (AVs) to patients and their close contacts. This observational household cohort study describes the effect of AVs on household transmission. We followed 285 confirmed primary cases in 259 households with 761 contacts. At 2 weeks, the confirmed secondary attack rate (SAR) was 8.1% (62/761) and significantly higher in persons <16 years of age than in those >50 years of age (18.9% vs. 1.2%, p<0.001). Early (≤48 hours) treatment of primary case-patients reduced SAR (4.5% vs. 10.6%, p = 0.003). The SAR in child contacts was 33.3% (10/30) when the primary contact was a woman and 2.9% (1/34) when the primary contact was a man (p = 0.010). Of 53 confirmed secondary case-patients, 45 had not received AV prophylaxis. The effectiveness of AV prophylaxis in preventing infection was 92%.

Following emergence of pandemic influenza A (H1N1) 2009 in North America in spring 2009 (*1*,*2*), global spread of the virus was rapid (*3*,*4*). In the United Kingdom, the first confirmed cases were detected in travelers returning from Mexico (*5*). The United Kingdom implemented a containment strategy until July 2009 that involved rapid case ascertainment, early treatment with antiviral drugs (AVs), and postexposure prophylaxis of patients' close contacts.

One key uncertainty was the transmissibility of the virus in household settings. Household-based studies of avian influenza previously provided a measure of transmissibility of newly emerging influenza viruses and also of the effectiveness of AVs in reducing spread (*6*). Early reports on pandemic (H1N1) 2009 have provided information on household transmission (*7*–*11*). Although most are from settings where AVs were not used (*8*,*10*) or where only a limited number of households were recruited (*7*,*9*), early work suggests that AVs had some effect on spread (*11*,*12*).

A detailed investigation of the first few 100 (FF100) case-patients and their close contacts (*13*) was undertaken across the United Kingdom beginning in April 2009 to gain an early understanding of the clinical and epidemiologic parameters of pandemic (H1N1) 2009 (*14*). Following the publication of early FF100 findings (*5*,*11*,*15*), we report the final results from ≈300 UK households of key household transmission characteristics.

Methods

The FF100 study has been described in detail (*15*,*16*). This study was a prospective investigation of the first

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laboratory-confirmed cases and patients' household contacts to determine key parameters such as virologic and clinical secondary attack rates (SARs) and effectiveness of AVs.

Definitions

Three case definitions were used: 1) virologically confirmed cases were persons testing positive for pandemic (H1N1) 2009 virus by specific reverse transcription PCR (RT-PCR) on respiratory swab; 2) influenza-like illness (ILI) cases were persons experiencing history of fever and ≥1 respiratory symptom (dry cough, productive cough, coryza, shortness of breath, or sneezing) within 2 weeks of onset of the confirmed household primary case; and 3) acute respiratory infection (ARI) cases were persons experiencing ≥1 respiratory symptom (as defined above) and/or fever within 2 weeks of onset of the confirmed household primary case. A household contact was any person who lived in the same household as a confirmed primary case-patient and ≥1 overnight stay after onset of illness in the person who was the primary case-patient (*16*).

A household was defined as the primary case-patient plus all household contacts. For a household, a virologically confirmed primary case was the case-patient with first date of onset within that household. A secondary case was any case-patient with date of onset >24 hours after date of onset of primary case. If a patient's onset of illness was <24 hours of onset of the primary case, it was classified as co-primary. A similar approach was followed for clinically confirmed secondary cases, with clinical co-primary cases excluded.

Secondary cases were defined as case-patients who had received prophylaxis if AVs were administered ≤24 hours before illness onset. Any asymptomatic contact who received AVs was classified as having prophylaxis. For a small number of contacts with non–case-defining symptoms before starting AVs, it was not possible to distinguish prophylaxis and treatment. These contacts were excluded for AV analyses.

Case Ascertainment

Initially, all patients with virologically confirmed cases detected in the United Kingdom were included in the FF100 dataset, and their households were followed up. As case numbers grew rapidly, convenience sampling was undertaken before closure of FF100 on June 21, 2009.

Collection of Epidemiologic Information

Information on case-patients was collected at 2 time points. Initial information was collected as soon as possible after a positive laboratory result was reported. Data were collected directly from case-patients or their parent or guardian by public health workers in person or by telephone interview. Information collected included demographics, clinical history (date of illness onset, signs and symptoms), medical history (including 2008–09 seasonal trivalent influenza vaccine or AV use), and underlying medical conditions. Inactivated trivalent influenza vaccines from various manufacturers are used in the United Kingdom with composition determined by World Health Organization recommendations.

Case-patients provided details of close household contacts. At initial interview, contacts were asked about their contact history with the primary case-patient; clinical history, including recent respiratory symptoms with dates of onset and treatment; medical history, including underlying medical conditions; and use of AVs with dates of administration.

Daily telephone follow-up of contacts was undertaken for 7 days. If any respiratory symptoms developed, contacts were instructed to speak to their general practitioners for prompt investigation, including collection of respiratory swab specimens. Swab samples were also inadvertently obtained from several contacts who did not have case-defining illness. To ensure that all contacts testing positive for pandemic (H1N1) 2009 virus were identified, the FF100 database and Health Protection Agency (HPA) laboratory reports of confirmed cases were compared.

Final follow-up of case-patients and household contacts was undertaken >2 weeks after to gather information on possible complications, final outcome (e.g., illness, death, and recovery), and use of AVs and antimicrobial drugs. For scheduled telephone follow-up, calls were attempted for a minimum of 3 consecutive days before the patient was classified as lost to follow-up. Information was gathered on a hard-copy questionnaire or entered directly into a Web-enabled database. Data verification and quality assurance were undertaken through standard data entry checks, double entry, and internal and external consistency checks.

Statistical Analysis

Single-person households were excluded from household analysis. SAR was calculated for clinical illness (ILI and ARI) and confirmed infection. The cumulative household SAR was defined as the total number of secondary cases in a household divided by number of household members at risk (excluding primary and co-primary cases) 14 days after onset in the primary case-patient. Household SAR was calculated by age group (<16 years, 16–49 years [reference group], ≥50 years), gender, AV prophylaxis (yes or no), and timing of treatment for the primary case-patient (≤48 hours vs. >48 hours) through univariate logistic regression analyses for the different endpoints. Multivariate analyses were also performed, adjusted for the aforementioned variables, and model fit assessed by using the Hosmer-Lemeshow goodness-of-fit test. Because confirmed SAR may be affected by failure

to obtain swabs from symptomatic contacts, observed positivity rates in the ARI and nonsymptomatic groups were used to adjust for this possibility.

A survival analysis was undertaken to determine the effect of prophylaxis on household SAR while accounting for timing of administration. A contact enters the model with time zero at index onset, and survival time is defined up until onset of disease in the contact (failure), or excluded at the end of the 2-week follow-up period. AV prophylaxis exposure was treated as a time-varying covariate, and for each contact, survival time was split into pre-AV and AV prophylaxis periods. The hazard ratio of becoming a secondary case-patient when AV prophylaxis was given was estimated by using Cox regression, adjusted for age, sex, and AV treatment of the primary case-patient ≤48 hours. This approach accounted for prophylaxis not usually being given to contacts until the case-patient was identified by health services.

Laboratory Confirmation

Respiratory samples from influenza patients were analyzed for pandemic influenza A (H1N1) 2009 and seasonal influenza viruses by RT-PCR. Combined nose and throat swab specimens were collected from patients who had signs and symptoms of suspected infection. These specimens were sent to a designated UK laboratory performing real-time RT-PCR for pandemic (H1N1) 2009 virus. Pandemic (H1N1) 2009 diagnosis was confirmed before June 2009 by sequencing the influenza A PCR amplicon (*17*), and from June onwards by real-time PCR of a swine lineage N1 (*18*).

Ethical Considerations

This observational study was undertaken as part of management of a national outbreak. The work was done under National Health Service Act 2006 (section 251), which provides statutory support for disclosure of such data by NHS and data processing data by HPA for communicable disease control. Health Protection Scotland remains embedded as part of NHS, and outbreak and investigation data were shared as part of the coordination of national outbreaks.

Results

Recruitment and Follow-up of Households

A total of 322 confirmed primary and co-primary case-patients were identified in 296 households (Figure 1). Of these 296 households, 37 were single-person. Case-patients from single-person households were older (mean age 27.4 vs. 19.7 years in other households; p = 0.003) with a nonsignificant trend toward males (64.9% vs. 50.2%; p = 0.092). Single-person households were excluded from further analysis, leaving 259 primary and 26 co-primary case-patients in 259 households (Figure 1).

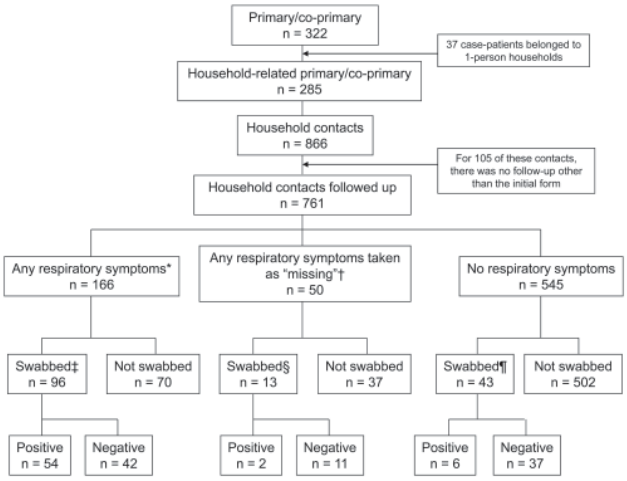

Figure 1. Flowchart of pandemic (H1N1) 2009 case-patients and household contacts, including contacts with respiratory symptoms, contacts from whom swab specimens were collected, and PCR result, United Kingdom, 2009. *Symptom onset date <2 weeks after index case-patient symptom onset; †46 persons had symptom onset date >2 weeks after index case-patient and 4 had missing symptom onset date; ‡5 persons had swabs taken >2 weeks after index case-patient symptom onset and 3 had positive test results; §2 persons (neither positive) had swabs taken >2 weeks after index case-patient symptom onset; ¶3 persons (none positive) had swabs taken >2 weeks after index case-patient symptom onset.

The total number of household contacts identified was 866. Of these, 105 (12.1%) declined to participate or were lost to follow-up (Figure 1), with no significant differences in age (p = 0.32) and sex (p = 0.47) between those followed and not followed up. Distribution of household sizes, primary cases, contacts, and secondary cases is shown in Table 1.

Household and Primary Case-Patient Characteristics

Average household size was 4 people (SD = 2.1), with a median size of 4 (interquartile range [IQR] 3–5) (Table 1). A comparison of age, gender, and AV use of primary case-patients, co-primary case-patients, and contacts is provided in Table 2.

Of the primary case-patients, 245 (95.7%) had received AV treatment (of whom 116/118 with information had received oseltamivir). Among treated case-patients, 104 (42.4%) had started treatment ≤48 hours of disease onset, with median time to AV treatment of 3 days (IQR 1–5).

Household Close Contacts

The age and gender distribution of the 761 followed-up household contacts are summarized in Table 2. Information on AV prophylaxis was available for 587 contacts (Tables

Table 1. Household size of case-patients with pandemic (H1N1) 2009 virus infection, United Kingdom, 2009

No. persons in household	No. households	No. primary and co-primary case patients	No. contacts	No. secondary case-patients
2	42	44	40	2
3	46	51	87	6
4	76	81	223	15
5	28	31	109	7
6	18	20	88	2
7	12	17	67	13
8	7	10	46	5
9	4	4	32	1
10	4	5	35	6
11	2	2	20	2
15	1	1	14	3
Total	240	266	761	62

2, 3); of the 444 contacts who named the AV they received, 435 received oseltamivir. Mean number of days from onset in the primary case-patient to starting prophylaxis in contacts was 4.4 days (SD 4.9, median 4 days, IQR 2–6 days) (Figure 2). Compliance for use of AVs found 255 contacts with information on prophylaxis start and end dates, with a median time to receiving AV of 9 days (IQR 8–10). Only 8 contacts received treatment for <5 days.

Household Secondary Attack Rates

Household contacts in whom respiratory symptoms developed within 2 weeks and from whom swab samples were collected are summarized in Figure 1. Overall, of 761 household contacts, 166 had ARI symptoms, 62 of whom were confirmed secondary case-patients, with a SAR of 8.1% (Table 4). Among those without ARI, 43 provided swab samples, 6 of whom had positive test results. The positivity rate in those with and without ARI that were tested was projected onto non-swabbed ARI patients to give an adjusted confirmed SAR of 13.8%. The SAR, adjusted for age and sex, was 16.7%.

Univariate analysis revealed a significantly higher confirmed SAR for patients aged <16 years and for those 16–49 years, compared to those ≥50 years. The SAR in male patients was higher than female patients, but the difference was not significant (Table 4). Most secondary case-patients (86.8%, 45/53) had not received prophylaxis; contacts who had not received AV prophylaxis had a significantly higher confirmed SAR than those who had (Table 4). Contacts who received prophylaxis ≤2 days after onset in the primary case-patient had a nonsignificantly higher SAR than those who received therapy later (Table 3), although the study did not have sufficient statistical power to detect such differences. The confirmed SAR was significantly lower in contacts whose primary case-patient had received treatment ≤48 hours of onset rather than after 48 hours (Table 4).

The confirmed SAR by age of primary case-patients is shown in Table 5. Confirmed SAR was high among those <16 years of age, whether the primary case-patient was a child or an adult. Similarly, SAR was low among adults, whether the primary case-patient was a child or an adult (Table 5). When transmission from adults to children was analyzed by gender, a significant difference was found for SARs in children according to sex of the adult primary case-patient: 33.3% (10/30, 95% confidence interval [CI] 17.3–52.8) for female primary case-patients and 2.9% (1/34, 95% CI 0.1–15.3) for men (odds ratio 16.5, 95% CI 2.0–138.8; p = 0.010).

Multivariate analysis shows the adjusted odds for a virologically confirmed secondary case were significantly higher for children <16 years of age than for adults. In addition, contacts who received AV prophylaxis had a significantly reduced risk of confirmed infection than those not treated (Table 4). Finally, the adjusted odds of a secondary case-patient were significantly lower when the primary case-patient had received treatment <48 hours of onset.

SAR for Clinically Confirmed Cases of ILI and ARI

For the ILI outcome, 259 households yielded an additional 16 cases defined as co-primaries. Seventy-eight clinically confirmed secondary cases occurred among

Table 2. Primary and co-primary confirmed case-patients with pandemic (H1N1) 2009 virus infection and household contacts, by sex, age, and prophylaxis status, United Kingdom, 2009*

Variable	No. (%) primary and co-primary case-patients	No. (%) contacts
Sex, n = 1,030		
M	143 (50.2)	364 (48.9)
F	142 (49.8)	381 (51.1)
Age, y		
<16	154 (54.0)	212 (27.9%)
16–49	114 (40.0)	378 (49.7)
≥50	17 (6.0)	171 (22.5)
Prophylaxis, n = 843		
No	253 (98.8)	132 (22.5)
Yes	3 (1.2)	455 (77.5)
Total	285	761

*n = 1,046 except as indicated.

Table 3. Confirmed SAR of pandemic (H1N1) 2009 virus infection, according to time antiviral drug prophylaxis began after onset of illness in primary case-patient, plus timing of secondary cases after onset of primary case, United Kingdom, 2009*

Timing	No. contacts	No. secondary case-patients at 14 d	SAR, % (95% CI)	No. (%) secondary case-patients			
				2 d	3–4 d	5–7 d	>7 d
No prophylaxis	143	45	31.5 (24.0–39.8)	15	12	10	8
Day 0	57	1	1.8 (0.0–9.4)	0	0	1	0
Days 1–2 (≤48 h)	81	4	4.9 (1.4–12.2)	0	3	1	0
Day 3–7 (inclusive)	214	3	1.4 (0.3–4.0)	NA	0	3	0
>7 d	92	0	0.0 (0.0-3.9)	NA	NA	NA	0
Total case-patients	587	53	9.0 (6.8–11.7)	15 (2.6)	15 (2.6)	15 (2.6)	8 (1.4)

*SAR, secondary attack rate; CI, confidence interval; NA, not applicable.

745 contacts for an overall household ILI SAR of 10.5% (Table 6).

For the ARI outcome, a further 26 ARI cases were defined as co-primaries. In the 259 households, of 719 contacts, 120 secondary case-patients resulted for an ARI SAR of 16.7% (Table 7). The effect of age, AV prophylaxis of contacts, and early treatment of case-patients were generally similar for both ILI and ARI clinical endpoints compared to virologically confirmed endpoints in both univariate and adjusted analysis (Tables 6, 7).

Survival Analysis of Prophylaxis

The hazard ratio (HR) of becoming a confirmed secondary case-patient when receiving AV drugs was 0.08 (95% CI 0.02–0.27). Results were similar after adjusting for AV treatment of the primary case-patient, age, and sex (HR 0.09, 95% CI 0.03–0.32). When looking at ILI endpoint, the unadjusted HR was 0.27 (95% CI 0.13–0.56) and adjusted HR was 0.27 (95% CI 0.13–0.57) and for ARI, the unadjusted HR was 0.31 (95% CI 0.18–0.52) and adjusted was 0.27 (95% CI 0.15–0.48). The Kaplan-Meier plots for the 3 endpoints are shown in Figure 3 and multivariate survival analysis results in Table 8.

In most households, either all members received prophylaxis (122/206, 59.2%) or none at all (30/206, 14.6%).

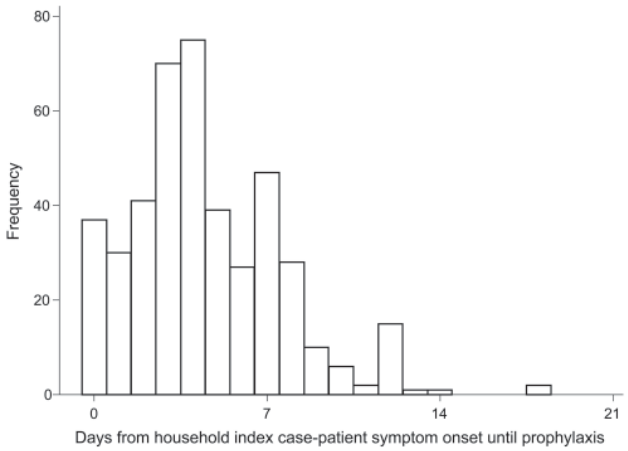

Figure 2. Days from symptom onset date of household primary case-patient with pandemic (H1N1) 2009 virus infection until antiviral prophylaxis started, N = 352, United Kingdom, 2009.

In discordant households, where some received prophylaxis and some did not (54/206, 26.2%), virologically confirmed SAR was similar to the main analysis: the SAR was 41.7% (95% CI 30.8%–53.4%) in those not receiving and 3.2% (95% CI 0.9%–7.9%) in those receiving prophylaxis. Survival analyses were repeated to allow for clustering within households, with the CIs being marginally wider.

Discussion

This study involved the prospective follow-up of households during the UK containment phase for pandemic (H1N1) 2009. We found a moderately high, virologically confirmed SAR with higher clinical (ILI and ARI) endpoints. Age-specific differences for SARs were significant; the SAR was highest among children. The SARs for child contacts were higher when adult women were the primary case-patients than when men were. Finally, most secondary case-patients had not received AV prophylaxis, and AV administration to household contacts substantially reduced the risk for infection.

This study found an overall virologically confirmed household SAR of 8%, similar to results for an earlier study involving the FF100 (*11*): SAR reached 34% among contacts who did not receive AV prophylaxis. The SAR increased further for clinical endpoints. These SARs for those who did not receive AVs compare to results of a study in Kenya which reported a confirmed household SAR of 26% (*10*) in a population without widespread use of AV prophylaxis. Another study in Japan (*7*), where >90% of contacts had received AV prophylaxis, reported a virologically confirmed SAR of only 5%. Other studies have used clinical endpoints, such as in the United States (*8*), where a clinical SAR of 10% was reported after 7 days. These findings compare to household SARs found for seasonal influenza in historical studies, ranging from 18% (*19*) to 22% (*20*). Although these studies had similar design, there are several possible explanations for our results, such as differences in case definition, a different period of follow-up, differences in ascertainment of secondary cases, and differences in AV use. Our observed SAR among those who did not receive prophylaxis is higher than that previously observed for seasonal influenza and suggests

Table 4. Univariate and multivariate analysis of pandemic (H1N1) 2009 virus infection SAR for virologically confirmed cases of pandemic (H1N1) 2009 virus infection, by gender, age group, and prophylaxis, United Kingdom, 2009*

Variable	No. contacts†	No. secondary case-patients	Univariate analysis		Multivariate analysis	
			SAR, % (95% CI)	p value‡	OR (95% CI)	p value
Sex, n = 745						
M	364	37	10.2 (7.5–13.7)		1.0, baseline	
F	381	25	6.6 (4.0–10.7)	0.08	1.0 (0.5–2.0)	0.96
Age, y						
<16	212	40	18.9 (14.2–24.7)		18.2 (3.9–85.5)	
16–49	378	20	5.3 (3.1–9.0)		3.5 (0.7–16.2)	
≥50	171	2	1.2 (0.3–4.7)	<0.001	1.0, baseline	<0.001
Prophylaxis, n = 587						
No	143	45	31.5 (24.4–39.5)		1.0, baseline	
Yes	444	8	1.8 (0.8–3.9)	<0.001	0.05 (0.02–0.09)	<0.001
Primary case-patient treatment						
>48 h	453	48	10.6 (8.1–13.8)		1.0, baseline	
≤48 h	308	14	4.5 (2.5–8.1)	0.003	0.30 (0.13–0.68)	0.004
Total	761	62	8.1 (6.4–10.3)			

*n = 761 except as indicated. Hosmer-Lemeshow goodness-of-fit test for multivariate model, p = 0.751. SAR, secondary attack rate; CI, confidence interval; OR, odds ratio.
†Excludes co-primary cases.
‡Indicates overall p value for differences by group.

a substantial proportion of close contacts were infected with pandemic (H1N1) 2009 virus. Serologic studies will provide important insights into the rates of infection (both symptomatic and asymptomatic in a household setting).

Recent publications have explored the possibility of using household data to estimate AV effectiveness for seasonal influenza (*21*,*22*). Our study provides evidence that AV prophylaxis of household contacts significantly reduces SAR for all endpoints, updating earlier work (*11*). Most secondary cases occurred in contacts who had not yet received AV prophylaxis after onset of illness in the primary case-patient, with a very high SAR observed in those that had not received AV for all endpoints, due to the delay for many before prophylaxis was started. The adjusted survival analysis took into account the confounding effect of time to prophylaxis and demonstrated that AVs are effective for all endpoints. Other studies in Japan (*7*), the United States (*9*,*12*), Hong Kong, China (*23*), and Germany (*24*) have attempted to determine the effectiveness of postexposure prophylaxis for pandemic influenza. Most show a statistically nonsignificant positive effect of AVs (*7*,*9*). Studies concerning AV effectiveness for seasonal influenza, in particular a large placebo-controlled household study, found that postexposure prophylaxis reduced the incidence of infection in close household contacts by 89% (*25*). Our study demonstrates that timely administration of AVs to close contacts provides significant protection against clinical disease.

Our study found clear age-specific differences in SAR, with a much higher household SAR in children than in the elderly. This age-specific pattern is also replicated, at least partially, by seasonal influenza: Longini reported a SAR of 24% in those <18 years of age and a rate of 14% in those >18 years (*26*). The high household SARs in children in the present study, illustrates the susceptibility of this subgroup and is consistent with general practice consultation data, laboratory surveillance data, and results of school outbreak investigations (*27*,*28*). The observation of very low SAR in those >50 years, who have also had household exposure to a confirmed case, demonstrates protection afforded by cross reacting H1N1 influenza antibodies from prior exposure to H1N1 subtypes circulating in the period before 1957 (*29*,*30*).

This study found that SAR was significantly lower when the primary case-patient had received rapid AV treatment, before and after adjustment for prophylaxis of contacts. The observation is biologically plausible as studies demonstrate early AV use reduces virus shedding (*31*). This may translate into reduced likelihood of secondary transmission and supports rapid treatment of patients to reduce household transmission. The observation that SARs from child to child and from adult to child (>20%) were similar, yet at least 4-fold higher than from child to adult or adult to adult, is also consistent with the increasing prevalence of cross-reacting antibodies against pandemic (H1N1) 2009 virus with age (*32*). Children are

Table 5. Pandemic (H1N1) 2009 virus infection SAR, by age of patient with virologically confirmed primary case, United Kingdom, 2009*

Transmission†	No. contacts	No. secondary case-patients	SAR, % (95% CI)
Child to child	148	29	19.6 (13.5–26.9)
Child to adult	318	9	2.8 (1.3–5.3)
Adult to adult	231	13	5.6 (3.0–9.4)
Adult to child	64	11	17.2 (8.9–28.7)

*SAR, secondary attack rate; CI, confidence interval.
†Primary case-patient to contact.

Table 6. Univariate and multivariate analysis of pandemic (H1N1) 2009 virus infection SAR for clinically confirmed cases of influenza-like illness, by gender, age group, and prophylaxis, United Kingdom, 2009*

Variable	No. contacts†	No. secondary case-patients	Univariate analysis		Multivariate analysis	
			SAR % (95% CI)	p value‡	OR (95% CI)	p value
Sex, n = 730						
M	357	33	9.2 (6.7–12.7)		1.00, baseline	
F	373	45	12.1 (7.9–18.1)	0.22	2.6 (1.4–4.9)	0.003
Age group, y						
<16	204	38	18.6 (13.9–24.6)		7.8 (2.7–22.1)	
16–49	371	32	8.6 (5.4–13.5)		2.7 (1.0–7.4)	
≥50	170	8	4.7 (2.2–9.8)	<0.001	1.00, baseline	<0.001
Prophylaxis, n = 573						
No	129	56	43.4 (35.1–52.1)		1.0, baseline	
Yes	444	18	4.1 (2.3–7.1)	<0.001	0.05 (0.02–0.09)	<0.001
Primary case-patient treatment						
>48 h	445	55	12.4 (9.6–15.8)		1.0, baseline	
≤48 h	300	23	7.7 (4.7–12.2)	0.040	0.78 (0.42–1.48)	0.458
Total	745	78	10.5 (8.5–12.9)			

*n = 745 except as indicated. Hosmer-Lemeshow goodness-of-fit test for multivariable model, p = 0.291. SAR, secondary attack rate; CI, confidence interval; OR, odds ratio.
†Excludes co-primary case-patients.
‡Indicates overall p value for differences by group.

known to excrete influenza virus in higher titers and for a longer period than adults (*33*,*34*), and social play between children often entails very close contact, so an SAR of 21% from child-to-child is expected. The SAR, however, for adult-to-child transmission was just as high, particularly among female primary case-patients, which suggests that despite lower virus titers and shorter duration of excretion, women transmitted pandemic (H1N1) 2009 infection as efficiently as child primary case-patients. This suggests adult respiratory hygiene is suboptimal in the home environment.

This study has several strengths: this is one of the largest pandemic influenza household studies published to date, and active follow-up was undertaken with daily telephone calls to ensure timely clinical investigation with swab collection to maximize case ascertainment. There are, however, limitations. First, not all those who had respiratory symptoms develop had throat swabs done, leading to under-ascertainment of confirmed secondary case-patients. Adjustments have been made to account for this. Second, case finding was based on a screening algorithm requiring fever. Thus, primary cases of pandemic influenza without fever would have been excluded; however, all clinical endpoints were gathered from secondary case-patients. Third, this article presents information only on clinical and virologic endpoints. There is now evidence that a substantial proportion of persons exposed to a primary case-patient will have asymptomatic or very mildly symptomatic infection.

Table 7. Univariate and multivariate analysis of pandemic (H1N1) 2009 SAR infection for acute respiratory infection, by gender, age group and prophylaxis, United Kingdom, 2009*

Variable	No. contacts†	No. secondary case-patients	Univariate analysis		Multivariate analysis	
			SAR, % (95% CI)	p value‡	OR (95% CI)	p value
Sex, n = 704						
M	339	56	16.5 (12.9–20.9)		1.0, baseline	
F	365	64	17.5 (12.5–24)	0.72	1.9 (1.0–3.5)	0.04
Age, y						
<16	194	49	25.3 (15.4–38.6)		7.0 (3.0–21.0)	
16–49	359	56	15.6 (9.2–25.2)		3.6 (1.5–8.8)	
≥50	166	15	9.0 (5.5–14.4)	<0.001	1.0, baseline	0.001
Prophylaxis, n = 549						
No	106	80	75.5 (66.4–82.7)		1, baseline	
Yes	443	34	7.7 (4.5–12.7)	<0.001	0.02 (0.01–0.03)	<0.001
Primary case-patient treatment						
>48 h	435	79	18.2 (14.8–22.1)		1, baseline	
≤48 h	284	41	14.4 (10.1–20.3)	0.019	1.7 (0.9–3.1)	0.11
Total	719	120	16.7 (14.1–19.6)			

*n = 719 except as indicated. Hosmer-Lemeshow goodness-of-fit test for multivariate model, p = 0.392. SAR, secondary attack rate; CI, confidence interval; OR, odds ratio.
†Excludes coprimary case-patients.
‡Indicates overall p-value for differences by group.

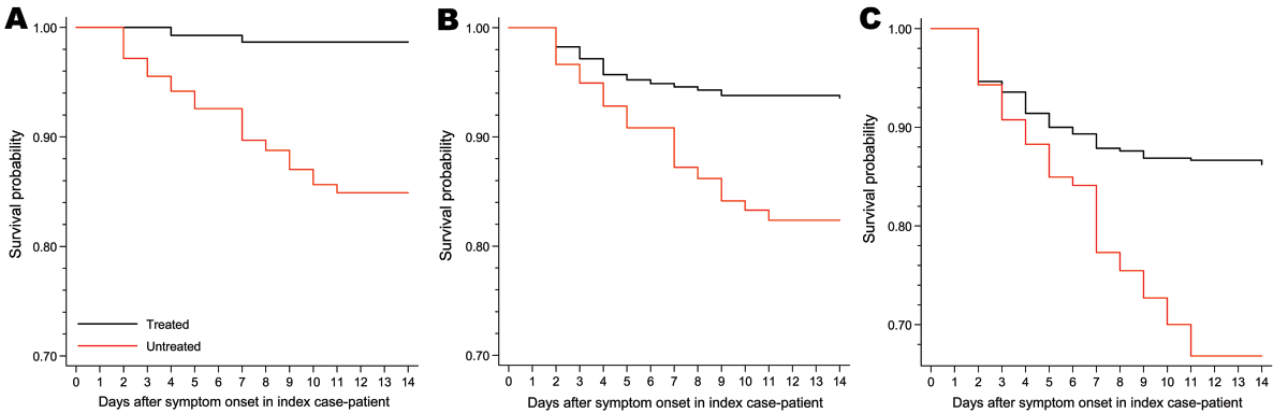

Figure 3. Kaplan-Meier graphs of days from symptom onset in index case-patient until onset of symptoms in secondary case-patients, United Kingdom, 2009. A) Virologically confirmed pandemic (H1N1) 2009; B) clinical influenza-like illness; C) acute respiratory infection.

This requires serologic investigation (*30*). Fourth, because data were captured as part of the acute public health response, data gathering was undertaken through multiple interviewers. Missing data were minimized by final follow-up of case-patients and contacts, and the demographic profile was not indicative of a systematic bias that might invalidate the results. Fifth, if a primary case-patient was confirmed quickly, their contacts may have avoided further contact, whereas if the primary case-patient was identified later, close contact may not have been avoided. However, a time-varying survival analysis found no significant difference for contacts not receiving AV. Sixth, information concerning prior respiratory disease in contacts was not gathered, and some persons may have had prior exposure to pandemic (H1N1) 2009. However, this is unlikely because pandemic transmission was not yet widespread when our data were collected, and this should not have been a major potential confounding factor. Finally, we assumed household secondary case-patients acquired their infection after contact with a defined primary case-patient in the household, rather than in the community. Although more advanced statistical methods do exist to take into account these competing transmission risks (*26*,*35*), this study was undertaken at a stage when community transmission was limited so this contribution is assumed to be minimal.

In conclusion, we demonstrate transmission of pandemic influenza in the household setting in the United Kingdom during the containment phase. Household SARs were generally higher than those of seasonal influenza. Timely AV treatment of primary case-patients and prophylaxis was effective in protecting household contacts, although delayed administration of AV did allow spread. Prompt AV administration (either as treatment or prophylaxis) reduces symptomatic SARs.

Table 8. Multivariable survival analysis of for pandemic (H1N1) 2009 virus infection SAR with virologic, influenza-like-illness, and acute respiratory infection endpoints, by gender, age group, and prophylaxis, United Kingdom, 2009*

	Hazard ratio (95% confidence interval)		
Variable	Virologic	Influenza-like illness	Acute respiratory infection
Sex			
M	1 (reference)	1 (reference)	1 (reference)
F	0.97 (0.56–1.69)	1.77 (1.08–2.89)	1.30 (0.90–1.90)
Age, y			
<16	4.23 (2.35–7.62)	2.78 (1.68–4.61)	1.90 (1.29–2.81)
16–49	1 (reference)	1 (reference)	1 (reference)
≥50	0.33 (0.08–1.42)	0.47 (0.18–1.22)	0.54 (0.29–1.00)
Antiviral drug prophylaxis			
Untreated	1 (reference)	1 (reference)	1 (reference)
Treated	0.09 (0.03–0.32)	0.27 (0.13–0.57)	0.27 (0.15– 0.48)
Index case-patient treatment			
>48 h	1 (reference)	1 (reference)	1 (reference)
≤48 h	0.45 (0.23–0.87)	0.72 (0.42–1.23)	0.99 (0.66–1.50)

*SAR, secondary attack rate.

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Pandemic (H1N1) 2009 Risk for Frontline Health Care Workers

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To determine whether frontline health care workers (HCWs) are at greater risk for contracting pandemic (H1N1) 2009 than nonclinical staff, we conducted a study of 231 HCWs and 215 controls. Overall, 79 (17.7%) of 446 had a positive antibody titer by hemagglutination inhibition, with 46 (19.9%) of 231 HCWs and 33 (15.3%) of 215 controls positive (OR 1.37, 95% confidence interval 0.84–2.22). Of 87 participants who provided a second serum sample, 1 showed a 4-fold rise in antibody titer; of 45 patients who had a nose swab sample taken during a respiratory illness, 7 had positive results. Higher numbers of children in a participant's family and working in an intensive care unit were risk factors for infection; increasing age, working at hospital 2, and wearing gloves were protective factors. This highly exposed group of frontline HCWs was no more likely to contract pandemic (H1N1) 2009 influenza infection than nonclinical staff, which suggests that personal protective measures were adequate in preventing transmission.

Australia was affected early in the (H1N1) 2009 influenza pandemic with 37,636 cases and 191 deaths reported. The state of Victoria was the first to observe a substantial peak in the number of persons infected (*1*). The pandemic was managed within the framework of the Australian Health Management Plan for Pandemic Influenza (*2*). Guidelines for use of personal protective equipment (PPE) were established in the Victorian Health Management Plan for Pandemic Influenza (*3*). Recommendations included use of N95 masks, gloves, protective eyewear, and long-sleeved gowns.

Influenza in health care workers (HCWs) is common, and acquisition in the workplace is well documented. An uncontrolled study found that after an influenza epidemic in Glasgow, Scotland, 120 (23.2%) of 518 HCWs seroconverted (*4*). Early in 2009, twelve HCWs with probable or possible work place acquisition of pandemic influenza were reported in the United States. None had worn full PPE (*5*).

That HCWs may be concerned about attending work during a potentially serious influenza pandemic is not surprising. During the severe acute respiratory syndrome outbreak of 2003, some HCWs reportedly stayed at home for fear of becoming infected and transmitting infection to family members. A number of surveys have found that 16%–33% of HCWs may not report to work in the event of an influenza pandemic (*6*–*9*).

HCWs need to know the transmission risks to make rational decisions about working during an influenza pandemic. Because HCWs are exposed in the community as well as the workplace, they should know about the additional risks for contracting influenza at work. This information is also imperative for pandemic workforce planning.

We sought to determine whether frontline HCWs were at greater risk for contracting pandemic (H1N1) 2009 influenza than the control group. Additionally, we sought information on factors that may have increased or decreased the risk for infection.

Methods

We conducted a cohort study, comparing frontline HCWs with intensive patient contact (clinical) and staff with no patient contact (nonclinical). Frontline HCWs

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were defined as those who worked ≥1 shift per week and had likely exposure to patients with pandemic influenza infection. These workers included doctors, nurses, and physiotherapists, as well as others in the emergency department, intensive care unit, infectious diseases units, and respiratory and other wards where patients with suspected pandemic influenza were housed. Staff members who had no clinical contact were chosen as a convenient surrogate for a community control group. These workers included university and hospital staff in nonpatient contact areas such as the library, information technology, and administration. This study was approved by the Human Research Ethics Committees at each of the hospitals and all participants gave written informed consent. The study was conducted from August 24, 2009, through December 16, 2009.

Four tertiary referral hospitals in metropolitan Melbourne were involved: Royal Melbourne, St Vincent's, Austin, and Alfred Hospitals. At all sites, patients with suspected or confirmed pandemic influenza infection were cared for in negative pressure isolation rooms when they were available, and in private rooms when they were not. Institutional infection control policies directed that gloves, gowns, goggles, and masks be used when caring for these patients. Use of N95 masks was initially recommended in all hospitals, although hospital 1 changed to surgical masks after June 16, 2009. Hand hygiene with an alcohol-based product and respiratory etiquette were promoted at all hospitals.

The progression of the pandemic in Victoria is shown in Figure 1. The original research plan was to obtain 2 serum samples, 3 months apart, from all participants to test for seroconversion and also to obtain weekly nose swabs for pandemic influenza detection by using real-time PCR. By the time the study commenced, the pandemic was waning, influenza cases were decreasing in Victoria, and following the original study plan was not considered feasible.

The plan was thus modified. An initial serum sample was obtained from all participants to measure for pandemic influenza antibodies. At study entry, participants completed a Web- or paper-based questionnaire that requested information on demographic characteristics, known influenza exposures outside the workplace, and any history of fever or respiratory symptoms occurring during the pandemic but before the study. In addition, the clinical group was asked about work exposure to patients with suspected pandemic influenza and their usual use of PPE when caring for these patients. Participants were also asked about use of neuraminidase inhibitors (NIs) and specifically whether they received prophylaxis after exposure to a patient with confirmed influenza.

Participants were instructed to provide nose swab specimens for viral testing if they experienced signs and symptoms, including cough, sore throat, rhinorrhea, laryngitis, fever, myalgias, or headache. All were asked to complete a weekly questionnaire regarding symptoms, influenza exposure, and use of NIs. If a participant reported respiratory illness, a second serum sample was requested for antibody testing to document possible seroconversion.

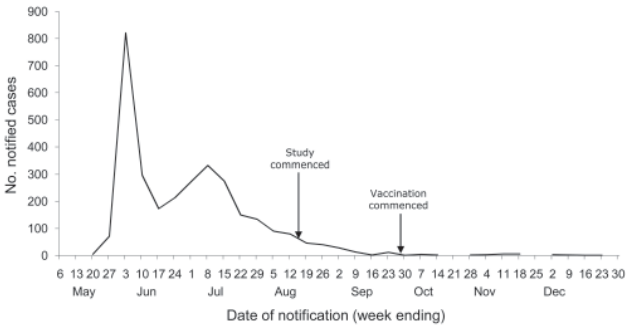

Figure 1. Notified cases of laboratory confirmed pandemic (H1N1) 2009, by week, Victoria, Australia, 2009. Arrows indicate dates when this study and vaccination commenced. Data provided by Victorian Department of Health, 2010.

Serum was tested for antibodies to pandemic (H1N1) 2009 influenza virus by using the hemagglutination inhibition assay with A/California/7/2009 virus and turkey red blood cells (*10*). A titer of <40 was defined as negative and ≥40 as positive. Nucleic acid detection was performed on nasal swabs by using reverse transcription PCR (RT-PCR) for influenza-specific and pandemic (H1N1) 2009 virus–specific sequences on swabs; kits were provided by the Centers for Disease Control and Prevention (Atlanta, GA, USA) (*11*) and an ABI-7500FAST instrument at the World Health Organization (WHO) Collaborating Centre for Reference and Research on Influenza in Melbourne.

Statistical Analysis

On the basis of early estimates of antibody positivity to pandemic influenza virus in the community, we assumed 20% infection rates in clinical staff and 10% rates in nonclinical staff. We calculated that 438 participants were required to achieve 80% power to detect this difference using a 0.05 two-tailed significance level. The primary outcome was the presence of a positive antibody titer in the first serum sample, indicating likely pandemic influenza infection.

We performed 2 separate univariate and multivariate analyses to delineate putative risk and protective factors (1 included all participants and the other included clinical participants only) to investigate any association between health care–specific risk factors and pandemic influenza. Multivariate analysis was performed by using forward and backward stepwise logistic regression, including all variables in the model initially and a p value for removal

of 0.1 and for entry of 0.2. Data were analyzed by using StataIC10 (StataCorp., College Station, TX, USA).

Results

The study took place from August 24, 2009, through December 16, 2009, largely before release of the pandemic influenza vaccine, and no participant was vaccinated during the study. Table 1 shows the number of patients who had confirmed pandemic influenza infection (by PCR) and were treated in each of the hospitals. Characteristics of study participants are shown in Table 2.

A total of 446 HCWs participated in the study, 231 in the clinical group and 215 in the nonclinical group. Overall, 79 (17.7%) of 446 demonstrated evidence of infection on the basis of a positive antibody titer of ≥40, 46 (19.9%) of 231 in the clinical group, and 33 (15.3%) of 215 in the nonclinical group; the difference was not statistically significant (odds ratio [OR] = 1.37, p = 0.21, 95% confidence interval [CI] 0.84–2.22).

The median participant age was 38 years (range 18–74 years); 27% were <30 years of age, 20% were 30–39 years of age, 25% were 40–49 years of age, and 20% were ≥50 years of age. Figure 2 shows the reverse cumulative distribution of first serum antibody titers, according to age. We found no statistically significant difference between the curves (p = 0.11 by ordinal logistic regression).

On multivariate logistic regression, the only factor associated with a higher risk for pandemic influenza among all participants was younger age (OR 0.96, 95% CI 0.94–0.99) after adjustment for participant status (clinical vs. nonclinical), age, gender, hospital, seasonal influenza vaccination, confirmed pandemic influenza, reported respiratory illness, community contact with influenza, oseltamivir prophylaxis, number of children in the household <18 years of age, and hours worked per week. On univariate analysis, the only factors that were significantly associated with protection against infection in the clinical group were use of any mask (OR 0.16, 95% CI 0.03–0.97) and use of gloves (OR 0.09, 95% CI 0.02–0.5) for patients in droplet precautions. Adjusted odds ratios are shown in Table 3.

Table 1. Number of patients with pandemic (H1N1) 2009 influenza virus infection at each of 4 hospitals, Australia, August 24–December 16, 2009*

Hospital no.	No. patients with confirmed pandemic (H1N1) 2009	No. inpatients	No. ICU patients	No. deaths
1	57	36	10	0
2	85	35	8	3
3	97	43	9	2
4	33	27	10	3

*ICU, intensive care unit.

Serology and Swab Test Results

Of the 395 participants, 140 (35%) reported a respiratory illness and 46 had nose swabs taken. Seven were positive for pandemic (H1N1) 2009 virus by PCR, 1 for subtype H3N2 influenza, and 38 were negative. One of the 46 had 2 swabs taken during different illnesses; the first was positive and the second was negative for pandemic (H1N1) 2009 virus. PCR cycle threshold values for swab specimens were from 30 to 40, indicating low viral loads. This finding may indicate that poor swabbing techniques were used, that the sample had been taken as infection was waning, or that level of infection was low (data not shown).

For 87 participants, a second serum sample was taken because of a reported respiratory illness. The average number of days between the first and second sample was 60 days (range 28 to 122 days, median 54) days. Thirty-six participants who had nose swabs performed also had a second serum sample taken. Seroconversion occurred in only 1/87 workers, with an initial titer of <10 and a subsequent titer of 40 (76 days later). This participant had a nose swab taken during a respiratory infection, which was negative for influenza virus. Seroconversion did not occur in any of the participants with a positive nose swab specimen. The mean number of days from obtaining a

Table 2. Characteristics of clinical and nonclinical participants at 4 hospitals at study entry (unless otherwise specified) who were infected with pandemic (H1N1) 2009, Australia, August 24–December 16, 2009*

Factor	Clinical participants, n = 231	Nonclinical participants, n = 215
Antibody titer ≥40	46 (19.9)	33 (15.3)
Mean age, y (range)	35.1 (19.8–56.6)	43.2 (18.5–74.1)
Female gender	157 (68.0)	153 (71.2)
Seasonal vaccination 2009	163 (70.1)	141 (65.6)
Previous seasonal vaccination	187 (80.0)	152 (70.7)
Reported confirmed pandemic (H1N1) 2009 influenza virus infection	1 (0.4)	0
Other influenza-like illness	155 (67.1)	118 (54.9)
Oseltamivir prophylaxis	13 (5.6)	1 (0.5)
Community contact with influenza	42 (18.2)	46 (21.4)
Median no. children <18 years in household (range)	0 (0–7)	0 (0–3)
Nasal swab taken during study	30 (12.9)	16 (7.4)
Mean no. hours worked per week (range)	39.2 (8–90)	37.9 (6–86)

*Values are no. (%) except as indicated.

positive nose swab specimen to the second serum sample was 44 days (actual number: 14, 21, 27, 43, 45, 114 days). One participant with a positive nose swab sample did not have a second serum sample taken. None of the participants with a positive nose swab or seroconversion reported taking NIs in their weekly survey.

Four of the 7 participants with a positive PCR result and the 1 in whom seroconversion occurred were in the clinical group (3 doctors, 1 pharmacist, 1 nurse, 1 physiotherapist). The participant who showed seroconversion was 29 years of age; participants with a positive PCR result ranged from 24–63 years of age. Two of the participants with a positive PCR result worked on the infectious disease ward, 2 in the emergency department, and 1 in the intensive care unit; seroconversion occurred in the participant who worked in a medical ward. Five of the participants with positive PCR results and the participant in whom seroconversion occurred had received the 2009 and previous seasonal influenza vaccines. None of the participants with confirmed influenza reported taking oseltamivir for either prophylaxis or treatment.

Weekly Questionnaires

In total, 395 participants completed 1–13 weekly questionnaires each. Eighty-nine clinical and 51 nonclinical participants reported 139 and 91 respiratory illnesses, respectively. No participant reported having laboratory-confirmed pandemic (H1N1) 2009 influenza. Six reported community contact with someone who had laboratory-confirmed infection. One reported taking oseltamivir after contact with an infected person in the workplace. This person had 2 serum samples taken 88 days apart; both had an antibody titer of <10.

Discussion

In this study, we evaluated the risk for pandemic (H1N1) 2009 in HCWs compared with the risk for such infection in a control group, as well as the factors associated with infection. HCWs had slightly higher rates of seropositivity than nonclinical staff; however, this difference was not statistically significant. Our data are supported by results of another recent study, which found that being a HCW was not a risk factor for serologically confirmed seasonal influenza virus infection and that the risk of HCWs acquiring influenza was more strongly associated with household than workplace exposure (*12*). That study found a seroconversion rate of 11.2% in HCWs and 10.3% in non-HCWs. However, it examined only doctors and nurses, whereas our study included other types of frontline HCWs. Another study reported a seroprevalence for pandemic (H1N1) 2009 of 26.7% in HCWs, which was not significantly different from the seroprevalence of the general population (*13*). Neither of these studies examined use of PPE.

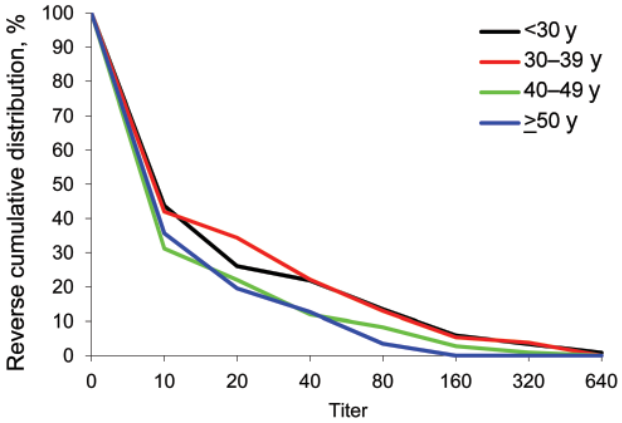

Figure 2. Reverse cumulative distribution of first serum antibody titer for pandemic (H1N1) 2009, by patient age, Victoria, Australia, 2009.

Overall, we found that 17.7% of participants had serologic evidence of pandemic (H1N1) 2009 virus infection after the peak of the outbreak. This proportion reflects the observed 16% seroprevalence in adults in Melbourne (*14*). These rates are lower, however, than the 31.7% antibody positivity found in South Australia during a prelicensure study of pandemic influenza vaccine in July 2009, which excluded subjects with confirmed or suspected pandemic (H1N1) 2009 influenza (*15*). This difference in titers may have reflected geographic differences in infection rates or differences between the populations sampled.

In the analysis of all participants, we found that older age was associated with lower rates of pandemic (H1N1) 2009 influenza infection. We did not observe higher levels of preexisting antibodies against pandemic (H1N1) 2009 influenza with increasing age, which has previously been reported. However, results of other studies examining the relationship between seroprevalence and increasing age are conflicting (*15–18*). Immune mechanisms other than type-specific antibodies may be providing protection for older participants. Other possibilities are that older persons have older children who may be less likely to acquire or transmit influenza or that older participants were more conscientious with respiratory etiquette and hand hygiene; attempts to measure these factors were not included in this study.

Among the HCWs we studied, working at hospital 2 conferred protection against pandemic (H1N1) 2009 virus infection. This hospital was in a geographic area with fewer cases than the others, but if this were the explanation, then a similar finding might have been expected in the nonclinical group, which was not demonstrated. Furthermore, at least as many cases of confirmed pandemic (H1N1) 2009 influenza were seen at hospital 2 as were seen at the other hospitals (Table 1). Factors such as reported compliance with PPE, were adjusted for in the multivariable analysis

Table 3. Factors significantly associated with positive titer for pandemic (H1N1) 2009 in HCWs at 4 hospitals, Australia, August 24–December 16, 2009*

Factor	Antibody positive, n = 46	Antibody negative, n = 185	Adjusted OR (95% CI)
Mean age (range), y	33.0 (19.8–49.7)	35.6 (21.2–56.6)	0.92 (0.87–0.98)
Workplace, no. HCWs			
Emergency department	13	56	1
Infectious diseases ward	1	31	0.17 (0.02–1.48)
Intensive care unit	19	45	2.53 (1.05–6.09)
Medical ward	5	16	–
Other	6	22	–
Respiratory ward	2	15	–
Hospital no., no HCWs			
1	11	54	1
2	3	48	0.26 (0.07–0.98)
3	15	41	–
4	17	42	–
Community contact with influenza, no. HCWs	4	38	0.25 (0.07–0.92)
Gloves for DP, no. using/no. responses	40/45	182/184	0.06 (0.01–0.46)
Median no. children <18 y in household (range)	0 (0–7)	0 (0–4)	1.83 (1.18–2.82)

*HCWs, health care workers; OR, odds ratio; CI, confidence interval; –, no result (comparator group); DP, droplet precautions. Results are adjusted for HCW status (clinical vs. nonclinical), gender, receipt of seasonal influenza vaccine, confirmed pandemic (H1N1) 2009, reported respiratory illness, oseltamivir prophylaxis, hours worked per week, work type (doctor, nurse, physiotherapist, other), work contact with influenza virus infection, mask/eye protection/gown/gloves for patients in droplet precautions, aerosol-generating procedures, and wearing an N95 mask or eye protection.

to reduce the effect of hospital type on influenza risk. The reason for the lower risk associated with hospital 2 has not been identified but may relate to other unmeasured factors, such as compliance with hand hygiene procedures.

Wearing gloves while caring for patients as part of droplet precautions was strongly associated with a lower risk of having had pandemic (H1N1) 2009 virus infection. Use of gloves was highly correlated with use of gowns, masks, and eye protection on logistic regression (results not shown). This finding confirms the great importance of PPE in preventing transmission of respiratory viruses in the health care setting and may explain why HCWs with definite exposure to influenza in the workplace, in addition to probable exposure in the community, do not have higher rates of infection than those with only community exposure.

The risk for pandemic (H1N1) 2009 virus infection increased with the number of children <18 years of age living in the participant's household, which has previously been reported as a risk factor (*12*). In Victoria, the median age of persons with reported pandemic (H1N1) 2009 virus infection was 15 years, with 67% of all notified case-patients being 5–17 years of age (*1*). Miller et al. also found that children were predominantly infected (*17*). This finding, coupled with the difficulties of maintaining good respiratory etiquette in young children, is a plausible explanation for the effect of child number on infection risk.

Working in the ICU was also identified as a risk factor for pandemic influenza; patients in ICU may be severely ill, with high viral loads, and staff may be heavily exposed during multiple aerosol-generating procedures. In addition, use of PPE and hand hygiene compliance may have been lower than in other wards or patients with pandemic influenza may have been unrecognized and therefore appropriate PPE not used.

Exposure of HCWs to suspected or proven pandemic influenza in the community was protective against having a positive antibody test result. This finding is counterintuitive and difficult to explain. One hypothesis is that HCWs who knew that they had had community exposure may have been more attentive to hand hygiene and other infection control precautions while at work or were more likely to enact social distancing.

We found only 1 instance of seroconversion among the 87 participants (including the 6 with PCR-confirmed infection), each of whom had 2 serum samples taken for antibody measurement. Miller et al. reported that 89.1% of participants with pandemic (H1N1) 2009 had an antibody titer of >32 three weeks after infection, although a baseline serum sample was not taken; therefore, seroconversion could not be demonstrated (*17*). None of the participants with positive PCR results reported taking NIs, and all had serum samples taken ≥2 weeks after the positive nose swab specimen, allowing sufficient time for seroconversion. Our results are likely to be true positives, as all swabs were only taken when patients were symptomatic. Previously, virus isolation has been the gold standard for influenza detection but RT-PCR is now considered to be more sensitive and specific. A previous study by some of the current authors has shown that seroconversion occurs in 80%–90% of serum samples if they are tested a sufficient time after infection (confirmed by RT-PCR) (*19*). Nasal swabs are a relatively peripheral type of sample (*20*). If viral load is low in the nose, the sample may be insufficient as an antigenic stimulus to induce a detectable level of seroconversion

in the serum. This may be the explanation for the lack of seroconversion seen in some PCR-positive cases in this study.

Because the number of pandemic (H1N1) 2009 cases in Victoria was low by the time this study commenced, we used a single antibody measurement for diagnosis in most patients. This is not ideal, because some participants may have had preexisting cross-reactive antibodies, as reported by others (*15*,*16*). However, this cross-reactivity has been most commonly found in older persons >65 years of age, a population which was underrepresented in our study. The explanation given for presence of cross-reactive antibodies in older persons has been past exposure to other antigenically similar viruses or a lifetime exposure to influenza A virus (*17*). Because this exposure could not have occurred in our younger study participants (median age 38 years) and serum samples were collected toward the end of the pandemic wave when many would have already been exposed, reactivity likely was specific to pandemic (H1N1) 2009. These factors support the use of a single antibody measurement for diagnosis.

This study relied on self-reported symptoms and risk factors, including use of PPE, making it subject to recall bias. This is a particular problem potentially for recall of exposures (e.g., to others with influenza or for use of PPE). However, many of the predictor variables were not subject to recall bias (e.g., clinical or nonclinical status, work place, age, gender, occupation, and number of children in the household). In addition, in order to influence the results, the 2 exposure groups would have had to exhibit differential recall. Although it could be postulated that HCWs may have perceived that they were at greater risk for exposure and may have therefore been more conscientious when filling out questionnaires, we believe that because of the large amount of public awareness of pandemic (H1N1) 2009 at that time, it is unlikely that this group would have been more conscientious than the nonclinical group.

In conclusion, we found that HCWs did not have a substantially increased risk of contracting pandemic (H1N1) 2009 in a health care setting with high availability of PPE. We conclude that use of PPE was highly protective against acquiring pandemic (H1N1) 2009 virus infection, and we therefore encourage its use, along with scrupulous hand hygiene and respiratory etiquette.

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Marked Campylobacteriosis Decline after Interventions Aimed at Poultry, New Zealand

Ann Sears, Michael G. Baker, Nick Wilson, Jonathan Marshall, Petra Muellner, Donald M. Campbell, Robin J. Lake, and Nigel P. French

Beginning in the 1980s, New Zealand experienced rising annual rates of campylobacteriosis that peaked in 2006. We analyzed notification, hospitalization, and other data to explore the 2007–2008 drop in campylobacteriosis incidence. Source attribution techniques based on genotyping of *Campylobacter jejuni* isolates from patients and environmental sources were also used to examine the decline. In 2008, the annual campylobacteriosis notification rate was 161.5/100,000 population, representing a 54% decline compared with the average annual rate of 353.8/100,000 for 2002–2006. A similar decline was seen for hospitalizations. Source attribution findings demonstrated a 74% (95% credible interval 49%–94%) reduction in the number of cases attributed to poultry. These reductions coincided with the introduction of a range of voluntary and regulatory interventions to reduce *Campylobacter* spp. contamination of poultry. The apparent success of these interventions may inform approaches other countries could consider to help control foodborne campylobacteriosis.

Campylobacteriosis is a common bacterial gastroenteritis reported in New Zealand and many other industrialized countries, with most cases caused by *Campylobacter jejuni* (*1*,*2*). Campylobacteriosis has been a notifiable disease in New Zealand since 1980, and medical practitioners are required to report confirmed or suspected cases to their local public health service (*3*). Campylobacteriosis notifications rose steadily after campylobacteriosis first became notifiable and peaked in 2006 at >380 per 100,000 population (*4*). A concomitant increase in campylobacteriosis hospitalizations has been noted, which suggests this rise in notifications is unlikely to be artifactual (*3*,*5*).

To help inform prevention and control strategies, research efforts have been directed at establishing the likely contributors to this rise in campylobacteriosis incidence. Consistent with international findings (*6*–*8*), New Zealand investigations implicated poultry meat as a significant source of foodborne sporadic campylobacteriosis (*9*–*13*). A relatively small case–control study in Christchurch in 1992–1993 reported several poultry-associated risk factors, including consumption of undercooked poultry (*10*). A larger national case–control study in 1994–1995 reported similar findings, with a combined population-attributable risk of poultry-related exposures >50% (*9*). A systematic review also concluded that poultry consumption was a prominent risk factor for sporadic campylobacteriosis in New Zealand (*11*). Reports noted the rise in campylobacteriosis was closely correlated with an increase in consumption of fresh poultry (*14*).

Microbiological source attribution approaches have also been used to estimate the contribution of different sources and transmission pathways of campylobacteriosis in New Zealand. These techniques involve examining the epidemiology of campylobacteriosis at the genotype level by comparing *C. jejuni* genotypes from humans with those found in a range of food and environmental sources. In 2005, a major source attribution study for campylobacteriosis was initiated at a sentinel surveillance

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site in the Manawatu region of New Zealand (*12*). *C. jejuni* isolates from cases notified in the region were genotyped by using multilocus sequence typing (MLST) and compared with isolates recovered from food and environmental sources (*12*,*13*). Statistical modeling was used to apportion human cases to potential disease sources, thereby estimating each source's relative importance (*13*,*15*,*16*). This modeling revealed that >50% of sporadic campylobacteriosis cases in the region were attributable to poultry (*12*,*13*).

On the basis of these findings, public health professionals advocated for more rigorous controls on foodborne pathways of campylobacteriosis, particularly for poultry (*5*,*14*). One intervention promoted was the freezing of fresh poultry meat to reduce levels of *Campylobacter* spp. contamination, with fresh poultry allowed to be sold only when it could be shown to pose a low risk to human health (*5*,*14*). In late 2006, the New Zealand Food Safety Authority (NZFSA) released a risk management strategy for reducing incidence of poultry-associated foodborne campylobacteriosis.

New Zealand has a highly integrated, closed system of poultry production, with all poultry meat available for retail sale being of domestic origin. Processors of poultry meat control most aspects of production, processing, and distribution; 3 processing companies supply >90% of all chicken meat consumed in New Zealand (*2*). As a result, interventions applied to the local poultry industry affect all domestically consumed poultry.

A marked decline in campylobacteriosis notifications was observed during 2007 and 2008 (*17*). We investigated this decline to assess whether it was causally related to the poultry-focused food safety interventions.

Methods

Descriptive Epidemiology

Historic notification and hospitalization data were used to calculate annual rates of campylobacteriosis in New Zealand during 1980–2009 for notifications and 1996–2009 for hospitalizations. A detailed descriptive analysis was then undertaken to examine the epidemiology of campylobacteriosis for the 12-year period 1997–2008 on the basis of notified and hospitalized cases.

Campylobacteriosis notification data are collated nationally by the Institute of Environmental Science and Research Ltd from notifications made by medical practitioners to their local public health service. During the study period, >96% of these notifications were culture-confirmed cases, with the remainder being epidemiologically linked to confirmed cases. Hospitalization data are collated by the Ministry of Health from information supplied by public hospitals. Analysis of hospitalized cases was based on patients with a principal diagnosis code for *Campylobacter* enteritis (International Classification of Diseases, 9th Revision, Clinical Modification, code 008.43, or International Classification of Diseases, 10th Revision, code A04.5). These data were further selected to exclude hospital transfers, readmissions within 30 days, and day cases (i.e., patients assessed in hospital for a short time but not requiring an overnight stay). Admissions to private hospitals were excluded because few patients with infectious diseases are admitted to such institutions and documentation is inconsistent.

In the detailed descriptive analysis, temporal trends in disease incidence and distribution were examined according to patient age, sex, socioeconomic status, ethnicity, urban versus rural dwelling, region (health board area), and season. Case-patients were assigned rurality and deprivation scores on the basis of their home domicile. For rurality assignment, we used a Statistics New Zealand classification system, which defines 7 grades of rurality on the basis of population size and employment address (*18*). Socioeconomic status was measured by deprivation scores assigned according to the New Zealand Deprivation Index, an area-based measure of socioeconomic position derived from the 5-year Census of Population and Dwellings (*19*).

The main descriptive analysis rates were calculated by using interpolated and extrapolated Census Usually Resident population data from 1996, 2001, and 2006. Rates for 2007 and 2008 (with 2007 being the transition year, on the basis of the gradual implementation of interventions) were compared with the average annual rates for 2 baseline periods (1997–2001 and 2002–2006). For the longer time-trend analysis, rates were calculated by using mid-year population estimates derived by Statistics New Zealand (*20*).

To examine the stability of the notification system for enteric diseases during the period of interest, we compared rates for campylobacteriosis notification and hospitalization with rates for 3 other notifiable enteric diseases (salmonellosis, yersiniosis, and cryptosporidiosis). Ethical approval for this study was obtained from the Multi-Region Ethics Committee, Wellington, New Zealand.

Source Attribution

During March 2005–December 2008, *C. jejuni* isolates from human case-patients and environmental and food sources were collected in the Manawatu area and genotyped (sequence-typed) by using MLST (*12*,*16*). Food samples were collected from fresh meat (poultry, beef, lamb) in retail stores, and environmental water samples were collected from swimming locations in rivers. Sheep and cattle feces were sampled from farms adjacent to the catchments of these rivers.

Two models were used to apportion human cases to sources on the basis of sequence types: the modified Hald model and the Island model (*12*,*15*). The modified Hald model combines the prevalence of each *C. jejuni* sequence type among the sources with the observed number of human isolates of that type by using a Bayesian framework (*15*). This model includes source-specific and type-specific factors, and accounts for variation in the estimated prevalence. The source-specific factor gives a measure of the ability of a source to act as a vehicle for human infection, whereas the type-specific factor yields a measure of the ability of a particular sequence type to cause disease.

The Island model uses an evolutionary model to assign sequence types to a particular source "island" or population (*12*). Mutation, recombination, and migration rates for isolates within and between each island are estimated by using the source isolates, and the posterior distribution of these estimates are then used to infer the origin of human isolates (*12*,*13*). To account for variations in food-processing practices that may affect the likelihood of human infection from each food source, we further extended both models to examine whether changes had occurred over time in the relative contribution of different sources to human campylobacteriosis (dynamic modeling) (*21*).

Key Informant Interviews and Policy Review

Key informants (n = 12), including industry and food safety experts, were interviewed to obtain information on interventions implemented to reduce *Campylobacter* spp. contamination in poultry. We used information from these interviews together with a review of policy documents from NZFSA and the poultry industry to formulate a summary of the interventions implemented from 2006 through 2008.

Results

Descriptive Epidemiology

The time-trend analysis of annual notification and hospitalization rates demonstrates a steady rise and then a marked decline in the incidence of campylobacteriosis (Figure 1). In the detailed descriptive analysis covering 1997–2008, the 2008 annual rate for campylobacteriosis notifications was 161.5/100,000 population, representing a 54% decline compared with the average annual rate of 353.8 for 2002–2006 (online Technical Appendix, www.cdc.gov/EID/content/17/6/1007-Techapp.pdf). The 2008 campylobacteriosis hospitalization rate of 7.6/100,000 population represented a 56% decline compared with the average annual rate for 2002–2006 of 17.3/100,000 population (Figure 1).

Statistically significant declines in notifications were evident across all analyzed population subgroups, although the magnitude of the declines varied (online Technical Appendix). Similarly, significant decreases were seen for most subgroups for campylobacteriosis hospitalizations (results not shown).

For the 2002–2006 period (before the decline), a trend for lower notification rates was shown among those residing in more rural areas compared with those living in main urban areas (rate ratios [RR] <1 where the reference is "main urban areas") (Figure 2). In contrast, significantly higher notification rates were observed among those residing in more rural areas compared to those living in main urban areas in 2008 (Figure 2; online Technical Appendix), indicating greater declines in incidence occurred in urban areas than in rural areas during 2007–2008.

The largest declines in campylobacteriosis notification rates between the average annual rate for 2002–2006 and the 2008 rate were seen in winter months (RR 0.38, 95% confidence interval [CI] 0.36–0.40), in urban populations (RR 0.42, 95% CI 0.41–0.43), in the age groups 20–29 years and 30–39 years (RR 0.40, 95% CIs 0.38–0.43 and 0.37–0.43, respectively) and in the Asian ethnic group (RR 0.26, 95% CI 0.22–0.31) (online Technical Appendix).

Conversely, the smallest declines in notification rates comparing the 2008 rate with the average annual rate for 2002–2006 were seen in rural populations (RR 0.66, 95% CI 0.62–0.70), in the 0–4 and the ≥80 years-of-age groups (RR 0.63, 95% CI 0.59–0.67, and RR 0.61, 95% CI 0.53–0.70 respectively) and in Māori, the indigenous people of New Zealand (RR 0.49, 95% CI 0.44–0.55) (online Technical Appendix).

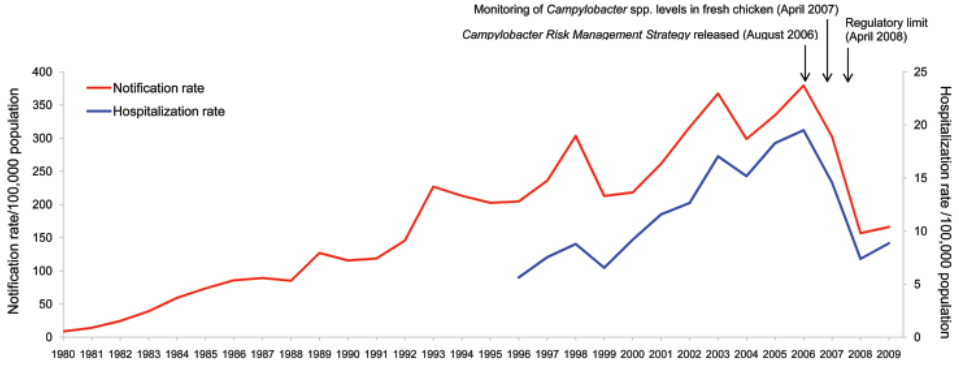

Figure 1. Campylobacteriosis notification rates per 100,000 population by year, 1980–2009, and hospitalization rates per 100,000 population by year, 1996–2009, New Zealand. Arrows indicate key interventions.

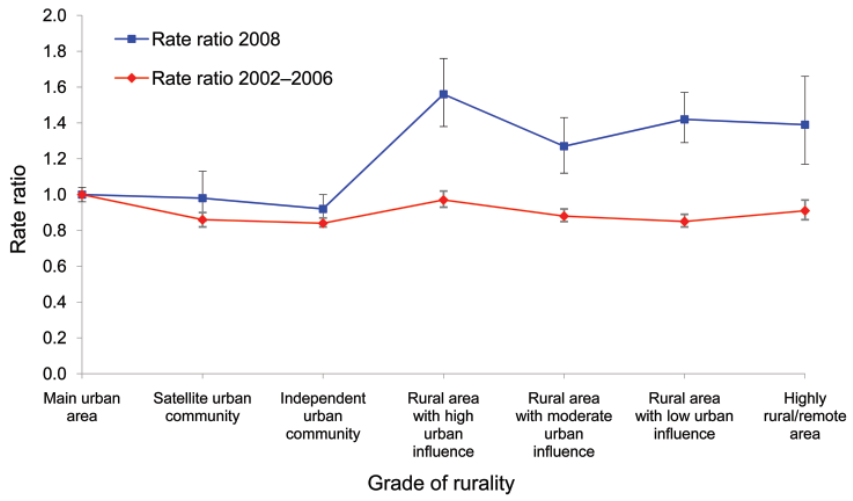

Figure 2. Rate ratios of campylobacteriosis notifications in New Zealand by grade of rurality for 2002–2006 and 2008. Main urban area was used as reference value for rate ratios. Error bars indicate 95% confidence intervals.

Figure 3 shows the temporal relationship between campylobacteriosis notification rates for 1997–2008 and 3 other notifiable enteric diseases. The marked decline in campylobacteriosis notifications during 2007–2008 is evident, while over this same period, salmonellosis, cryptosporidiosis, and yersiniosis rates remained relatively stable

Source Attribution

During the study period 2005–2008, 572 human *C. jejuni* isolates and 811 food and environmental isolates were collected (and had complete MLST profiles available). The estimated number of cases attributable to each source over time (based on the dynamic modified Hald model) is shown in Figure 4. These data show that compared with the baseline period (2005–2006), the number of cases in the Manawatu region attributed to poultry declined by 74% (95% credible interval 49%–94%) in 2008. No evidence was found for a decline in cases attributed to nonpoultry sources over the same period (p>0.5) (Figure 4). Similar results were obtained for the dynamic version of the Island model (results not shown).

Summary of Interventions

Specific food safety and poultry industry interventions were implemented beginning in 2006, in line with NZFSA's strategy for reducing the incidence of foodborne campylobacteriosis (Table). From April 2007, poultry processors monitored and reported to the NZFSA-administered National Microbiological Database *Campylobacter* spp. prevalence in poultry flocks by using presence/absence cecal testing and *Campylobacter* spp. contamination levels in poultry carcass rinsates at the end of primary processing (Table).

In April 2008, mandatory *Campylobacter* spp. performance targets were introduced based on enumerated levels of *Campylobacter* spp. contamination on poultry carcasses at the end of primary processing, with escalating regulatory responses if targets were not met (*22*). NZFSA has subsequently released an updated *Campylobacter* Risk Management Strategy (*23*).

Key informants noted that attention to detail with hygienic practices throughout production and primary processing and alterations to the immersion-chiller conditions were key areas in which improvements were made. Furthermore, the monitoring of *Campylobacter* spp. contamination levels in poultry carcass rinsates at the end of primary processing and setting mandatory *Campylobacter* spp. performance targets (rather than mandating specific interventions) were viewed by both industry and regulator informants as key facilitators of the strategy's success.

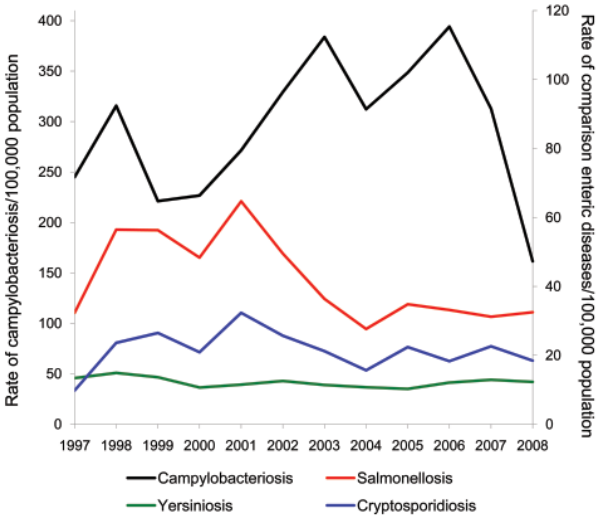

Figure 3. Annual campylobacteriosis notification rates per 100,000 population compared with annual notification rates per 100,000 population for salmonellosis, cryptosporidiosis, and yersiniosis, New Zealand, 1997–2008.

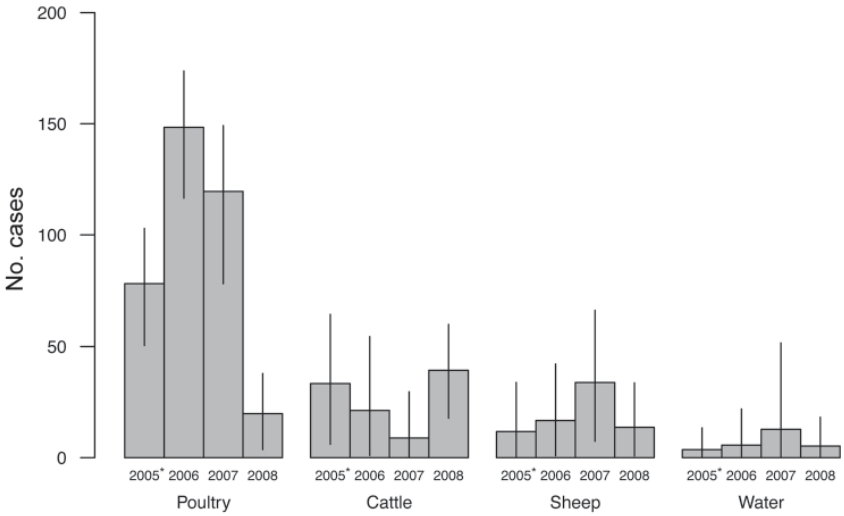

Figure 4. Number of cases attributed to source by year as determined by the modified Hald model in the Manawatu region of New Zealand. Error bars indicate 95% credible intervals. *2005 data are March through December only.

Discussion

New Zealand experienced a marked decline in campylobacteriosis incidence during 2007, with the 2008 notifications and hospitalization rates >50% lower than the averages for 2002–2006. This decline was sustained in 2009 (Figure 1). This decreased incidence implies 70,000 fewer community cases in New Zealand in 2008 compared with the peak in 2006, on the basis of the widely used multiplier of 7.6× the number of notified cases occurring in the community (*24*).

This reduction in incidence corresponds closely in time to the introduction of voluntary and regulatory interventions to reduce contamination of poultry with *Campylobacter* spp. Furthermore, patterns of the decline in disease incidence by population subgroup and area, along with the lack of plausible alternative explanations, suggest a causal effect from the poultry-focused interventions. The greater decline in campylobacteriosis in urban populations compared to the decline in rural populations (Figure 2) suggests that changes in foodborne transmission pathways were a key driver of the decline, compared with exposure pathways more likely to be encountered in rural settings (e.g., direct contact with contaminated environments or animals).

Source attribution modeling also provides supportive evidence that the decline in human campylobacteriosis can be largely attributed to a reduction in infection arising from poultry. The attribution study suggested a 74% decline in cases originating from poultry sources in 2008 compared with the baseline for 2005–2006. No statistically significant declines in attribution were found for any other sources (Figure 4).

It is difficult to attribute the decline in poultry-associated human disease to any single intervention, because a range of food safety and poultry industry interventions were implemented since 2006. However, key informants identified the monitoring and reporting of *Campylobacter* spp. enumeration levels on poultry carcasses at the end of primary processing as particularly important, as well as the setting of mandatory performance targets.

The fall in campylobacteriosis rates in New Zealand is unusual in terms of the size and speed of the decline, and the regulatory measures that were used. Internationally, a small number of countries have reported declines in campylobacteriosis incidence following the implementation of control strategies focusing on poultry (*25*–*28*). These countries have used various interventions, but a commonality has been strengthening on-farm biosecurity and monitoring the prevalence of *Campylobacter* spp.–positive flocks.

Although substantial evidence exists that poultry industry interventions contributed to the decline in campylobacteriosis incidence in New Zealand, several alternative explanations should be considered. These include the possibility of surveillance artifact, declining poultry consumption, declining disease associated with other foods or drinking water, effects of climate, and changes in consumer behavior.

Surveillance artifact is unlikely to have contributed significantly to the decline, however, given the magnitude of the reduction, the similarity of temporal trends in hospitalization and notification data (Figure 1), the decline occurring across all population subgroups, and the lack of similar declines for the comparison group of notifiable enteric diseases (Figure 3). Furthermore, the decline in campylobacteriosis in 2007 and 2008 was observed for all geographic areas (albeit to varying degrees), which suggests a change in a ubiquitous and common exposure. Salmonellosis rates may also have been expected to fall because of the potential concomitant effects of the interventions on *Salmonella* spp. contamination of poultry.

However, the lack of decline in salmonellosis is not surprising in the New Zealand context because *Salmonella* spp. contamination levels were very low in poultry before the implementation of these interventions (*29*).

To assess the possible impact of poultry consumption on the decline in campylobacteriosis, we examined poultry production data. In New Zealand, poultry production approximates poultry consumption because of the closed

Table. Key regulator and industry interventions and activities introduced in 2006–2008 to reduce poultry-associated foodborne campylobacteriosis, New Zealand*

Step	Initiative	Aim	Comments
Primary production	Development of voluntary Broiler Growing Biosecurity Manual by industry, building on existing industry biosecurity manuals and codes of practice	Identify effective on-farm biosecurity procedures in the New Zealand context; set industry best practice for on-farm biosecurity to help prevent *Campylobacter* spp. infection of flocks	Implemented in August 2007; developed by industry based on evaluation of existing on-farm biosecurity procedures and review of national and international best practice†
	Improvements in procedures for catching and transporting birds and for cleaning/drying of transport crates	Reduce possible cross infection between infected and non-infected birds during transport	
	Monitoring and reporting prevalence of *Campylobacter* spp. in cecal samples taken from birds from each growing shed each time birds are sent for processing	Determine the proportion of infected flocks; aid investigation of risk factors for flock infection; identify poor-performing farms	Implemented April 2007; reported to National Microbiological Database, administered by NZFSA‡
Processing	Monitoring and reporting enumerated levels of *Campylobacter* spp. from rinsates of bird carcasses exiting the immersion-chiller (at the end of primary processing)	Assess the effectiveness of risk mitigation strategies implemented on-farm and during processing in reducing *Campylobacter* spp levels; inform development of national targets for *Campylobacter* spp. contamination at the end of primary processing	Implemented April 2007; reported to the National Microbiological Database, administered by NZFSA
	Industry exchange of information and implementation of improvements during primary processing (particularly immersion-chiller conditions)	Identify cost-effective processing interventions that reduce the levels of *Campylobacter* spp. on broilers at completion of primary processing; inform an updated industry Code of Practice for primary poultry processing	2006–2008
	Implementation of an updated industry Code of Practice for primary processing of poultry (slaughter and dressing)	Set industry best practice for primary processing based on knowledge gained from previous processing trials	Issued August 24, 2007; implemented March 2008§
	Mandatory targets for *Campylobacter* spp. contamination levels on poultry carcasses after primary processing	Enable regulatory action to occur if poultry processors exceed a certain level of *Campylobacter* spp. contamination on broiler carcasses at the end of primary processing (on exiting the immersion-chiller)	Implemented April 2008; reported to the National Microbiological Database administered by NZFSA
Retail	Voluntary use of leak-proof packaging	Reduce potential for cross-contamination from contaminated packaging in retail and home settings	Introduced for whole carcasses by most primary processors. Introduced for portion packs by some supermarkets
	Intermittent monitoring of *Campylobacter* spp. contamination of retail poultry	Assess *Campylobacter* spp. levels in retail packs purchased by consumers; inform interventions and code of practice for secondary processing	Reflects *Campylobacter* spp. levels at primary processing and subsequent changes due to secondary processing, storage, distribution, and processing/handling at the retail outlet
Consumer	Enhanced consumer education	Increase public awareness of food safety risk mitigation behaviors	Initially instigated in 1998 by NZFSA and the existing New Zealand Food Safety Partnership
Other	Enhanced human campylobacteriosis surveillance and source attribution research	Monitor source attribution of human campylobacteriosis to guide future interventions	Source attribution work is ongoing to monitor the proportion of human campylobacteriosis cases attributable to different sources and transmission pathways

*NZFSA, New Zealand Food Safety Authority.
†www.pianz.org.nz/Documents/Version_1.pdf.
‡Mandatory cecal testing was discontinued July 2009.
§www.foodsafety.govt.nz/elibrary/industry/Code_Practice-Zealand_Food.htm.

nature of the production system. Over the period of the marked decline in campylobacteriosis incidence (2006–2008), fresh poultry production waned by only 5.8% (*30*). While this fall in production could have affected the incidence of poultry-associated foodborne campylobacteriosis, it is unlikely to be sufficient to explain the >50% drop in campylobacteriosis notifications occurring over this period.

Several foodborne pathways of campylobacteriosis (other than poultry) have been identified, including red meat and raw milk consumption (*9*,*31*). The contribution of these pathways to sporadic campylobacteriosis in New Zealand has been estimated to be markedly less than that of poultry (*9*,*12*). The magnitude of the decrease seen in 2008 is such that even if the contributions from food sources other than poultry had been eliminated in their entirety, they likely could not account for the observed decline in campylobacteriosis.

Contaminated water and other environmental sources have been implicated as a transmission pathway of human campylobacteriosis (*32*,*33*). Although water is found to be contaminated with *Campylobacter* spp., molecular epidemiologic studies have shown a low similarity between these genotypes and those found in human case-patients, suggesting that the strains detected in water are relatively apathogenic or that humans have limited exposure to them (*12*). Furthermore, a high proportion of New Zealanders receive treated community water supplies, with only small gradual increases in the proportion receiving water that meets microbiological quality criteria (*34*).

Changes in consumer behavior (e.g., hygiene, food preparation, eating out) could have plausibly contributed to the decline. However, challenges in altering consumer behavior have been acknowledged (*35*), and, given the rapidity of the decline in incidence, it is unlikely a sudden, marked change in consumer behavior could have been a key driver of the decline.

The effect of climate was considered as a possible driver of the decline. Despite the seasonal pattern observed for campylobacteriosis, the main drivers of the association between climate and campylobacteriosis remain elusive (*36*). However, the rapidity of the fall in incidence suggests that global climate change factors are unlikely to be key drivers.

A strength of this study is the multiple data sources that were accessed and analyzed, including source attribution techniques and key informant interviews. Nevertheless, a limitation of this study in determining the likely cause of the recent decline in campylobacteriosis is the descriptive nature of the epidemiologic analysis and the complex epidemiology of campylobacteriosis, which means that not all factors that might influence the disease's incidence were examined explicitly. Although validated by studies in 2 other regions, the source attribution analyses were from 1 sentinel site only, and this work also has its own limitations (*12*,*13*,*15*). A further weakness is that details of specific industry-level interventions to reduce poultry contamination are not in the public domain, and therefore cannot be examined in detail. We were also unable to examine in detail data on *Campylobacter* spp. contamination levels in poultry. However, summary microbiological data on *Campylobacter* spp. contamination levels from the national database for 2007 and 2008 as published in the updated *Campylobacter* Risk Management Strategy (*23*) support a reduction in *Campylobacter* spp. prevalence and counts on poultry over the period of the decline.

Rates of campylobacteriosis have shown marked annual variations in the past, so it will be important to assess medium- to long-term trends in disease and its attribution to assess the effects of NZFSA's strategy. Notification and hospitalization data for 2009 indicate that the decline in incidence seen in 2008 has been largely sustained (Figure 1). Despite the 2009 rates being slightly higher than those of 2008, they still represent a substantial decline compared with the average for 2002–2006 (48% for notifications and 50% for hospitalizations).

Although there are costs associated with implementing industry regulation, these are likely to be offset by both the direct and indirect savings from reduced disease effects and lost productivity, conservatively estimated to have cost NZ$600 per campylobacteriosis case in 2005 (*37*). Given an estimated 70,000 fewer cases of campylobacteriosis in the community in 2008 than in 2006, this decline represents notable savings to New Zealand society. While progress has been made in responding to New Zealand's campylobacteriosis epidemic, the costs and effects are still significant. As such, further research (including evaluating additional interventions) is desirable from a public health perspective to enable continued reductions of the still high burden posed by campylobacteriosis.

The findings of this study provide evidence of a successful population-level food safety response to a serious public health issue. New Zealand has experienced a prolonged national epidemic of campylobacteriosis. Fresh poultry was implicated as the dominant source, and a range of voluntary and regulatory interventions were introduced to reduce *Campylobacter* contamination of poultry. The apparent success of these interventions demonstrates approaches other countries could consider for controlling infectious disease epidemics linked to specific food sources. This example highlights the importance of integrated public health surveillance that includes upstream hazards as well as disease (*38*). Finally, the success of the response shows the value of collaboration between industry, food safety regulators, and public health researchers in addressing important food safety issues.

Acknowledgments

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Wild Birds and Increased Transmission of Highly Pathogenic Avian Influenza (H5N1) among Poultry, Thailand

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Since the outbreaks of highly pathogenic avian influenza (HPAI) subtype H5N1 virus, wild birds have been suspected of transmitting this virus to poultry. On January 23, 2004, the Ministry of Public Health in Thailand informed the World Health Organization of an avian influenza A (H5N1) outbreak. To determine the epidemiology of this viral infection and its relation to poultry outbreaks in Thailand from 2004 through 2007, we investigated how wild birds play a role in transmission. A total of 24,712 swab samples were collected from migratory and resident wild birds. Reverse transcription PCR showed a 0.7% HPAI (H5N1) prevalence. The highest prevalence was observed during January–February 2004 and March–June 2004, predominantly in central Thailand, which harbors most of the country's poultry flocks. Analysis of the relationship between poultry and wild bird outbreaks was done by using a nonhomogeneous birth and death statistical model. Transmission efficiency among poultry flocks was 1.7× higher in regions with infected wild birds in the given or preceding month. The joint presence of wild birds and poultry is associated with increased spread among poultry flocks.

Avian influenza is a viral disease of poultry and is distributed worldwide. The virus is classified based on 2 surface proteins, the hemagglutinin (HA) protein (H1–H16) and the neuraminidase (NA) protein (N1–N9), which can be found in numerous combinations (*1*). All H and N subtypes can be found as low pathogenic avian influenza virus strains in aquatic wild birds, which are assumed to be the main reservoirs outside poultry (*2*,*3*). Occasionally, low pathogenic avian influenza virus strains are introduced into domestic poultry flocks with no clinical signs or only mild clinical consequences, but strains carrying the H5 or H7 gene can mutate into highly pathogenic avian influenza (HPAI) strains that cause high death rates in domestic poultry (*4*) and, occasionally, in migratory birds (*5*,*6*). Because of the devastating effect of HPAI outbreaks in commercial poultry, all outbreaks caused by H5 and H7 subtypes are notifiable (*7*).

Currently, a HPAI virus strain of subtype H5N1 is circulating in many countries in Eurasia and Africa, causing high death rates in poultry, substantial economic losses, and human deaths. The strain was first identified in Southeast Asia in 1996 and has since spread to 63 countries in Asia, Europe, Africa, and the Middle East (*7*). Millions of domestic poultry died from the effects of the disease or from culling efforts to control the spread of the virus (*1*,*2*,*8*,*9*). The spread of the HPAI (H5N1) virus from Southeast Asia to Russia, Europe, and Africa was assumed to originate from a virus source at Qinghai Lake, People's Republic of China (*6*,*10*). Therefore, migratory birds were considered to be responsible for long distance dispersal of the virus (*11*–*13*).

In Thailand, 7 waves of HPAI (H5N1) virus outbreaks have occurred since January 2004. Poultry and wild bird populations in 1,417 villages in 60 of the 76 provinces were affected, and >62 million birds died or were culled to prevent further transmission (*14*–*16*). Introduction of the virus into poultry flocks is considered to be possible

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through infected wild birds. Additional insight on the basis of quantitative data into the role of wild birds would be necessary to further develop control measures and surveillance programs.

Relatively little effort has been made to quantify the association between infection in wild birds and outbreaks in poultry flocks, most likely because of the lack of data on infection in wild birds. Recently, a preliminary study was carried out that analyzed the prevalence of HPAI (H5N1) infection in wild birds in Thailand (*14*). In that study, 6,263 pooled surveillance samples from wild birds in Thailand, collected from January 2004 through December 2007, were tested for evidence of infection. Testing indicated that prevalence patterns in wild birds mirrored outbreaks among poultry; however, the association was not proven or quantified. We studied extensive data on 24,712 wild birds, sampled and analyzed from 2004 through 2007 in Thailand, to quantify the possible effect of infection in wild birds on the spread of the infection among poultry flocks.

Materials and Methods

Data Collection

Data about subtype H5N1 infections in wild bird populations were provided by the National Institute of Animal Health of Thailand, Regional Veterinary Research and Development Centers, the Veterinary Science faculty of Mahidol University, and the Department of Livestock Development, Thailand. A total of 24,712 wild bird samples were collected from January 2004 through December 2007. During 2004–2006, sampling was part of a general countrywide surveillance program; in 2007, sampling was targeted specifically at areas where outbreaks in poultry had occurred.

Sampling methods have been described previously (*14,16,17*). Wild birds were either trapped by using baited traps, hand nets, or mist nets, or shot. Tracheal/oropharyngeal swabs and cloacal swabs of live birds and bird carcasses were collected from active surveillance (sampling of healthy wild birds) and passive surveillance (sampling of sick or dead birds). Swab samples were collected in viral transport media, stored at 4°C, and shipped to the laboratory, where they were stored at −80°C until further analysis could be done.

Virus Detection

Methods used for antigen detection have been described by Tiensin et al. (*16*) and Siengsanan et al. (*14*). Supernatants from homogenized tissue and swab samples were filtrated and inoculated in 11-day-old embryonated chicken eggs or MDCK cell cultures. After incubation at 37°C for 3 days, allantoic fluid was harvested. The inoculated MDCK cell culture was observed daily for cytopathic effect, and supernatant fluid was harvested by day 4, even if no cytopathic effect was observed. Viruses were initially identified in allantoic fluids or culture supernatants by the HA assay according to World Health Organization recommendations (*14*). Negative samples were inoculated 2 additional times in embryonated chicken eggs before specimens were confirmed as negative.

RNA from positive samples acquired from virus culture was extracted by using a viral RNA extraction kit (QIAGEN, Valencia, California, USA), according to the manufacturer's instructions. Reverse transcription PCR (RT-PCR) was performed by using a 1-step RT-PCR kit (QIAGEN) to identify the subtype, according to the manufacturer's instructions. Primers for RT of viral genome and all HA, NA, and matrix (M) genes for virus subtype and influenza A virus identification have been published elsewhere (*14,17–19*). PCR products were processed with 1% agarose gel electrophoresis and were purified by QIAquick PCR purification kit (OIAGEN). Sequencing was performed by using the H5 and N1 specific primers, and sequence data were edited following methods previously described (*14,17,18*).

Statistical Analysis

For each identified bird species, geographic location and season were recorded. Bird species were divided into 3 groups: 1) resident birds (nonmigratory populations), present year-round in Thailand; 2) migratory (visitor) birds, bird populations moving between Russia or China to Thailand during September/October and March/April; and 3) breeding visitor birds, which migrate to Thailand for breeding in different periods of the year.

To study the relevance between the regions and subtype H5N1 outbreaks in wild birds, we divided Thailand into 4 major geographic regions (northern, northeastern, central, and southern) on the basis of the former administrative region grouping system used by the Ministry of Interior, Thailand. Because of the high number of outbreaks in the Central region (*14,17,20*), it was further divided into 6 parts: central–northwest, central–north, central–central, central–east, central–southeast, and central–southwest. On the basis of procedures established by the Thai Meteorological Department, the seasons were divided into 3 periods: summer (March–June), the rainy season (July–October), and winter (November–February).

Prevalence of HPAI (H5N1) infection and 95% confidence intervals (CIs) were calculated for each group of bird species, sampling region, and season. Three variables associated with HPAI (H5N1) prevalence were analyzed by binary logistic regression. Overall significance of the model was assessed by the likelihood ratio χ^2 test. The goodness-of-fit was calculated by using the Hosmer-Lemeshow goodness-of-fit test. Statistical significance of

the regression coefficients was tested by using the Wald likelihood ratio test. Odds ratios (OR) and respective 95% CI were calculated. For multiple comparisons, the Bonferroni multiple comparison correction was applied to demonstrate statistical significance ($p<0.001$). Statistical analysis was performed by using statistical software SPSS version 17 (SPSS Inc., Chicago, IL, USA).

Data on outbreaks among poultry were taken from Tiensin et al. (*16*). We used their definition of poultry, which encompasses all farmed avian species in Thailand, including backyard chickens and ducks. Different species or types of production systems were not differentiated in the data. Using a nonhomogeneous birth model (*21*), we investigated the association between subtype H5N1 presence in infected poultry flocks and wild birds. Prevalence data from the 9 different regions were modeled independently and conditioned on the number of infected birds during the first month of detected infection for each region. Time lapse was measured in months from the first month infection was detected. To analyze the association between presence of subtype H5N1 in wild birds and outbreaks in poultry, we pooled data for the 3 wild bird groups (resident birds, migratory visitor birds, and breeding visitor birds) to increase power.

In most regions, sampling among wild birds was only done systematically after a poultry outbreak in that region, except in the central–northwest, central–north, and central–central regions. We could therefore only use the latter 3 regions to investigate whether the presence of infected wild birds was related to the poultry outbreak.

The nonhomogeneous birth model depends on the so-called reproductive power, which statistically quantified (in our setting) the ability of infected poultry flocks to spread infection to susceptible poultry flocks. For the statistical model, we used probability distributions from the Burr family. Distribution functions Burr XII and Burr III were fitted by using a conditional fitting procedure (*21*). For every region, we determined whether infected wild birds were detected during a particular month. A wild-bird infected month was defined as a month in which there was detection of infected wild birds or which showed wild-bird infection in the preceding month. We investigated whether wild-bird infection affected the reproductive power for the poultry outbreak in the same region. Reproductive power for wild-bird infected months was compared with that in non–wild-bird infected months for the central–northwest, central–north, and central–central regions. For comparison, we also calculated the reproductive power for poultry outbreaks for the 6 other regions of Thailand by using previously described methods (*22*). Model selection was done by using Akaike's Information Criterion (AIC) (www.modelselection.org/aic).

Results

Descriptive Statistics

Infected poultry flocks and wild birds were found in all 9 regions during the study period. In online Appendix Figure 1 (www.cdc.gov/EID/content/17/6/1016-appF1.htm), we present the numbers of wild birds sampled per month for each of the 9 regions and outbreak data of subtype H5N1 in poultry flocks. A total of 24,712 wild birds were sampled, consisting of 303 species, 64 families, and 20 orders (online Appendix Table 1, www.cdc.gov/EID/content/17/6/1016-appT1.htm). Of these, 192 samples were positive for subtype H5N1, resulting in an overall prevalence of 0.78% (95% CI 0.67%–0.89%) (online Appendix Table 1). Positive samples were found in 35 species of 12 orders (online Appendix Table 2, www.cdc.gov/EID/content/17/6/1016-appT2.htm). Prevalence differed significantly among the group of wild bird species ($p<0.001$), with a prevalence of 0.187% (95% CI 0.01%–0.21%) in migratory birds ($n = 2,142$), 0.829% (95% CI 0.66%–0.94%) in resident birds ($n = 16,633$), and 0.814% (95% CI 0.61%–0.99%) in breeding visitor birds ($n = 6,143$). The highest prevalence of virus-positive birds was found in resident and breeding visitor birds ($p<0.001$) (online Appendix Table 3, www.cdc.gov/EID/content/17/6/1016-appT3.htm).

The aggregated data from online Appendix Figure 1, presented for Thailand as a whole in Figure 1, show a marked increase in the number of infected poultry flocks detected from September through December 2004. A relatively high number of wild birds positive for subtype H5N1 were detected from January 2004 through May 2004, before the poultry outbreaks in June 2004. Infections in wild birds were consistently detected after the poultry outbreaks had ended, except during April and May in 2005, 2006, and 2007.

The spatial distribution and size classes of infected poultry flocks, as well as numbers of infected wild birds detected, are shown in Figure 2. In 2004 and 2005, infected wild birds were reported in the same locations where infected poultry flocks were found, especially in the central region. No infected poultry flocks were found in 2006 and 2007 in these areas. Subtype H5N1 prevalence in wild birds differed by sampling location. Central Thailand had the highest overall prevalence of 0.9% (95% CI 0.77%–1.03%), compared with other regions ($p<0.001$); the Northwest-Central region in central Thailand had a significantly higher prevalence ($p<0.001$) (online Appendix Table 3).

The percentages of wild birds positive for subtype H5N1 in each season are also shown in online Appendix Table 3. Prevalence differed significantly during January and February 2004 (7.92%; 95% CI 5.8–10.4; $p<0.001$) and in the summer of 2004 (11.79%; 95% CI 8.7%–15.8%; $p<0.001$), compared with the other seasons.

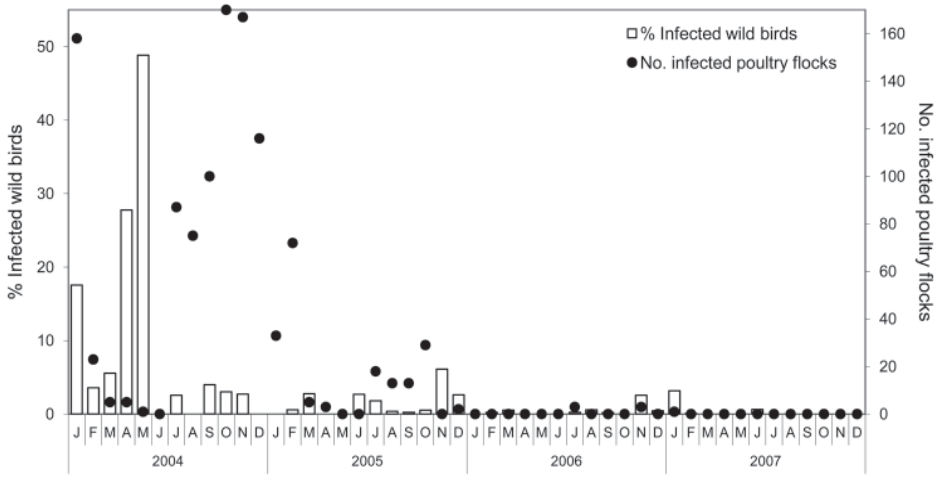

Figure 1. Epidemic curve of the number of highly pathogenic avian influenza (H5N1) virus infections in poultry flocks and percentage of infected wild birds during January 2004–December 2007, Thailand.

Association between Outbreaks in Poultry and Infection in Wild Birds

The Burr XII and Burr III distributions each have 5 parameters. These distributions were used to model the observed poultry outbreak data for each of the 9 regions, taking into account wild-bird infection. The AIC, when we used the Burr XII model to fit the observed data, was 5,628.6, substantially lower than that for the Burr III distribution, which gave an AIC of 5,829.8. We therefore chose the Burr XII distribution to model the data (online Appendix Figure 2, www.cdc.gov/EID/content/17/6/1016-appF2.htm, gives the fit to the data for all 9 regions). The model fits the data rather well.

We also fitted the Burr XII distribution to model the observed poultry outbreak data in non–wild-bird infected months, leading to an AIC of 5,677.7. Because the model

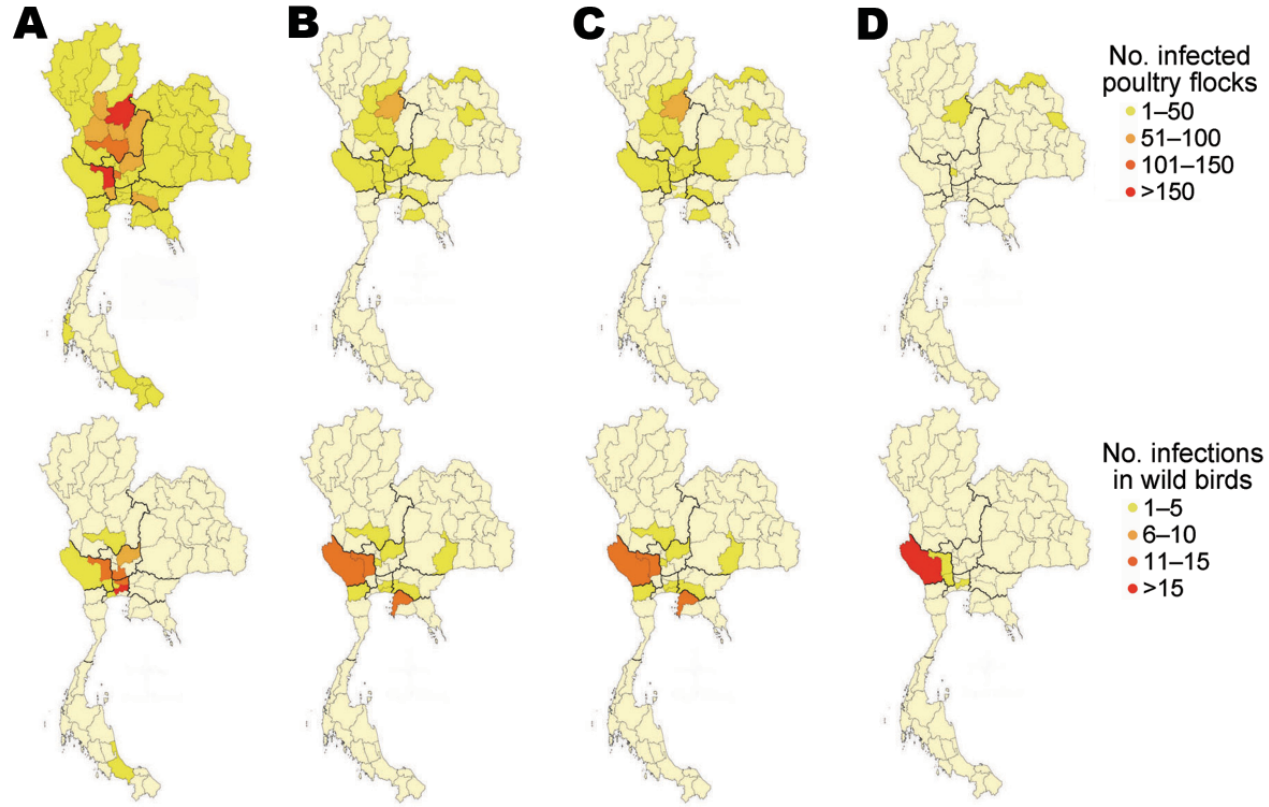

Figure 2. Distribution of highly pathogenic avian influenza (HPAI) subtype H5N1 infections in poultry flocks (top) and wild birds (bottom), Thailand. A) 2004, B) 2005, C) 2006, and D) 2007.

with wild-bird infection has a lower AIC, data clearly show that the reproductive power of poultry flocks in wild-bird infected months was higher than in non–wild-bird infected months. Parameter estimates for the model are shown in the Table. The log of the proportionality ($\ln[c_3]$) is 0.523, corresponding to a proportionality factor of ≈1.67, indicating that the reproductive power in wild-bird infected months is ≈1.7× higher than that in non–wild-bird infected months (Figure 3, where we give the reproductive power for the associated period). In Figure 3, we have also plotted the reproductive power for the 6 regions for which we could not do the wild-bird related comparison (regions 1 and 5–9). The reproductive power as a function of time was almost indistinguishable from the curve for the non–wild-bird infected months in regions 2, 3, and 4.

Table. Parameter estimation of the nonhomogeneous birth model using the Burr XII distribution for documenting data on HPAI (H5N1) outbreak in poultry, Thailand, 2004–2007

Parameter	Estimate	SE
$\ln(b_1)$	0.772	0.0777
$\ln(a)$	1.142	0.0627
$\ln(c_1)$	–1.574	0.0746
$\ln(c_2)$	–0.045	0.0627
$\ln(c_3)$	0.523	0.073

*HPAI, highly pathogenic avian influenza.

Discussion

We analyzed one of the largest datasets available of wild birds sampled for HPAI (H5N1) infection in Thailand, a country where several outbreaks of the disease have occurred in poultry flocks. Our aim was to determine the prevalence and distribution of HPAI (H5N1) in wild birds and to determine whether an association exists between outbreaks in poultry flocks and in wild birds within different regions in Thailand. We calculated the reproductive power in poultry flocks, a measure for the ability of a poultry flock to infect other susceptible poultry flocks. Notably, reproductive power was 1.7× higher in so-called wild-bird infected months, compared with poultry outbreaks in non–wild-bird infected months, suggesting a strong association of spread among poultry flocks and the presence of the infection in wild birds.

Poultry flocks in this study represent several avian species, which were considered as a single group with equal infectiousness, susceptibility, and other characteristics, in the absence of more precise information. Domestic ducks, which normally manifest a subtype H5N1 infection subclinically, were included in the poultry group. Ducks were not sampled according to criteria related to clinical signs. Available data do not allow a more differentiated analysis.

To quantify the association with outbreaks in poultry, we regarded wild birds as 1 group. We can therefore not differentiate the quantification of interaction to the level of specific wild-bird groups. In our additional analyses, however, most cases of HPAI (H5N1) infection in wild birds were found in resident birds, as compared with migratory and breeding visitor birds. Therefore, resident wild birds may be responsible for the association that we quantified. Our results can possibly be explained by the difference in exposure time of the wild birds. We partially confirmed, but more importantly expanded and added detail to, the conclusions reached by Siengsanan et al. (*14*), on the basis of pooled samples for a smaller part of the database. Bird species seemed to differ in susceptibility for infection. In our study, H5N1 virus infection was detected in many resident bird species, but we did not have a sufficient number of birds to differentiate in the quantitative analysis between different species. Species do differ, however, in terms of potential contact to poultry, especially birds considered to be peridomestic species of the Columbiformes, Cuculiformes, and Passeriformes orders, which are commonly associated with poultry environments. Transmission of subtype H5N1 to poultry populations by this group of resident bird species is more likely than transmission by other resident birds, including those belonging to the Galliformes, Gruiformes, Piciformes, Psittaciformes, and Struthioniformes orders. The habitats of these birds are not located near poultry areas. Previous experimental studies have shown that infected individuals of peridomestic species such as sparrow and starling can shed subtype H5N1 after infection, but they die quickly (*23*,*24*). Therefore, these birds are unlikely to be long-term reservoirs but may be a higher risk to poultry than other resident bird species. Pigeons were found to be less susceptible to severe neurologic signs and death from HPAI (H5N1) infection (*24*). Infected pigeons appeared to shed low amounts of virus, thereby limiting virus transmission to sentinel birds (*23*–*29*). Our data showed a relatively high prevalence of HPAI (H5N1) in herons and storks (commonly

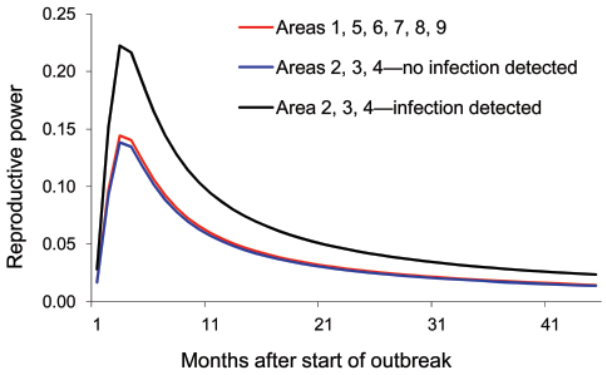

Figure 3. Reproductive powers of highly pathogenic avian influenza (H5N1)–infected poultry flocks in wild-bird infected months and in non–wild-bird infected months within different regions of Thailand, 2004–2007.

known as scavengers and hunters of juvenile aquatic birds), which suggests that these birds are predominantly infected by contact with infected poultry flocks.

The prevalence of HPAI (H5N1) infections in resident birds was higher in areas with poultry flocks. We could not determine whether wild birds became infected because of spillover from poultry flocks or whether wild birds were the origin of outbreaks in poultry flocks. The association we found is not necessarily one of cause and effect. The 2 populations may have been affected by the same factors that increase transmission between flocks, e.g., contaminated water, movement between poultry flocks, or even increased transmission through fomites.

Even though data results are from the largest sampling effort available, the lack of a clear sampling strategy in the collection of wild-bird data precludes a definite answer to whether poultry flocks were infected with HPAI (H5N1) from infected wild birds or vice versa. Siengsanan et al. (*14*) suggested that poultry outbreaks precede detection of the infection in wild birds, but we have found no evidence either for or against that claim, again because of the sampling strategy used. One could argue the fact that infected poultry flocks produce massive amounts of virus, which supports the view that infection in wild birds is mostly seeded from poultry. A study carried out by Bavinck et al. (*29*) suggested that small backyard flocks did not contribute to the spread of subtype H7N7 infection in the Netherlands during 2003.

Seasonal bird migration, as well as enhanced movement and trade of poultry in the winter period caused by major social events occurring at the end of the year, may play a role in virus spread (*30*). Our data show increased prevalence among wild birds in all winter periods, with the exception of 2007 in which neither poultry farm outbreaks nor wild bird infections were detected. The actual sources of new introductions of HPAI (H5N1) into the commercial poultry flocks in Thailand could not be elucidated by our analysis.

From January through October 2004, a relatively small number of wild-bird samples was collected, compared with the number of samples collected from November 2004 to December 2007. Selection bias may have occurred during this period. Despite a bias in sampling numbers, HPAI (H5N1)–infected wild birds were detected during April–May 2004 just before the onset of the 2004 outbreak, but were not observed in that same period during 2005–2007 despite larger sampling numbers.

Variation in geographic distribution of HPAI (H5N1) infections in wild birds was observed over different areas. The central region of Thailand with dense poultry populations and large populations of birds living in the surrounding wetlands can be considered a hotspot for HPAI (H5N1) outbreaks. Our dataset shows high prevalence rates of the virus in the central region, corresponding with previous studies of HPAI (H5N1) surveillance in wild birds (*14*), in poultry flocks during 2004–2005 (*16*,*17*), and in cases of HPAI (H5N1) infection among humans during 2004 (*31*).

Associating these observations to our statistical model is interesting, because the reproductive power of poultry flocks in regions 1, 5, 6, 7, 8, and 9 was almost identical to that in regions 2, 3, and 4 during non–wild-bird infected months (Figure 3); regions 1, 5, 6, 7, 8, and 9 experienced no outbreaks in wild birds. It is however impossible to conclude from the current data that absolutely no wild birds were infected because, in these regions, relatively few samples were collected during the appropriate periods (online Appendix Figure 1).

By determining the reproductive power in poultry, which is the ability of infected poultry flocks to spread infection to susceptible poultry flocks, we quantified the association between wild bird infection and outbreaks in poultry. We also attempted to take the reproductive power in wild birds, during poultry-infected months, as our starting point. However, too few infected wild birds were available for a reliable analysis.

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Methicillin-Resistant *Staphylococcus aureus*, Samoa, 2007–2008

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Little is known about the epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) in most Pacific Island nations. Relatively high rates of MRSA have been reported in Polynesian people living outside the Pacific Islands. To determine the prevalence and characteristics of MRSA, we assessed wound swabs from 399 persons with skin and soft tissue infection living in Samoa. MRSA was isolated from 9% of study participants; 34 of the 196 *S. aureus* isolates were MRSA. Five MRSA genotypes were identified; the 3 most common were USA300, the Queensland clone, and a sequence type 1 MRSA strain that shares <85% homology with the sequence type 1 MRSA strain common in the region (WA MRSA-1). The Southwest Pacific MRSA clone was identified but accounted for only 12% of MRSA isolates. The high prevalence of MRSA in Samoa provides impetus for initiatives to improve antimicrobial drug resistance surveillance, infection control, and antimicrobial drug use in Pacific Island nations.

Infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) have become a global health concern during the past 2–3 decades. The epidemiology of MRSA has demonstrated marked geographic variation in the prevalence and genotypes of MRSA (*1*,*2*), and recent reports from many parts of the world indicate that the prevalence and diversity of MRSA continue to increase (*3*,*4*). Studies of the global epidemiology frequently have not included MRSA obtained from persons living in developing nations. The prevalence and genetic variation of MRSA infection in most Pacific Island nations remain unknown. The only study of MRSA in the Pacific was conducted after a report in 2004 of an increased incidence of MRSA infection in Polynesian people in Hawaii (*5*). This study showed that most MRSA infections in Hawaii were caused by the USA300 MRSA strain (*6*).

Relatively high rates of MRSA infection have been reported in Polynesian people living outside the Pacific Islands region, including in Alaska, Australia, and New Zealand, and have been attributed to infection with the Southwest Pacific clone of MRSA (sequence type [ST] 30 SCC*mec* type IV, also referred to as Western Samoan phage pattern [WSPP] MRSA or the Oceania strain) (*7*–*10*). The Southwest Pacific clone of community-associated MRSA was identified in New Zealand in 1992 (*11*). The first isolate and many subsequent isolates of this strain were from persons in New Zealand who had some association with Samoa. The Southwest Pacific MRSA clone has now spread as far as Europe and South America (*4*,*12*). This MRSA strain has been postulated to have arisen from a pandemic penicillin-resistant *S. aureus* strain, known as phage type 80/81, that caused serious hospital- and community-acquired infections during the 1950s (*13*).

Samoa is an independent nation in the Southwest Pacific, with a population of ≈180,000 persons (Samoan Statistics Department, www.spc.int/prism/wstest/index.htm; Figure). Samoa has a small and developing economy predominantly comprising remittances from Samoan persons living overseas, agriculture, and tourism. The gross domestic product per capita was US $2,987.90 in 2008 (http://data.un.org). Life expectancy in Samoa is 74.9 years for women and 68.5 years for men, and the infant mortality rate is ≈22.3 per 1,000 live-born infants. The publicly funded National Health Service is based at the national

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Figure. Map of Samoa, showing the 2 main islands, Upolu and Savai'i, and the capital Apia. Reproduced with permission from Oxford Cartographers (www.oxfordcartographers.com).

referral hospital in Apia; in addition, Samoa has 7 district hospitals (*14*). Several outreach and integrated community health services provide primary health care services, such as clinics and vaccinations.

Samoa gained independence from New Zealand in 1962; a large Samoan population resides in New Zealand. In New Zealand, infections caused by *S. aureus*, whether methicillin susceptible or resistant, are more common in Maori and Polynesian persons than in other ethnic groups (*15–17*). Antimicrobial drug resistance is routinely monitored in New Zealand through testing of bacterial isolates from specimens taken for diagnostic purposes. In 2008, 9% of *S. aureus* isolates were methicillin resistant, but this rate varied considerably among different parts of the country, with rates as high as 13%–16% in hospitals in the Auckland area (*18*).

In Samoa, as in many other developing countries, antimicrobial drug resistance is not systematically monitored. The microbiology service at the main public hospital in Apia performs antimicrobial drug susceptibility testing only when specifically requested by the treating doctor. This testing is infrequent, and the results are not regularly collated (V. Kini, pers. comm.). Testing for antimicrobial drug susceptibility requires equipment, resources, and a high level of expertise and quality assurance—requirements that are beyond the means of many laboratories in the developing world. If a high rate of antimicrobial drug resistance exists in Samoa, it would substantially affect this relatively poor country with relatively high rates of infectious diseases. We performed a systematic study to assess the prevalence and characteristics of MRSA isolated from wound swabs from persons with skin and soft tissue infection living in Samoa.

Methods

During the summer of 2007–2008, a study investigator (J.A.S.) collected *S. aureus* isolates from persons with skin and soft tissue infection. Review of patient notes was used to identify hospital patients who had skin lesions. All ambulatory patients who attended a clinic during the visit by J.A.S. and any family members who accompanied them were asked if they had a skin lesion. All persons with a skin lesion were invited to participate in the study; <5 persons declined to participate. At hospitals, participants included inpatients and outpatients, but this information was not recorded. No attempt was made to categorize infections as hospital acquired or community acquired, although the source of the wound was recorded.

All participants had skin and soft tissue infection, but this infection was not always the primary reason for contact with health services. Skin and soft tissue infection was defined as increasing pain, warmth, induration, erythema, or purulent discharge. Swabs were collected twice from patients who had separate lesions on the upper and lower body. If a patient had >1 infected site in the same region (e.g., upper body), only the larger lesion was swabbed.

Patient data collected included demographic information (age, sex, occupation, and village of residence) and recent exposure to health services (use of antimicrobial drugs in the last month and contact with health services in last 3 months). We attempted to include persons of different ages, both sexes, and various occupations.

The Lower South Ethics Committee in New Zealand, and the Health Research Council in Samoa gave ethical approval for the study. Participants were given an information sheet about the research and a small card explaining, in Samoan, how to take care of wounds. The study investigator explained the purpose of the study in Samoan, and the consent form was printed in Samoan and in English.

Swabs were taken after the wound was cleaned with sterile water. Swabs were placed into Amies transport media (Fort Richard Laboratories, Auckland, New Zealand), stored on ice, and transported to the laboratory at 1 of the 2 main hospitals. All swabs were processed within 12 hours after collection. Swabs were spread onto sheep blood agar containing aztreonam (6 mg/L) and incubated for 16–24 hours. A catalase test was performed on colonies suspected to be *S. aureus*, and catalase-positive colonies were identified by using a latex agglutination test (BBL Staphyloslide Latex Test, Becton Dickinson, Sparks, MD, USA). *S. aureus* isolates were transported on a nutrient agar slope to LabPLUS (Auckland, New Zealand).

In New Zealand, antimicrobial drug susceptibility testing was performed by using disk diffusion for penicillin, and agar dilution breakpoint testing for cotrimoxazole, doxycycline, erythromycin, gentamicin, oxacillin, and

vancomycin in accordance with Clinical and Laboratory Standards Institute methods and interpretive standards (*19*–*21*). MRSA isolates were tested by disk diffusion for susceptibility to ciprofloxacin and clindamycin, including inducible clindamycin resistance. Additional fusidic acid and mupirocin disk diffusion susceptibility testing was performed when required to assist with identification of MRSA strains.

MRSA isolates were tested for the genes encoding for Panton-Valentine leukocidin (PVL) by PCR (*22*). Multilocus sequence typing (*23*); *spa* typing (*24*); and if necessary, pulsed-field gel electrophoresis (PFGE) of *Sma*I-digested genomic DNA were used to identify and characterize MRSA strains. StaphType software version 1.5 (Ridom GmbH, Würzburg, Germany) was used to assign *spa* types. We compared *spa* types by using the BURP (based upon repeat pattern) algorithm and excluding *spa* types with <5 repeats and setting a maximum cost of 4 between members of a *spa* group cluster. PFGE banding patterns were analyzed with BioNumerics software version 5.1 (Applied Maths, Saint-Martens-Latern, Belgium) by using the Dice coefficient and unweighted pair group method with arithmetic averages, at settings of 0.5% optimization and 1.5% position tolerance.

Categorical data are expressed as proportions and 95% confidence intervals (CIs), calculated by the modified Wald method. Statistical significance of comparative categorical data was examined by using the χ^2 test.

Results

A total of 423 swabs were obtained from skin and soft tissue infections of 399 persons at 8 locations on the 2 main islands of Samoa (Upolu and Savai'i) and a smaller island, Manono. The 8 locations were the main public hospital in Apia (the capital on Upolu), the main public hospital on Savai'i, 5 district hospitals around the main islands, and community-based clinics on Manono.

Infections in skin and soft tissue were in wounds from falls, cuts, dog bites, car accidents, and burns; as well as boils and carbuncles. Many were described as *po'u*, a Samoan term for idiopathic tropical ulcer. Twenty-four persons each had swabs from 2 separate lesions. Even though we did not categorize infections as hospital acquired or community acquired, we were able to infer likely place of acquisition from the description of the wound: 20 (5%) of 399 represented infections of surgical sites, which were hospital acquired; 335 (84%) represented community-acquired infections, such as impetigo; *po'u*; boils; or infections of wounds resulting from dog bites, machete cuts, or assaults. We were not able to further classify the remaining 44 (11%) infections.

S. aureus was isolated from 196 (46%, 95% CI 42%–51%) of the 423 wound swabs and from 187 (47%, 95% CI 42%–52%) of the 399 study participants. *S. aureus* was more likely to be obtained from children <5 years of age than from persons in other age groups (37 [70%] of 53 compared with 150 [43%] of 346; p<0.001) and less likely in persons currently or recently treated with antimicrobial drugs (84 [38%] of 224, compared with 103 [59%] of 175 persons who were not; p<0.001) (Table 1).

Thirty-four (17%, 95% CI 13%–23%) of the 196 *S. aureus* isolates were methicillin resistant. MRSA was isolated from 8% (95% CI 6%–11%) of the 423 wound swabs and from 9% (95% CI 6%–12%) of the 399 study participants. Almost 1 in 5 of the 187 participants with *S. aureus* had MRSA (34/187, 18%, 95% CI 13%–24%). The basic demographic features of persons with MRSA did not differ substantially from those with methicillin-susceptible *S. aureus* (MSSA). Recent antimicrobial drug use and recent health care were not associated with an increase in MRSA infections. For example, the proportion of persons with recent health care exposure from whom MRSA was isolated (23/262, 9%) did not differ significantly from the proportion of persons without recent health care exposure (11/137, 8%).

Participants lived in 165 villages around Samoa. We detected *S. aureus* in participants from 102 villages. In terms of place of domicile, MRSA was widespread

Table 1. Demographic characteristics of study participants and prevalence of MSSA and MRSA, Samoa, summer 2007–2008*

Characteristic	Total study population	No. (%, 95% CI)		
		S. aureus positive	MSSA	MRSA
No. participants	399	187 (47, 42–52)	153 (38, 34–43)	34 (9, 6–12)
Male sex	263	121 (46, 40–52)	95 (36, 31–42)	26 (10, 7–14)
Age, y†				
<5	53	37 (70, 56–81)‡	33 (62, 49–74)	4 (8, 3–18)
5–15	93	50 (54, 44–64)	44 (47, 38–57)	6 (7, 3–14)
16–59	195	79 (34–48)	60 (31, 25–38)	19 (10, 6–15)
≥60	58	21 (36, 25–49)	16 (28, 18–40)	5 (9, 3–19)
Antimicrobial drug treatment in previous month	224	84 (38, 31–44)§	67 (30, 24–36)	17 (8, 5–12)
Health care contact in previous 3 months	262	108 (41, 35–47)	85 (32, 27–38)	23 (9, 6–13)

*MSSA, methicillin-susceptible *Staphylococcus aureus*, MRSA, methicillin-resistant *S. aureus*; CI, confidence interval.
†Median age, y (range) of study participants: All, 32 (0–89); *S. aureus* positive, 24 (0–84); MSSA positive, 26 (0–84); MRSA positive, 36 (0–76).
‡p<0.001, *S. aureus* positive vs. study population >5 y of age.
§p<0.001, *S. aureus* positive vs. no antimicrobial drug treatment in the previous month.

throughout Samoa and was isolated from persons from 27 villages. There did not appear to be any geographic clustering associated with residence close to the main public hospital in Apia, although MRSA was more likely to be isolated from residents of Upolu, the main island on which Apia is located, than from residents of Savai'i, the less-populated and less-developed island. MRSA was isolated from 11% of participants from Upolu, but from only 3% of participants from Savai'i.

Of the 153 MSSA isolates from 187 participants, 124 (81%) isolates were resistant to penicillin, but resistance to any other antimicrobial drug was uncommon. Of the 34 MRSA isolates, 22 were resistant only to β-lactams. Of the remaining 12 MRSA, 8 were ciprofloxacin and erythromycin resistant, 2 were erythromycin resistant with inducible clindamycin resistance, and 2 were ciprofloxacin resistant. All MRSA isolates were susceptible to cotrimoxazole, doxycycline, gentamicin, and vancomycin, and none had constitutive clindamycin resistance.

Except for the MRSA isolates identified as type ST1 by multilocus sequence typing, all other MRSA belonged to a known MRSA strain (Table 2). Seven of the 9 ST1 isolates had indistinguishable PFGE profiles. The ST1 MRSA isolates had 82% homology by PFGE typing with another ST1 MRSA strain common in the region (the Australian WA MRSA-1).

Antimicrobial drug resistance profile was associated with strain (Table 2). All isolates of the USA300 strain were ciprofloxacin resistant, and most also were erythromycin resistant. All of the Queensland clone and Southwest Pacific clone MRSA, and 8 of the 9 ST1 MRSA isolates, were resistant only to β-lactams.

Discussion

Considerable concern exists internationally about increasing levels of antimicrobial drug resistance (*25*). Consequences include treatment failure, the need to use newer antimicrobial drugs to achieve treatment goals, the adverse effects frequently associated with these newer drugs, increased expenditure, and longer hospital stays. In developing countries, such as Samoa, an additional concern exists: when resistance to less expensive antimicrobial drugs becomes widespread, the more expensive antimicrobial drugs are simply not available (*26*). As in many other developing countries (*27*), use of antimicrobial drugs is extremely high in Samoa. More than 60% of all prescriptions dispensed in hospitals and private pharmacies include an antimicrobial drug (*28*). No current data are available on the prevalence of antimicrobial drug resistance in Samoa. In other countries, high levels of antimicrobial drug use correlate with high levels of resistance (*29*). In addition, inadequate dosing and poor adherence by patients may also increase the development of resistance (*25*). Anecdotal evidence indicates these practices also are common in Samoa.

In Samoa, children <5 years of age were more likely than persons in other age groups to have skin and soft tissue infection caused by *S. aureus*; however, the prevalence of MRSA infections were similar in children, adults, and elderly persons. Participants who had reported recent antimicrobial drug use were less likely to have *S. aureus*, but their prevalence of MRSA did not differ significantly from those who did not report recent antimicrobial drug use. The lack of association between MRSA prevalence and health care exposure or antimicrobial drug use was surprising; however, the data on antimicrobial drug use

Table 2. Strains identified among the MRSA isolates, Samoa, summer 2007–2008*

No. (%) isolates	Strain†	MLST type	No. (%) PVL-positive isolates	*spa* type (% of strain)	Antimicrobial drug susceptibility (% of strain)
10 (29)	USA300	ST8	9 (90)	t008 (100)	Resistant to ciprofloxacin and erythromycin (80); resistant to ciprofloxacin (20)
9 (26)	Queensland clone	ST93	9 (100)	t3949 (56), t202 (44)‡	Resistant only to β-lactams (100)
9§ (26)	–	ST1	0	t1853 (78), t6080 (11)¶	Resistant only to β-lactams (89); resistant to erythromycin (11)
4 (12)	Southwest Pacific/ WSPP/Oceania clone	ST30	4 (100)	t019 (100)	Resistant only to β-lactams (100)
2 (6)	AK3	ST5	0	t002 (50), t1265 (50)#	Resistant to erythromycin** (50); resistant only to β-lactams (50)

*MRSA, methicillin-resistant *S. aureus*; MLST, multilocus sequence typing; PVL, Panton-Valentine leukocidin; WSPP, Western Samoa Phage Pattern.
†International MRSA strain designations, except for AK3, which is a New Zealand designation for a community-associated MRSA strain common in New Zealand.
‡*spa* type t3949 (11–17–23–17–17–**17**–16–16–25) is a single-repeat variant of t202 (11–17–23–17–17–16–16–25). The extra repeat is shown in **boldface**.
§Only 8/9 ST1 MRSA isolates were available for PVL PCR, spa typing, and pulsed-field gel electrophoresis (PFGE) typing. Seven of the 8 isolates had indistinguishable PFGE profiles, and the eighth shared 87% homology.
¶*spa* type t6080 (07–23–21–17–13–**13**–34–16–13–33–13) is a single-repeat variant of t1853 (07–23–21–17–13–34–16–13–33–13). The extra repeat is shown in **boldface**.
#*spa* type t1265 (26–23–17–34–17–20–17–12–**12**–**12**–16) is a variant of t002 (26–23–17–34–17–20–17–12–**17**–16). The differing repeats are shown in **boldface**.
**This isolate also had inducible clindamycin resistance.

may not be entirely reliable. A previous study found that many Samoans are unclear about which medicines were antimicrobial drugs (*30*). Most of the health care exposure reported in our study related to primary care exposure, and most of the MRSA strains isolated are typically associated with community acquisition (*31*). Even though most participants had community-onset skin and soft tissue infection, more detailed information about diagnosis and place of acquisition would have been valuable.

Few data are available on the prevalence of MRSA in the community in nonindustrialized countries. The World Health Organization has identified a need to strengthen monitoring of antimicrobial drug prescriptions and resistance and has funded pilot programs to achieve this (*25*,*32*). In this study in Samoa, MRSA was isolated from 9% of all participants, and 18% of participants from whom *S. aureus* was isolated had MRSA. Although we attempted to include a wide range of persons in the study, we cannot be sure that some groups in our sample were not overrepresented or underrepresented. Carrying out such research is difficult in a country such as Samoa, where research and laboratory infrastructure are not ideal. Ability to speak Samoan, knowledge of Samoan culture, and previous experience of living in Samoa were essential for the success of the project.

We found that the diversity of MRSA isolates in Samoa that caused skin and soft tissue infection was similar to that in Denmark, a country of >5 million persons (*3*). We expected that isolates of the Southwest Pacific clone MRSA would be the predominant MRSA strain, but these were in the minority. The high prevalence of USA300 and Queensland clone MRSA might reflect the amount of travel between Samoa and the United States and Australia, respectively. The Queensland clone is now common in the Australian states of New South Wales and the Northern Territory in addition to Queensland (*33*). However, the large number of isolates of the Samoa ST1 MRSA clone and the isolation of almost equal numbers of 3 different MRSA clones suggest that the situation may not be that simple.

The Samoa ST1 MRSA isolates were distinct by both PFGE and *spa* typing from the community-associated ST1 MRSA strain commonly found in Australia and New Zealand, WA MRSA-1 (*33*). The Samoa ST1 MRSA isolates shared only 82% homology by PFGE with WA MRSA-1. WA MRSA-1 isolates are typically *spa* type t127, which does not cluster by BURP analysis with the *spa* types (t1853 and t6080) of the Samoa ST1 MRSA. In addition, the WA MRSA-1 strain is characterized by fusidic acid resistance and often also mupirocin or erythromycin resistance, whereas the Samoa ST1 MRSA isolates generally were resistant only to β-lactams. The Samoa ST1 MRSA isolates also were distinct from the USA400 ST1 MRSA strain with which they shared only 80% homology by PFGE. The Samoa ST1 MRSA strain might have originated in the Pacific. However, MRSA with *spa* type t1853 have also been isolated in New Zealand, mainly from patients in the Auckland area, since at least 2008 (*34*).

Three of the 5 strains that we identified among MRSA isolates in Samoa (the USA300, Queensland, and Southwest Pacific strains) typically are associated with community acquisition (*31*). The Samoa ST1 MRSA strain is also likely to be predominantly associated with community-acquired infections. ST1 is recognized as a prominent genetic background of community-associated MRSA (*35*). The Samoa ST1 MRSA shares ≥80% homology by PFGE typing with 2 other ST1 community-associated MRSA strains: WA MRSA-1 and USA400. WA MRSA-1 caused the first cases of community-associated MRSA infection in previously healthy persons in Western Australia in the early 1990s, and USA400 was the strain isolated from the first cases of community-acquired MRSA in the United States (*31*). The Samoa ST1 MRSA strains are not multiresistant, another feature typical of community-associated MRSA strains. On the other hand, none of the isolates of this strain carried the PVL genes, which are commonly found in community-associated MRSA. However, WA MRSA-1 is also PVL negative. Clearly, further characterization of the Samoa ST1 MRSA is warranted.

Evidence suggests that community-associated MRSA strains, particularly USA300, are more easily transmitted and might be more virulent than other *S. aureus* strains (*31*). In North America, approximately one third of persons with community-acquired USA300 MRSA infection require hospital admission (*36*). Moreover, community-associated MRSA is no longer just a problem in the community; it also has become a common cause of health care–associated infections (*37*). Hospital-acquired USA300 infections are more likely than community-acquired USA300 infections to be invasive and be associated with treatment failures (*36*). Thus, increases in the prevalence, severity, and complexity of diseases caused by globally successful community-associated MRSA strains are likely to be associated with increased illness, death, and cost. Economic analyses have consistently demonstrated that MRSA infections are associated with higher cost than are MSSA infections; although these studies have focused primarily on the costs associated with hospital care (*38*). None of these studies have investigated the consequences of MRSA in the developing world, yet the effects of disease are considerable (*39*).

Although reducing MRSA infection in the Samoan community is desirable, no controlled trials have demonstrated effective means of reducing community-associated MRSA infections in a community setting.

Recommendations to reduce transmission of community-associated MRSA include washing hands, caring for and covering wounds, not sharing contaminated personal items, appropriately disposing of contaminated waste, and appropriately prescribing antimicrobial drugs (*40*). Any interventions have substantial resource implications for a developing nation but must start with reliable surveillance of antimicrobial drug susceptibility, which is essential to monitor, control, and manage antimicrobial drug resistance. Thus, there is a clear need to assist developing countries with performing quality antimicrobial drug susceptibility testing and surveillance.

The results of our study, together with future surveillance efforts, can be used to provide information for local prescribing; the prevalence of MRSA in Samoa is high, and empiric prescription of antimicrobial drugs needs to account for this high prevalence. For example, we advocate that any patient in Samoa suspected to have serious, invasive *S. aureus* infection have adequate cultures and antimicrobial drug susceptibility testing performed. *S. aureus* infection in such a patient should be treated with vancomycin and a β-lactamase–stable penicillin drug until laboratory results are available. Patients with uncomplicated skin and soft tissue infections requiring antimicrobial drug treatment should receive cotrimoxazole or, if the patient cannot tolerate sulfonamides, clindamycin. Boils or furuncles should be treated by drainage, infection control, and wound care, with antimicrobial drugs reserved for complications.

We identified a wide range of genotypes of MRSA that were causing wound infections in a small Pacific Island nation. Our hope is that this study will provide a starting point for future research into antimicrobial drug resistance in the Pacific and provide impetus for initiatives to improve antimicrobial drug use in Pacific Island nations. Antimicrobial drug resistance is a global concern that does not respect national boundaries; consequently, countries need to assist each other in addressing the problem.

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Taenia solium Tapeworm Infection, Oregon, 2006–2009

Seth O'Neal, John Noh, Patricia Wilkins, William Keene, William Lambert, James Anderson, Jenifer Compton Luman, and John Townes

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

- Describe the epidemiology of cysticercosis in Oregon as based on a surveillance study
- Describe morbidity and mortality associated with cysticercosis as based on that surveillance study
- Describe goals of public health interventions for cysticercosis in Oregon as based on that study

Editor

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Neurocysticercosis (NCC) is a parasitic infection of the central nervous system caused by *Taenia solium* larval cysts. Its epidemiology in cysticercosis-nonendemic regions is poorly understood, and the role of public health institutions is unclear. To determine the incidence of NCC and to pilot screening of household contacts for tapeworms, we conducted population-based active surveillance in Oregon. We screened for *T. solium* infection by examining hospital billing codes and medical charts for NCC diagnosed during January 1, 2006–December 31, 2009 and collecting fecal and blood samples from household contacts of recent case-patients. We identified 87 case-patients, for an annual incidence of 0.5 cases per 100,000 general population and 5.8 cases per 100,000 Hispanics. In 22 households, we confirmed 2 additional NCC case-patients but no current adult intestinal tapeworm infections. NCC is of clinical and public health concern in Oregon, particularly among Hispanics. Public health intervention should focus on family members because household investigations can identify additional case-patients.

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Neurocysticercosis (NCC) is a parasitic disease caused by central nervous system infection with *Taenia solium* larval cysts. It is the most common helminthic infection of the central nervous system and a leading cause of acquired epilepsy in Latin America, Southeast Asia, and central Africa (*1*,*2*). The disease also is increasingly of clinical and public health concern in the United States, primarily in immigrants and travelers from cysticercosis-endemic regions (*3*–*5*).

Cysticercosis is acquired through fecal–oral transmission of tapeworm eggs shed in the feces of a human carrying intestinal tapeworms. Ingested eggs release oncospheres, which invade the intestinal mucosa and disseminate throughout the body to form larval cysts. NCC occurs when cysts develop in the central nervous system and is the primary source of illness and death (*6*). The tapeworm's complete life cycle occurs in regions with poor sanitary infrastructure, where foraging pigs have access to human feces. Most NCC cases in the United States probably were acquired in cysticercosis-endemic areas by immigrants or travelers who entered the United States already infected with cysts (*3*). However, immigrants and travelers also can harbor intestinal tapeworms, and domestic transmission of NCC does occur (*7*,*8*).

Few states require reporting of cysticercosis; thus, population-based epidemiologic data in the United States are limited. Even in jurisdictions that require reporting, the clinical nature of NCC diagnosis complicates surveillance efforts because no single laboratory test definitively establishes the diagnosis. Surveillance therefore relies on clinician or institutional reporting. In 1989, California became the first state to require reporting; 112 cysticercosis cases were reported during the first year, for a crude annual incidence of 1.5 cases per 100,000 Hispanics (*9*). A retrospective case-series from Oregon based on hospital discharge diagnoses during 1995–2000 estimated an annual incidence of 0.2 cases per 100,000 general population and 3.1 cases per 100,000 Hispanics (*10*). In 5 cases, no exposure to a cysticercosis-endemic area was documented, which suggests the possibility of local transmission.

Oregon adopted administrative rules for *T. solium* reporting in 2002 after the coroner's examination implicated hydrocephalus secondary to obstructing ventricular cysts in 2 unexplained deaths (*10*). However, no subsequent efforts were undertaken to stimulate passive reporting or to actively find unreported cases. As a result, only 7 NCC cases, all in Hispanics, were reported to public health officials during the first 5 years of reporting. Oregon has a rapidly growing Hispanic population, which currently represents 11% of the total population. Approximately half of all Oregon Hispanics report birth outside the United States (*11*). In the context of an increasing population at risk, the small number of passively reported cases suggests inadequate surveillance.

Identification and treatment of tapeworm carriers in the United States could prevent additional NCC cases. However, intestinal tapeworm infection produces few symptoms, and the prevalence is typically <1%–2%, even in regions where cysticercosis is endemic (*12*). During the 1980s, Los Angeles (LA) County, California, adopted a program of screening for tapeworm carriers with some success. By screening household members of NCC case-patients using light microscopy on fecal samples, the county identified an intestinal tapeworm carrier in 7% of its overall investigations and in 22% of investigations involving domestically acquired NCC (*13*). Improved screening methods have been developed in the interim, including an ELISA for *Taenia* sp. coproantigens in feces and an enzyme-linked immunoelectrotransfer blot (EITB) for serum antibodies against *T. solium* tapeworm (*14*,*15*). Serologic methods are desirable because they are specific to *T. solium* intestinal infection and highly sensitive (99%) and avoid the collection and processing of potentially infectious feces (*15*).

Our objective was to evaluate the utility of public health surveillance for *T. solium* infection in Oregon. We implemented population-based active surveillance to determine the incidence of cysticercosis. We also piloted screening specifically for additional *T. solium* infection among affected households by using a combination of symptom screening, laboratory analysis of fecal and serum specimens, and radiographic imaging.

Methods

Case Definition and Surveillance Period

We used the cysticercosis case definition in State of Oregon communicable disease investigative guidelines (www.oregon.gov/DHS/ph/acd/reporting/guideln/taeniasis_cyst_guideline.pdf). This definition classifies cases as confirmed, presumptive, or suspected according to published consensus criteria (*2*). Because clinical criteria for a definitive diagnosis (pathologic specimen, radiographic imaging demonstrating the scolex or direct visualization of the parasite on fundscopic eye examination) are seldom available, a presumptive diagnosis is common. We defined cases as infection diagnosed initially during January 1, 2006–December 31, 2009. Cases in non-Oregon residents were excluded. The Institutional Review Boards at Oregon Health & Sciences University and the State of Oregon Public Health Division reviewed and approved this study.

Case Ascertainment

During the 2009 calendar year, we requested quarterly reports of International Classification of Diseases, 9th

Revision, billing codes for cysticercosis (123.1) from all Oregon hospital systems. The first request included identification of historical cases going back to January 1, 2006; subsequent reports included only cases for the current quarter. All hospitals reported inpatient admissions, and those with integrated electronic medical records systems reported outpatient visits as well. To identify additional cases, we queried the main regional reference laboratory for cysticercosis serologic testing in Oregon. We also searched Oregon vital statistics data for deaths related to cysticercosis under all listed causes of death, both by diagnosis code and by keyword (i.e., cysticercosis, neurocysticercosis, and taenia). We obtained medical charts for all reported cases to verify the diagnosis and to extract clinical and epidemiologic data.

To stimulate passive reporting, we sent a letter to clinicians likely to diagnose NCC, including migrant worker health providers, radiologists, pathologists, neurologists, neurosurgeons, and infectious disease and emergency department physicians. We also distributed a newsletter about *T. solium* infection and reporting requirements to all licensed Oregon physicians.

Household Investigations

Persons with confirmed and presumptive cases diagnosed after July 1, 2008, were eligible for household investigation. After obtaining informed consent from the case-patient and all household contacts, the study physician used a standard interview tool to gather demographic, clinical, and epidemiologic data (online Technical Appendix, www.cdc.gov/EID/content/17/6/1030-Techapp.pdf). From each participant, we collected 1 fecal sample preserved in 10% formalin and a finger-stick blood sample on quantifiable filter paper preserved in StabilZyme Select (SurModics, Eden Prairie, MN, USA) stabilizer. We offered noncontrast computed tomography (CT) scan of the head to any household contact with clinical history of seizures or severe or chronic headaches or with any positive finding from laboratory tests.

Laboratory Methods

Laboratory processing was conducted at the Centers for Disease Control and Prevention Parasitology Diagnostics Laboratory (Atlanta, GA, USA). Fecal samples were examined by light microscopy for *Taenia* spp. eggs or proglottids and by ELISA for *Taenia* spp. coproantigens (*14*). Serum samples were analyzed by EITB for antibodies against *T. solium* cysts (EITB lentil lectin–bound glycoprotein) and against *T. solium* adult tapeworms (recombinant EITB [rEITB]) (*15*,*16*). The EITB lentil lectin–bound glycoprotein uses a semipurified fraction of homogenized *T. solium* cysts containing 7 *T. solium* glycoprotein antigens (*16*). The rEITB for taeniasis is based on baculovirus expression–purified recombinant antigen rES33 (*15*). We defined active intestinal (adult) tapeworm infection by either a positive ELISA coproantigen or by *Taenia* spp. eggs or proglottids in the fecal sample. We interpreted a positive serum rEITB in participants with negative fecal findings to indicate cleared *T. solium* intestinal tapeworm infection.

Data Analysis

Annual incidence rates were expressed as the number of cases per 100,000 population, with denominator estimates obtained from American Community Survey yearly estimates (*11*). We analyzed data using STATA version 10 (Stata Corp., College Station, TX, USA). Continuous variables were assessed by using either Kruskal-Wallis or Mann-Whitney tests for differences among or between groups of interest. We used the Fisher exact test to compare distributions of proportions or to examine association between pairs of categorical measures. All tests are 2-sided, with significance set at 0.05.

Results

We found 143 unique reports with diagnosis code 123.1 for Oregon residents during the surveillance period. Of the 56 (39%) reports we excluded, insufficient chart information was available to verify diagnosis or incidence year for 18 cases, and 38 cases were diagnosed before 2006. Of the remaining 87 cases, 79 (91%) were identified through active surveillance, including 75 (86%) through hospital queries, 2 (2%) through laboratory queries, and 2 (2%) during household investigations. Eight (9%) cases were spontaneously reported by clinicians, of which 6 were from 1 infectious disease clinician in a tertiary care hospital.

Of the 87 remaining cases, 19 (22%) were confirmed, 53 (61%) presumptive, and 15 (17%) suspected. Confirmed and presumptive cases therefore accounted for 72 (83%) of the total. All case-patients had radiographic imaging of the head; 83 (95%) had a CT scan, and 52 (60%) had magnetic resonance imaging (MRI). Confirmed and presumptive case-patients were 8.5× more likely than suspected case-patients to have received an MRI (odds ratio 8.5, 95% confidence interval 2.0–50.2). Birth country and travel history were not recorded for 12 of the suspected case-patients; an epidemiologic link to a cysticercosis-endemic area would have changed the classification to presumptive in all 12. The other 3 suspected case-patients had radiologic evidence suggestive of cysticercosis or links to a cysticercosis-endemic area, but their symptoms could have been explained by other diagnoses. Suspected case-patients were more likely to be female ($p = 0.04$) and older ($p<0.01$) and to have calcified lesions ($p<0.01$) than were confirmed or presumptive case-patients (Table 1).

Table 1. Demographic and clinical characteristics of cysticercosis case-patients, Oregon, 2006–2009

Characteristic	No. confirmed/presumptive (%), n = 72	No. suspected (%), n = 15	p value*
Demographic†			
M	46 (64)	5 (33)	0.04
Foreign-born	47 (65)	4 (27)	1.0
Signs and symptoms			
Seizures	29 (40)	4 (27)	0.07
Headache	27 (38)	3 (20)	
Focal neurologic deficit	5 (7)	1 (7)	
Altered mental status	3 (4)	3 (20)	
Traumatic injury	2 (3)	2 (13)	
Other	6 (8)	2 (13)	
Lesions			
1	22 (31)	2 (13)	0.10
2–4	21 (29)	9 (60)	
≥5	29 (40)	4 (27)	
Lesion location			
Parenchymal	61 (85)	15 (100)	0.49
Extraparenchymal	3 (4)	0	
Mixed	8 (11)	0	
Lesion stage			
Cystic only	22 (31)	1 (7)	0.01
Calcified only	32 (44)	13 (87)	
Mixed	18 (25)	1 (7)	

*By Fisher exact test.
†Median age, y (interquartile range): confirmed/presumptive, 36 (28–43); suspected, 47 (35–66); p<0.01, Mann-Whitney test.

Of the 72 confirmed and presumptive case-patients, 41 (57%) were hospitalized at time of diagnosis. The median inpatient stay was 4 days (interquartile range [IQR] 3–9), accounting for a total of 292 hospital days during initial illness only. Of these 41 hospitalizations, intensive care was involved in 16 (39%). Suspected case-patients were less likely to receive treatment with antiparasitic drugs (p = 0.03) or corticosteroids (p = 0.03); otherwise, hospitalization and treatment did not differ between patients with confirmed or presumptive cases. No deaths occurred for which cysticercosis was listed as a contributing factor.

We excluded suspected cases from incidence calculations (Table 2). Sixty-nine (96%) cases occurred in Hispanics. Including the 12 suspected cases for whom epidemiologic data were unavailable would increase the estimated mean annual incidence to 0.6 cases per 100,000 Oregon residents and 6.7 per 100,000 Hispanic Oregon residents. More case reports occurred during the active study year (2009), but the number of reports was not significantly higher in 2009 than in previous years (p = 0.08).

Country of birth was documented in the medical charts of 55 case-patients (Table 3). Three (5%) were US born; all were Hispanic. One was a 49-year-old man who denied any international travel; he had 1 obstructing fourth ventricular cyst confirmed by surgical pathology. Another US-born case-patient was a 24-year-old man with new-onset seizures, a single cystic parenchymal lesion found on MRI, and positive serologic test results for *T. solium* cysts. His only international travel included 1 week in Mexico 17 years before diagnosis. For both of these case-patients, family members reported ongoing travel to and from Mexico. Travel history was not available for the final US-born case-patient, a 57-year-old man with multiple parenchymal calcifications, seizures, and psychosis.

Thirty-two confirmed or presumptive NCC cases were initially diagnosed after July 1, 2008, and were therefore eligible for household investigation. We investigated 22 (69%) cases (Table 4). Of the 10 cases that were not investigated, 7 patients could not be located with the contact information available in the chart, 2 were identified in other

Table 2. Incidence of confirmed and presumptive cysticercosis cases, Oregon, 2006–2009

Variable	2006, n = 14	2007, n = 18	2008, n = 14	2009, n = 26*	All cases, n = 72
Hispanic, no. (%)	13 (93)	18 (100)	14 (100)	24 (92)	69 (96)
Years since immigration, median (interquartile range)	8 (5–10)	6 (2–10)	10 (8–17)	10 (4–18)	9 (4–15)
Annual incidence					
Cases/100,000 overall population	0.4	0.5	0.4	0.7	0.5
Cases/100,000 Hispanic population	3.4	4.5	3.4	5.5	5.8

*Number of reported confirmed and presumptive cases during the active study year (2009) compared with prior years, p = 0.08 by Fisher exact test.

Table 3. Region and country of orgin for the 55 cysticercosis case-patients for whom information was known, Oregon, 2006–2009

Region	No. (%) case-patients	Country (no. case-patients)
Central America, Caribbean	47 (85)	Mexico (40), Guatemala (5), Nicaragua (1), Cuba (1)
North America	3 (5)	United States (3)
Southeast Asia	2 (4)	Myanmar [Burma] (1), Thailand (1)
South America	1 (2)	Ecuador (1)
Africa	1 (2)	Cameroon (1)
Europe	1 (2)	Germany (1)

household investigations, and 1 was unable to provide informed consent because of psychosis. No case-patient refused the offer for household investigation. We found no significant difference between the 22 cases we investigated and the 10 we did not with respect to patient demographic or clinical characteristics. We did not identify any significant difference between the 22 investigated cases and the 40 confirmed or presumptive cases diagnosed before July 1, 2008. For investigated case-patients, median time since immigration to the United States was 10 years (IQR 6–14 years) and median time from last international travel to a cysticercosis-endemic country was 5 years (IQR 2–10 years).

A median of 6 (IQR 4–7) persons resided in each household. Of 111 total contacts, 79 (71%) were foreign born, and 41 (37%) reported international travel within the past 2 years. All fecal samples were negative by light microscopy and ELISA for coproantigen. One household contact had serum antibodies against *T. solium* adult tapeworms in 2 (9%) separate household contact investigations. In 1 household, the seropositive person was the brother of the index NCC case-patient. In the other household, the seropositive person was the husband of the index NCC case-patient. Nine case-patients (41%) and 1 (1%) household contact had circulating antibodies against *T. solium* cysts.

We offered head CT scans to 11 household contacts, 3 on the basis of positive serologic test results and 8 on clinical history. Of 9 who accepted, 2 had parenchymal calcifications consistent with NCC. One was a 7-year-old child from Myanmar (Burma) who had resettled with his family in Oregon 1 year earlier. He had a 3-year history of recurrent, untreated, generalized seizures that had not been reported to his physician. His mother was the household index case-patient; she sought care initially for severe headache and new-onset seizure; she had positive serologic test results for *T. solium* cysts and >20 parenchymal cystic lesions. The boy's father had serum antibodies against *T. solium* intestinal tapeworm infection with negative results of fecal studies. The other NCC case-patient was an adult man from Mexico City, Mexico, with an occipital parenchymal calcification and chronic headaches; he had immigrated 21 years earlier and denied international travel since immigration. We found no other evidence of *T. solium* infection in his household, other than the original case-patient.

Discussion

In Oregon, *T. solium* causes illness, particularly among the Hispanic population, which maintains ongoing contact with cysticercosis-endemic Latin America through immigration and travel. The mean annual incidence among Hispanics of 5.8 cases per 100,000 population is the highest documented rate within the United States, 4× the estimates from California in the mid-1980s, and 2× the previous estimate for Oregon (*9*,*10*,*13*). Although we documented no deaths directly resulting from cysticercosis, the morbidity and associated use of the health care system are high. Hospitalization at time of diagnosis was common, and intensive care was required in more than one third of hospitalizations. We did not quantify the health resource

Table 4. Demographic and clinical characteristics of case-patients with investigated and noninvestigated cysticercosis , Oregon, 2006–2009*

Characteristic†	Eligible for household investigation		No. (%) not eligible, n = 40	p value‡
	No. (%) investigated, n = 22	No. (%) not Investigated, n = 10		
Male sex	14 (64)	9 (90)	23 (58)	0.16
Lesions				
1	6 (27)	2 (20)	14 (35)	0.55
>1	16 (73)	8 (80)	26 (65)	
Lesion location				
Parenchymal	17 (77)	9 (90)	35 (88)	0.21
Extraparenchymal	2 (9)	1 (10)	0	
Mixed	3 (14)	0	5 (13)	
Lesion stage				
Cystic	6 (27)	1 (10)	15 (38)	0.15
Calcified	8 (36)	8 (80)	16 (40)	
Mixed	8 (36)	1 (10)	9 (23)	
EITB LLGP positive	9 (41)	–	–	

*EITB LLGP, enzyme-linked immunoelectrotransfer blot for antibodies against lentil lectin–bound glycoprotein of *Taenia solium* cysts.
†Median age, y (interquartile range): investigated, 31 (28–37); not investigated, 42 (35–57); not eligible, 35 (25–43); p = 0.15 by Kruskal-Wallis χ^2 test.
‡By Fisher exact test.

use related to treatment and follow-up of cases, but we noted surgical complications, shunt failure, and side effects from prolonged steroid use.

The relatively high incidence of cysticercosis in this study probably reflects increased case ascertainment rather than any increase in the underlying risk. Prior studies have relied primarily on hospital discharge data for case finding, which do not capture emergency department visits unless they result in inpatient admission. By requesting quarterly reports based on hospital billing codes, we were able to capture emergency department diagnoses. Many of these appear to have been less clinically severe, including uncomplicated new-onset seizures and headaches from calcified or nonobstructing cysts. In others, we found subsequent inpatient stays for treatment complications or clinical deterioration. Oregon's comparatively high incidence could alternatively be explained by underlying migration patterns, specifically if preferential migration to Oregon occurred from the highly cysticercosis-endemic central Mexican highlands. However, we have no definitive evidence to either support or refute this hypothesis.

Despite improved case ascertainment, for several reasons the incidence reported in this study most likely underestimates the true incidence of NCC in Oregon. First, we excluded suspected cases from incidence calculations. Although clinical and demographic characteristics of patients with suspected cases were similar to those with confirmed and presumptive cases, a documented epidemiologic link to a cysticercosis-endemic area was not found in all medical charts. For most persons with suspected cases, epidemiologic evidence suggesting exposure to *T. solium* would have changed the classification to presumptive. Including these suspected cases increased the mean annual incidence to 6.7 cases per 100,000 persons among Hispanics. Second, although several hospital systems reported outpatient visits related to cysticercosis, most outpatient visits in the state were not captured. Because less clinically severe disease can be diagnosed and treated completely in the outpatient setting, we may have missed these cases. Finally, we suspect that underdiagnosis is common, particularly in patients seeking care for headache related to intermittent inflammation around degenerating or calcified parenchymal cysts. The threshold for obtaining neuroimaging in the primary care setting often is high for chronic or intermittent headaches. The prevalence and health-resource use of headache related to NCC have not been characterized in cysticercosis-endemic or -nonendemic areas.

Opportunity to prevent NCC within the United States is primarily limited to identifying and treating domestic carriers of *T. solium* tapeworms. The numerous reports of NCC among US-born persons who have never traveled implicate domestic exposure to *T. solium* eggs (*3*,*7*,*17*–*20*). We found only 1 such person during our surveillance period, although other population studies have described probable domestic transmission in 7%–10% of NCC cases (*9*,*13*). Although most infected foreign-born persons were likely to have acquired infection outside the United States, some foreign-born case-patients may have acquired their disease within the United States. We documented frequent travel to and from cysticercosis-endemic areas among NCC case-patients and their household members, which suggests ongoing risk for tapeworm acquisition.

Despite the use of highly sensitive methods for testing serum and feces, we were unable to detect current intestinal tapeworm infection by screening household members of NCC case-patients. However, our case definition for current adult tapeworm infection was conservative. We found 2 contacts with circulating serum antibodies and negative results of fecal analysis, and we chose to interpret this discordance as evidence of past but cleared infection. However, given the high sensitivity and specificity of the rEITB, the unknown duration of antibody persistence, and the fact that we tested just 1 stool sample, an alternate interpretation of this discordance might be active adult tapeworm infection with false-negative results of fecal analysis.

We may not have been able to replicate the prior success in LA County, California, where tapeworm carriers were identified in 7% of investigated households (*13*), for other reasons. First, our sample size in this pilot program was small, and chance alone could explain the difference. Second, cases in LA County were based on date of symptom onset rather than date of diagnosis. Because symptom onset can substantially predate diagnosis, the cases we investigated may have been systematically biased toward more remote exposure. We defined cases according to date of diagnosis because exact symptom onset can be difficult to determine, particularly for chronic or intermittent headaches. Third, substantial underlying differences in case-patients and household contacts could exist between Oregon and LA County. With LA County's proximity to the Mexico border, case-patients and household members may have traveled outside the United States more recently or more frequently. In Oregon, the median time since immigration for case-patients was 4 years longer than the median time since immigration for case-patients in the LA County study. Similarly, the surveillance or investigation results from each study may not be generalizable to other states or other countries in which cysticercosis is not endemic. Finally, *T. solium* control efforts have been initiated in many areas of Latin America, and the underlying prevalence of tapeworm infection among immigrants from those regions might have decreased in the 20 years since the LA County program was implemented.

The strategy of routine screening for tapeworm carriers among household contacts of a person with symptomatic NCC may be inherently limited because of the long latency

between exposure to *T. solium* eggs and development of symptoms. We did find evidence of past tapeworm infection and possible transmission to other household members. Specific clinical or demographic characteristics of an NCC case-patient might correlate with the presence of a tapeworm in a household member, such as young age, remote exposure in a cysticercosis-endemic area, and viable or multiple lesions. Our sample size was too small to evaluate the effectiveness of limiting investigations based on these variables.

Even though the role of public health in tapeworm screening to prevent domestic transmission remains unclear, the public health system has other functions related to *T. solium* infection. Primary among these is a focus on the health of household members who are at increased risk for *T. solium* infection. Improved selection criteria for household investigations may increase the likelihood of detecting current tapeworm infection. Early identification, referral, and surgical treatment of chronic headache caused by hydrocephalus could prevent serious complications. Education of household members also is crucial because they may travel frequently to and from cysticercosis-endemic areas. Recognition of the need to avoid eating undercooked pork and to maintain good hygiene can reduce infection among travelers. Finally, increasing clinician awareness about *T. solium* infection is a necessary public health function, particularly for clinicians who care for Hispanic and other immigrant populations. Public health intervention should focus on the health of household members and on increasing awareness of the disease among affected families and among clinicians.

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Learning Objectives

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

- Distinguish the prevalence of cefepime-resistant *P. aeruginosa*
- Analyze risk factors for the development of cefepime-resistant *P. aeruginosa*
- Evaluate the effects of cefepime-resistant *P. aeruginosa* on the risk for mortality.

Editor

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Resistance to extended-spectrum cephalosporins complicates treatment of *Pseudomonas aeruginosa* infections. To elucidate risk factors for cefepime-resistant *P. aeruginosa* and determine its association with patient death, we conducted a case–control study in Philadelphia, Pennsylvania. Among 2,529 patients hospitalized during 2001–2006, a total of 213 (8.4%) had cefepime-resistant *P. aeruginosa* infection. Independent risk factors were prior use of an extended-spectrum cephalosphorin (p<0.001), prior use of an extended-spectrum penicillin (p = 0.005), prior use of a quinolone (p<0.001), and transfer from an outside facility (p = 0.01). Among those hospitalized at least 30 days, mortality rates were higher for those with cefepime-resistant than with cefepime-susceptible *P. aeruginosa* infection (20.2% vs. 13.2%, p = 0.007). Cefepime-resistant *P. aeruginosa* was an independent risk factor for death only for patients for whom it could be isolated from blood (p = 0.001). Strategies to counter its emergence should focus on optimizing use of antipseudomonal drugs.

Pseudomonas aeruginosa is one of the most common gram-negative bacterial causes of health care–acquired infections (*1*–*3*). These infections result in high

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morbidity and mortality rates (*4*,*5*). When serious *P. aeruginosa* infections are suspected, early and appropriate antimicrobial drug therapy is crucial because inadequate drug selection has been associated with increased mortality rates (*6*,*7*). Complicating the empiric selection of adequate therapy is the increasing prevalence of antimicrobial drug resistance among *P. aeruginosa* (*8*–*10*). Even in initially susceptible strains, resistance can rapidly develop during treatment (*11*–*13*).

Cefepime, a fourth-generation cephalosphorin, is one of the few agents remaining that has reliable activity against *P. aeruginosa*. However, increased prevalence of resistance to cefepime among these organisms has been noted (*14*–*18*). As such, elucidating the epidemiology of cefepime-resistant *P. aeruginosa* is crucial to ensure that this agent remains a viable therapeutic option. Our goals were to identify risk factors for cefepime-resistant *P. aeruginosa* infections in the hospital setting and to describe the clinical effects of these infections.

Methods

The study was performed at the Hospital of the University of Pennsylvania (HUP), a 725-bed tertiary-care center, and Penn Presbyterian Medical Center (PPMC), a 344-bed urban community hospital. Each hospital is located in Philadelphia, Pennsylvania, USA, and is part of the University of Pennsylvania Health System. The study was reviewed and approved by the University of Pennsylvania Institutional Review Board.

Participants

To investigate risk factors for cefepime-resistant *P. aeruginosa*, we conducted a case–control study. We identified study participants through records obtained from the clinical microbiology laboratory at HUP, which performs bacterial cultures on all clinical specimens from HUP and PPMC. All adult patients with a positive *P. aeruginosa* culture result from January 1, 2001, through December 31, 2006, were eligible for inclusion. Each participant was included only one time; the first positive *P. aeruginosa* culture identified during the study period was used.

On the basis of our first study goal—identifying risk factors—we designated all participants with a cefepime-resistant *P. aeruginosa*–positive culture result as case-patients and all participants with a cefepime-susceptible *P. aeruginosa* culture result as controls. All eligible case-patients and controls were included according to the aforementioned eligibility criteria.

Variables

To assess risk factor variables, we used a comprehensive clinical and administrative University of Pennsylvania health system database, which contains data for all hospitalizations since January 1, 2001, and has been used successfully for similar studies of antimicrobial drug resistance (*19*–*21*). Data elements obtained were age, sex, race, hospital (HUP or PPMC), admission as a transfer from another facility (i.e., outside hospital, long-term care facility, rehabilitation center), location within the hospital at the time of culture (i.e., intensive care unit or not intensive care unit), length of hospital stay before culture, prior admission to HUP or PPMC within the past 30 days, Charlson index (*22*), and all-patient refined–diagnosis-related group (APR-DRG) classification. The following concurrent conditions were also noted: renal insufficiency (serum creatinine level >2.0 mg/dL or requirement for dialysis), malignancy, diabetes, cirrhosis, congestive heart failure, chronic pulmonary disease, immunosuppressive therapy, and HIV infection. These variables were based on International Classification of Diseases, Ninth Revision codes; laboratory data; and pharmacy data.

Drug Susceptibility Profiles

We documented antimicrobial drug susceptibility profiles, anatomic site of cultures, and any co-infections. Drug susceptibilities were conducted and interpreted by a semiautomated system (MicroScan WalkAway System, NC16 panel; Dade Behring, St. Louis, MO, USA) or disk-diffusion susceptibility testing in accordance with the criteria of the Clinical and Laboratory Standards Institute (*23*). Isolates with MIC = 16 (intermediate) or MIC ≥32 (resistant) were deemed resistant. A multidrug-resistant strain of *P. aeruginosa* was defined as a strain with resistance to ≥3 antimicrobial drug classes (*24*). We documented all antimicrobial drug treatment administered during the same inpatient admission for up to 30 days before the positive *P. aeruginosa* culture. We then categorized the drugs by individual agent, class, and spectrum of activity as follows: aminoglycosides (gentamicin, amikacin), quinolones (levofloxacin, ciprofloxacin), extended-spectrum penicillins (piperacillin-tazobactam), extended-spectrum cephalosporins (cefepime, ceftazidime), carbapenems (imipenem, meropenem), anaerobic therapy (amoxicillin/clavulanate, ampicillin/sulbactam, ceftriaxone, imipenem, meropenem, metronidazole, clindamycin), tetracyclines (doxycylcine), and macrolides (azithromycin, erythromycin) (*25*). During this study period, cefepime was the primary extended-spectrum cephalosporin used at HUP and PPMC, per formulary guidelines. For multivariable analyses, antimicrobial drugs were categorized by agent or class.

Mortality Rates

To assess the relationship between cefepime-resistant *P. aeruginosa* and mortality rates, we performed a retrospective cohort study, designating the participants with cefepime-resistant *P. aeruginosa* as the exposed

group and those with cefepime-susceptible *P. aeruginosa* as the unexposed group. We focused specifically on rates for those hospitalized at least 30 days.

Statistical Analyses

We calculated the overall and annual prevalence of cefepime-resistant *P. aeruginosa* among all isolates identified during the study period. We then evaluated the annual prevalence of cefepime resistance over time by performing the χ^2 test for trend (*26*).

To assess possible associations between potential risk factors and cefepime-resistant *P. aeruginosa*, we initially conducted bivariable analyses. Categorical variables were analyzed by using the Fisher exact test, and continuous variables were analyzed by using the Wilcoxon rank-sum test (*27*). The strength of each association was evaluated by calculating an odds ratio (OR) and a 95% confidence interval (CI). Multivariable analysis was performed by using forward stepwise multiple logistic regression (*28*). All variables with p<0.20 on bivariable analyses were considered for inclusion in the multivariable model. Backward stepwise multiple logistic regression was also performed to determine whether identification of risk factors varied with the approach to multivariable analysis. Because of the need to adjust for time at risk when investigating risk factors for antimicrobial drug resistance, we required the "duration of hospitalization prior to culture" variable to remain in the final model (*29*). We also analyzed the interaction between risk factor variables in the final model. Finally, to focus on those isolates likely to represent clinical infection, as per Centers for Disease Control and Prevention criteria, we repeated the analyses on blood isolates only (*30*).

To assess the association between cefepime-resistant *P. aeruginosa* and mortality rates for those hospitalized at least 30 days, we conducted bivariable and multivariable analyses in a similar fashion as for the case–control study. As we did for the case–control study, we repeated the analyses on blood isolates only.

We considered a 2-tailed p<0.05 significant. We used STATA version 10.0 (StataCorp, College Station, TX, USA) to perform the statistical analysis.

Results

During the study period, culture results were positive for *P. aeruginosa* , and cefepime susceptibility was tested for 2,529 patients. Median patient age was 61 years (95% CI 60–62), and 1,439 (56.9%) patients were male. Regarding race and/or ethnicity, 1,116 (44.4%) were white, 848 (33.7%) were African American, 30 (1.2%) were Asian, 29 (1.2%) were Hispanic, and the rest were identified as other or unknown. Among all participants, 1,984 (78.5%) were hospitalized at HUP and 545 (21.6%) were hospitalized at PPMC.

P. aeruginosa isolates came from the following anatomic sites: respiratory tract (247 [35.5%]), urine (763 [30.2%]), wound (467 [18.5%]), blood (248 [9.8%]), tissue (120 [4.7%]), and other (35 [1.3%]). Among the 2,529 isolates, 213 (8.4%) exhibited cefepime resistance and 339 (13.4%) exhibited multidrug resistance. Annual prevalence of *P. aeruginosa* cefepime resistance over time showed no significant trend (p = 0.99; Figure).

Using bivariate analysis to compare exposures, we found several differences between cefepime-resistant and cefepime-susceptible *P. aeruginosa* (Table 1). Specifically, participants with cefepime-resistant *P. aeruginosa* were more likely to have received an extended-spectrum cephalosporin, extended-spectrum penicillin, or quinolone. Multivariate analysis indicated that prior use of an extended-spectrum cephalosphorin had the strongest association with cefepime-resistant *P. aeruginosa* (adjusted OR 2.18, 95% CI 1.57–3.04; p<0.001) (Table 2). Independently associated with cefepime-resistant *P. aeruginosa* were prior use of an extended-spectrum penicillin or a quinolone and transfer from an outside facility (Table 2). No substantive differences were found in the final model when analyses were limited to blood isolates.

The overall mortality rate among participants was 13.8% (348/2,529). The mortality rate for participants with cefepime-resistant *P. aeruginosa* was 20.2% (43/213) and for participants with cefepime-susceptible *P. aeruginosa* was 13.2% (305/2,316) (relative risk [RR] 1.53, 95% CI 1.15– 2.04; p = 0.007). After controlling for significant confounders in the multivariate analysis, cefepime-resistant *P. aeruginosa* was no longer associated with death (Table 3). However, the association between cefepime-resistant *P. aeruginosa* and death varied significantly, depending on whether the isolate was from the blood or elsewhere. When

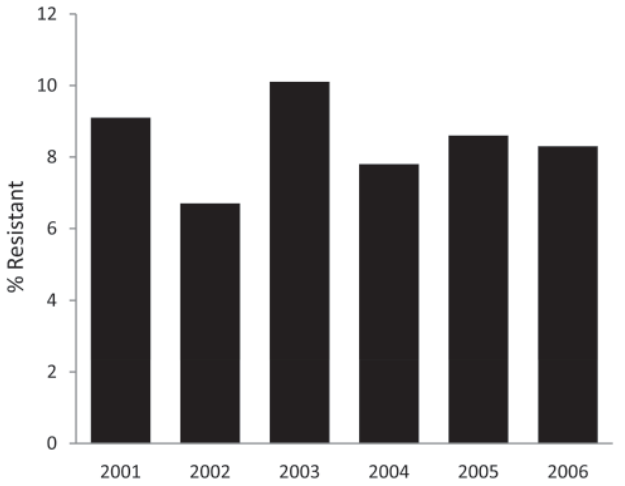

Figure. Prevalence of infection with cefepime-resistant *Pseudomonas aeruginosa*, Philadelphia, PA, USA, 2001–2006. p = 0.9946 for trend.

Table 1. Bivariable analysis comparing patient exposures to cefepime-resistant and cefepime-susceptible *Pseudomonas aeruginosa,* Philadelphia, PA, USA, 2001–2006*

Variable	No. (%) case-patients, n = 213	No. (%) controls, n = 2,316	OR (95% CI)	p value†
General				
Male sex	132 (62.0)	1,307 (56.4)	1.26 (0.94–1.70)	0.13
Race, white	95/207 (45.9)	1021/2,270 (45.0)	1.04 (0.77–1.39)	0.83
Hospital, PPMC	44 (20.7)	501 (21.6)	0.94 (0.65–1.34)	0.79
Transfer from another facility‡	73/212 (34.4)	509/2,304 (22.1)	1.85 (1.35–2.52)	<0.001
In ICU at time of culture	104/202 (51.5)	831/2,132 (39.0)	1.66 (1.23–2.24)	0.001
Prior hospitalization in past 30 d	60 (28.2)	526 (22.7)	1.33 (0.96–1.84)	0.07
APR-DRG§				
Concurrent illness	152 (71.4)	1,328 (57.5)	1.84 (1.34–2.55)	<0.001
Renal insufficiency	34 (16.0)	305 (13.2)	1.25 (0.82–1.86)	0.25
Malignancy	22 (10.3)	358 (15.5)	0.63 (0.38–0.99)	0.05
Diabetes	43 (20.2)	511 (22.1)	0.89 (0.62–1.28)	0.60
Liver disease	9 (4.2)	46 (2.0)	2.18 (0.92–4.58)	0.04
Congestive heart failure	2 (0.9)	37 (1.6)	0.58 (0.07–2.29)	0.77
Chronic pulmonary disease	52 (24.4)	453 (19.6)	1.33 (0.94–1.86)	0.11
Immunosuppressive therapy	39 (18.3)	256 (11.1)	1.80 (1.21–2.63)	0.004
HIV infection	5 (2.4)	54 (2.3)	1.01 (0.31–2.54)	>0.99
Antimicrobial drug use¶				
Any	150 (70.4)	1,458 (63.0)	1.40 (1.02–1.93)	0.03
Aminoglycoside	38 (17.8)	382 (16.5)	1.10 (0.74–1.60)	0.63
Quinolones	58 (27.2)	290 (12.5)	2.61 (1.85–3.65)	<0.001
Extended-spectrum penicillins	31 (14.6)	125 (5.4)	2.99 (1.89–4.60)	<0.001
Extended-spectrum cephalosporin	80 (37.6)	401 (17.3)	2.87 (2.10–3.90)	<0.001
Prior carbapenem	14 (6.6)	58 (2.5)	2.74 (1.38–5.08)	0.002
Prior anaerobic therapy	111 (52.1)	896 (38.7)	1.72 (1.29–2.31)	<0.001
Prior tetracyclines	1 (0.5)	18 (0.8)	0.60 (0.01–3.85)	>0.99
Prior macrolide	8 (3.8)	117 (5.1)	0.73 (0.31–1.52)	0.51

*OR, odds ratio; CI, confidence interval; PPMC, Penn Presbyterian Medical Center; ICU, intensive care unit; APR-DRG: all-patient refined-diagnosis–related group. Case-patients, those with cefepime-resistant *P. aeruginosa;* median (interquartile range) duration of stay before culture 8 (4–12) d; and Charlson index 2. Controls, those with cefepime-susceptible *P. aeruginosa* (interquartile range) duration of stay before culture 4 (4–5) d; and Charlson index 2.
†Fisher exact test for categorical variables; Wilcoxon rank-sum test for continuous variables.
‡Outside hospital, long-term care facility, or rehabilitation center.
§Patients in the extreme illness category.
¶Inpatient use within previous 30 d before culture during same hospitalization.

analyses were restricted to blood isolates, a significant independent association between cefepime-resistant *P. aeruginosa* and death was found (RR 15.55, 95% CI 3.10–77.89; p = 0.001] (Table 4).

Discussion

We found the following to be significant factors independently associated with isolation of a cefepime-resistant *P. aeruginosa* strain in culture of a clinical sample in the hospital setting: prior use of extended-spectrum cephalosphorins, extended-spectrum penicillins, or fluoroquinolones; and transfer from an outside facility. We also demonstrated that cefepime-resistant *P. aeruginosa* was independently associated with increased deaths among patients hospitalized for ≥30 days but only for those for whom *P. aeruginosa* was isolated from blood.

Table 2. Multivariable model of risk factors for cefepime-resistant *Pseudomonas aeruginosa* infection, Philadelphia, PA, USA, 2001–2006*

Variable	Unadjusted OR	Adjusted OR (95% CI)	p value
Prior use of extended-spectrum cephalosporin	2.87	2.18 (1.57–3.04)	<0.001
Prior use of extended-spectrum penicillin	2.99	1.91 (1.22–2.99)	0.005
Prior use of quinolone	2.61	1.96 (1.38–2.78)	<0.001
Prior use of carbapenem	2.74	1.70 (0.90–3.21)	0.10
Transfer from outside facility	1.85	1.49 (1.09–2.04)	0.01
Length of hospital stay before culture	NA	1.00 (0.99–1.01)†	0.81

*OR, odds ratio; CI, confidence interval; NA, not applicable. No substantive changes were found when above analyses were limited to bloodstream isolates only.
†Odds associated with each 1-day increase in hospital stay.

Table 3. Multivariable model of association between infection with cefepime-resistant *Pseudomonas aeruginosa* and death, Philadelphia, PA, USA, 2001–2006*

Variable	Adjusted OR (95% CI)	p value
Cefepime-resistant organism	1.28 (0.86–1.90)	0.232
Patient in ICU at time of culture	2.33 (1.75–3.10)	<0.001
APR-DRG	11.29 (6.53–19.50)	<0.001
Patient transfer from outside hospital	1.38 (1.05–1.81)	0.021
Length of hospital stay before culture	0.99 (0.98–1.00)	0.231

*OR, odds ratio; CI, confidence interval; ICU, intensive care unit; APR-DRG, all-patient refined-diagnosis–related group.

Past studies have found an association between use of an antipseudomonal agent and emergence of resistance to that same agent (*19*,*31*,*32*). Past studies have also demonstrated that *P. aeruginosa* resistance to 1 class of antimicrobial drugs is often associated with resistance to other classes (*11*,*33*). The tendency for health care–acquired *P. aeruginosa* to become resistant to drugs from multiple classes is well known, and several molecular mechanisms for its intrinsic and acquired resistance have been suggested (*10*). Our findings not only suggest that prior treatment with cefepime in itself is associated with subsequent emergence of cefepime-resistant *P. aeruginosa* but that even prior exposure to certain antipseudomonal agents in other classes is associated. Our results emphasize that to devise strategies that prevent further emergence of cefepime-resistant *P. aeruginosa*, recent prior use of antipseudomonal agents within the same class and from certain other classes must be recognized. The effect of curtailing use of non–β-lactam agents (i.e., fluoroquinolones) on cefepime-resistant *P. aeruginosa* prevalence should be formally assessed.

We also found that transfer from an outside facility was associated with cefepime-resistant *P. aeruginosa* infection. Because transferred patients potentially came from another hospital or from a long-term care facility, these patients might have been more likely to already be colonized with cefepime-resistant *P. aeruginosa* at the time of admission. That antimicrobial drug resistance is common in long-term care facilities is well known (*34*). Further work focusing specifically on antimicrobial drug–resistant *P. aeruginosa* infections in long-term care settings is warranted.

The significant association between cefepime-resistant *P. aeruginosa* infection and increased mortality rates (limited to those patients with *P. aeruginosa* bacteremia) may result from the fact that bacteremia is a more serious infection than, for example, a urinary tract infection. Alternatively, these results might be explained by noting that a blood isolate is more likely to represent a true infection than is an isolate from other anatomic sites, where isolates are more likely to represent colonization. Nonetheless, our results emphasize the potential serious effect of cefepime-resistant *P. aeruginosa* infection and the need for strategies to combat its further emergence.

This study has several potential limitations. The first is the ongoing, and appropriate, debate regarding the selection of the control group for case–control studies investigating the association between prior antimicrobial drug use and resistance. Like Harris and et al., we believe that selection of the control group depends on the study question (*29*,*35*). In our study, the main question was "What are the risk factors for cefepime resistance among all clinical isolates of *P. aeruginosa* in the hospital setting?" Thus, we selected patients with cefepime-susceptible *P. aeruginosa* infection as controls.

Another potential limitation is selection bias, which is always a concern in case–control studies. We believe that any such bias was minimized by the fact that every patient with a *P. aeruginosa* isolate was eligible for inclusion. Furthermore, all isolates were identified in the clinical microbiology laboratory at HUP, which processes all inpatient cultures at the participating study sites.

Misclassification bias is also a concern in case–control studies. However, the categorization of case-patients and controls and their exposure status was based entirely on preexisting clinical data from the clinical microbiology laboratory. The antimicrobial drug–susceptibility profiles were determined before study initiation, so determination of case and control status did not influence these profiles. Furthermore, case-patients and controls were selected without knowledge of their status regarding risk factors of

Table 4. Multivariable model of cefepime-resistant *Pseudomonas aeruginosa* infection and death (blood isolates only), Philadelphia, PA, USA, 2001–2006*

Variable	Adjusted OR (95% CI)	p value
Cefepimeiresistant organism	15.55 (3.10–77.89)	0.001
Patient in ICU at time of culture	3.22 (1.50–6.91)	0.003
APR-DRG	4.48 (1.60–12.60)	0.004
Patient transfer from outside hospital	1.26 (0.56–2.86)	0.57
Length of hospital stay before culture	1.01 (0.99–1.04)	0.80

*OR, odds ratio; CI, confidence interval; ICU, intensive care unit; APR-DRG, all-patient refined-diagnosis–related group.

interest. Thus, we believe any differential misclassification bias was unlikely.

Identification of participants in this study was based solely on clinical cultures. As such, that all of these cultures represented true infection is unlikely. For this reason, we performed additional analyses, focusing only on *P. aeruginosa* blood isolates because these would be expected to meet Centers for Disease Control and Prevention criteria for infection. The results of these secondary analyses did not differ substantively from the primary analyses investigating risk factors for cefepime-resistant *P. aeruginosa*. Finally, all patients in this study were admitted to either HUP or PPMC. Thus, our findings can only be generalized to similar academic centers. One must also keep in mind the differing resistance profiles at any given institution.

In conclusion, cefepime-resistant *P. aeruginosa* will negatively affect clinical outcomes, and strategies to counter its emergence are needed. Recognizing recent prior use of antipseudomonal agents, both within the same class and from certain other classes, is needed for devising successful interventions.

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Reflections on 30 Years of AIDS

Kevin M. De Cock, Harold W. Jaffe, and James W. Curran

June 2011 marks the 30th anniversary of the first description of what became known as HIV/AIDS, now one of history's worst pandemics. The basic public health tools of surveillance and epidemiologic investigation helped define the epidemic and led to initial prevention recommendations. Features of the epidemic, including the zoonotic origin of HIV and its spread through global travel, are central to the concept of emerging infectious diseases. As the epidemic expanded into developing countries, new models of global health and new global partnerships developed. Advocacy groups played a major role in mobilizing the response to the epidemic, having human rights as a central theme. Through the commitments of governments and private donors, modern HIV treatment has become available throughout the developing world. Although the end of the epidemic is not yet in sight and many challenges remain, the response has been remarkable and global health has changed for the better.

> We seem to think, with health problems as with other things, that science and technology will always save us, even though in the realm of human endeavor, it always comes (down) to people and our relationships—James Curran
>
> One of the saddest days of my life was when my mother told me Superman did not exist…. "'cause you just thought… he always shows up and he saves all the good people… and I was crying because there was no one… coming with enough power to save us."—Geoffrey Canada

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On June 5, 1981, the Morbidity and Mortality Weekly Report (MMWR), published by the Centers for Disease Control and Prevention (CDC), described *Pneumocystis carinii* (now *P. jiroveci*) pneumonia in 5 homosexual men in Los Angeles, California, USA, documenting for the first time what became known as acquired immunodeficiency syndrome (AIDS). The accompanying editorial suggested that the illness might be related to the men's sexual behavior. A month later, the MMWR reported additional diagnoses of *P. carinii* pneumonia, other opportunistic infections (OIs), and Kaposi sarcoma (KS) in homosexual men from New York City and California. These articles were sentinels for what became one of history's worst pandemics, with >60 million infections, 30 million deaths, and no end in sight.

This 30th anniversary year of the first description of AIDS is also the 15th anniversary of the introduction of highly active antiretroviral therapy (ART). Henceforth, AIDS will have been a treatable condition longer than it was the inevitably fatal disease first recognized. We offer highlights and reflections from a predominantly global perspective on 3 decades of collective experience with AIDS.

Early AIDS Surveillance and Epidemiology

To investigate this apparent outbreak, CDC investigators developed a simple surveillance case definition for what was first called KS/OI. The definition focused on certain OIs or KS in otherwise healthy persons and was used to establish a national reporting system. In light of new knowledge concerning AIDS and its underlying cause, the case definition was modified over time, but early surveillance indicated that an epidemic was under way and, in retrospect, had begun several years before the first reports. Retrospective testing of stored serum specimens from hepatitis patients in Los Angeles documented human immunodeficiency virus (HIV) infection as early as 1979.

The initial risk groups identified were men who have sex with men (MSM) and injection drug users (IDU). Field

investigations and surveillance activities demonstrated sexually linked cases in MSM and in persons with hemophilia and transfusion recipients, implicating transmission by male-to-male sexual contact as well as through blood and blood products. Cases in heterosexual persons and infants indicated that transmission could also occur through heterosexual contact and from mother to child.

Within <2 years, the essential epidemiology of AIDS—groups at risk and modes of transmission—was established, although debate about transmission through blood and blood products continued for several months after CDC believed the evidence was clear. In March 1983, the US Public Health Service published the first recommendations for AIDS prevention, including a recommendation that members of risk groups limit their numbers of sex partners and not donate blood or plasma. Although these recommendations were made before the etiologic agent, HIV, had been identified, they initiated AIDS prevention efforts and have largely stood the test of time, as has promotion of condom use.

As evidence accumulated that AIDS would not be confined to MSM and IDU, media interest grew and fears of contagion increased. Fear about transmission through casual contact led to discrimination against persons with AIDS, including barring HIV-infected children from school. During this time, CDC was recognized as a source of trustworthy information, and the agency gained respect by placing science above political considerations. Having devoted 71 articles to AIDS during 1981–1985, MMWR played a central role in dissemination of health information for rational policy decisions.

CDC regularly consulted with the World Health Organization (WHO), which published global data in its Weekly Epidemiological Record. While cases of AIDS in MSM and IDU began to be reported from other countries, several European countries reported cases in black Africans with no history of drug use or male-to-male sex. In the United States, cases also occurred in recent migrants from Haiti, subsequently designated as a risk group. Although this designation was useful for public health purposes, it resulted in discrimination against Haitian Americans. The subsequent explanation for AIDS in Africans and Haitians without other risk factors was heterosexual transmission of the causative agent.

In 1983, HIV was discovered, an accomplishment for which French scientists received the Nobel Prize for Medicine in 2008. In 1985, a serologic test for HIV became commercially available. Agreement on HIV as the causative agent and the availability of a diagnostic test were closing features of these early years. Despite the potential for hysteria and some examples of irrational responses, science and reason prevailed; epidemiology and surveillance served as the foundation of society's understanding and early response, as they would have to do repeatedly in future infectious disease epidemics.

AIDS as a Metaphor for Emerging Infections and the New Global Health

Social and environmental change, increased public health awareness, and improved diagnostic tools led to the emergence and recognition of several new pathogens in the last third of the 20th century. After a prolonged period of complacency with regard to infectious diseases, in 1992 the Institute of Medicine published an influential report on emerging infectious diseases. This term referred to conditions that were increasing in incidence in human populations or threatening to do so, were newly introduced or detected, or were recognized as being linked to a chronic disease or syndrome. No agent and disease better exemplify this concept than HIV and AIDS.

HIV type 1, group M (HIV-1), the predominant cause of the AIDS epidemic, evolved from a virus that crossed the species barrier from chimpanzees to humans. The earliest retrospective diagnosis of HIV-1 infection was made from a serum specimen collected in 1959 in Kinshasa, capital of what is now the Democratic Republic of Congo. Two additional but rare groups of HIV-1 (N and O) cause related zoonotic infections that are essentially restricted to central Africa. HIV-2, a second type of HIV rarely found outside western Africa, originated in sooty mangabeys.

Phylogenetic analysis of HIV-1 and SIVcpz (the simian immunodeficiency virus of chimpanzees closely related to HIV-1), combined with knowledge about the geographic range of the chimpanzee host, *Pan troglodytes troglodytes*, suggest that this cross-species transmission took place in central Africa early in the 20th century. The exact circumstances of cross-species transmission in central Africa are uncertain, but opportunities for human exposure to simian viruses through hunting and related activities are abundant. Over time, the virus presumably adapted to the human host and began to spread from person to person. At some unknown point, it was introduced into the Western Hemisphere, including Haiti and the United States.

Although the epidemic appears to have begun in central Africa, HIV prevalence is now highest in southern Africa; the Republic of South Africa alone is home to about one sixth of the world's HIV-infected persons. The reasons for this geographic distribution are not entirely clear, but biological factors, such as lack of male circumcision and rates of other genital (especially ulcerative) infections that facilitate HIV transmission, and social factors (some of which may have been influenced by the end of apartheid), such as frequent partner change and concurrent sexual partnerships, migration, and commercial sex, likely play a role. Whether infectiousness varies by virus subtype (subtype C is dominant in southern Africa) remains

debated. Under the South African presidency of Thabo Mbeki, AIDS denialism (the view that HIV is not the cause of AIDS) led to delayed implementation of ART and resulted in thousands of deaths.

Understanding the emergence and origins of HIV/AIDS will provide insight into global vulnerability to new infectious diseases. Without globalization and its central characteristic of increased movement of people, HIV might have remained in central Africa and the AIDS pandemic might have been delayed or might not have occurred. Although commerce and trade are as old as civilization, international air travel increased greatly in the latter half of the 20th century and enabled people to arrive at their destinations in greater numbers and within the incubation periods of many infectious diseases. The prolonged period between HIV infection and symptomatic AIDS, ≈11 years in adults, allowed widespread HIV transmission before recognition of the epidemic and any prevention attempts.

The response to HIV/AIDS epitomizes a new concept of global health. Essentials of global health today are its integration of core public health attributes (data and surveillance-based approaches, emphasis on populations, goals of social justice and equity, and prioritization of prevention), expansion into new areas such as treatment and health systems, and focus on emerging challenges beyond traditional priorities. New areas of emphasis include health security, chronic diseases (e.g., diabetes), and road traffic injuries. Global health is now about how the world deals with health rather than how a particular country addresses health problems in other countries.

AIDS and the Globalization of Science, Research, and Practice

A positive development in the response to AIDS has been its effect on science and the globalization of research and practice. Retrovirology and immunology became well-supported disciplines whose practitioners interacted productively with workers in other subjects such as epidemiology. Cohorts of physicians and scientists built their careers in basic as well as applied and clinical research. The frequency with which tuberculosis occurs in HIV-infected persons has led to a resurgence of interest in the diagnosis and treatment of this ancient disease, especially in Africa. Advances in the treatment of HIV-associated OIs have benefited other immunosuppressed persons. In addition, sexual and reproductive health gained renewed prominence.

Scientific advances resulted in the development of lifesaving, albeit not curative, treatment for HIV. Beginning with the approval of AZT (azidothymidine or zidovudine) in 1987, the development of antiretroviral drugs and the design of simple and standardized approaches for therapy in the developing world constituted a public health triumph. By the end of 2009, >5 million persons in low- and middle-income countries were accessing ART, unimaginable just a few years before and made possible through the use of generic drugs, price reductions for brand-name drugs, and efforts of international donors through initiatives such as the President's Emergency Plan for AIDS Relief and the Global Fund.

Research on the prevention of mother-to-child transmission of HIV has led to interventions with the potential to virtually eliminate HIV disease in children. Screening of donated blood and plasma for HIV and heat treatment of blood products have virtually eliminated transfusion-related HIV in high-income countries and vastly reduced its occurrence throughout low- and middle-income settings. Research has identified viable options for HIV prevention in IDU, such as needle and syringe exchange and opioid substitution therapy. Hospital hygiene and safe injection practices, previously neglected in much of the developing world, have become topics of global concern.

The earliest international collaborative field investigations on HIV/AIDS were in 1983 in Rwanda and the former Zaire, now the Democratic Republic of Congo. In 1984, Projet SIDA (French for AIDS Project) was established. This project, a joint venture between CDC, the National Institutes of Health, the Belgian Institute of Tropical Medicine, Mama Yemo Hospital (Kinshasa), and the then Zairian Department of Public Health, conducted landmark epidemiologic studies in central Africa. At one time Zaire had the highest citation index for AIDS research in the world. Subsequently, a second CDC-sponsored field station, Projet Retro-CI, contributed to the body of research from in western Africa, documenting lower pathogenicity and transmission rates for HIV-2, which indicated that although HIV-2 was a cause of AIDS, it was unlikely to result in a pandemic.

Numerous other international collaborations on HIV/AIDS had influence far beyond research publications. Investigators from low- and middle-income countries were trained, university exchanges arranged, and numerous careers influenced and internationalized with incalculable effects. Many US and European universities established HIV training and research collaborations with their counterparts in developing countries. These relationships have built platforms upon which new initiatives, in areas such as maternal and child health, could be built. One of the lasting contributions of international HIV/AIDS work may be the training and empowering of professionals in low- and middle-income countries to influence health in their own countries.

Despite the advances in HIV prevention and treatment, the challenges remain daunting. In 1984, the US Secretary of Health and Human Services famously predicted the

availability of an HIV vaccine within 2 years. Now, >25 years later, an effective vaccine remains elusive. Although billions of dollars have been expended on prevention research, an estimated 2.6 million persons acquire HIV annually. Only about a third of patients who qualify for treatment under the relatively conservative WHO guidelines actually receive it, and neither the optimal time for treatment initiation nor the optimal use of antiretroviral drugs to interrupt transmission have been determined. Tuberculosis remains a major killer of HIV-infected persons in Africa, our tools for combating it are outdated, and coordination between tuberculosis programs and HIV/AIDS programs remains less than optimal.

The AIDS Response, Nothing for Us without Us

Activism and advocacy profoundly influenced the response to HIV/AIDS. Outside the gay community, initial concern about HIV/AIDS was largely limited to scientists tracking the epidemic or searching for a cause. In the face of stigma, discrimination, and indifference to their friends dying, affected communities organized to provide prevention advice, care, and support. Community groups like Gay Men's Health Crisis sprung up, delivering services and engaging in political activities. Organizations such as ACT UP undertook acts of civil disobedience to influence the research agenda, improve access to HIV drugs, and lower the cost of treatment.

Although early activists were predominantly American MSM, their work influenced other affected communities. When the magnitude of the epidemic in Africa became apparent, activists from the Northern Hemisphere contributed to demands for treatment access in the Southern Hemisphere. Vulnerable groups, including sex workers and IDU, made themselves heard internationally in an unprecedented way. "Nothing for us without us" captures the insistence of affected communities that they participate in the design of programs and interventions.

A key figure in the global response was Jonathan Mann, an epidemiologist from CDC who served as the founding director of Projet SIDA and was appointed in 1986 as the first director of WHO's HIV/AIDS program. Mann recognized that the global spread of HIV/AIDS represented unequal vulnerability more than it did individual behavior, and he defined human rights as central to health and an effective HIV/AIDS response. He clashed with WHO leadership and bureaucracy and resigned in 1990. Tragically, Mann died in a plane crash in 1998.

Much has been written about the different ways that HIV/AIDS has been addressed, compared with other sexually transmitted infections, and the term AIDS exceptionalism has been coined. For example, specific consent forms and counseling were required before HIV testing, and limits were placed on sharing patient names between health jurisdictions for HIV surveillance purposes. These practices responded to concerns of affected communities that infected persons would be subject to discrimination such as termination of insurance or employment. Mandatory HIV testing, unhelpful and discriminatory, was largely prevented, but exceptionalist views may also have delayed expansion of HIV testing in clinical settings and thus access to care, including in the Southern Hemisphere. As HIV became treatable and surveillance practices successfully protected confidentiality, much of the exceptional approach to HIV gradually diminished.

Major human rights challenges persist, however. IDU and MSM suffer intense discrimination in many countries; their prevention needs are neglected and their very lives are sometimes in danger. Gender inequalities remain a driver of ill health. Increased attempts at criminalizing HIV transmission and continued travel restrictions for HIV-infected persons illustrate the enduring relevance of Jonathan Mann's message.

AIDS and the Architecture of Global Health

HIV/AIDS played a major role in shaping current global health architecture. The threat posed by HIV led WHO to establish a dedicated program in 1986. In 1996, the Joint United Nations Programme on HIV/AIDS was established to coordinate the multisectoral response. In 2001, the United Nations General Assembly Special Session on HIV/AIDS, the first high-level summit ever devoted to a disease, committed the world to specific targets. In 2002, the Global Fund to Fight AIDS, Tuberculosis and Malaria was created, and a year later, US President G.W. Bush announced the President's Emergency Plan for AIDS Relief, the largest bilateral health program ever undertaken. The scale-up of HIV/AIDS services has highlighted the need to focus on strengthening health systems and on other health-related Millennium Development Goals relating to maternal and child health.

The increase in actors in global health, including philanthropic organizations such as the Bill and Melinda Gates Foundation and the William J. Clinton Foundation, in part resulted from, but also coincided with, development of the global AIDS response. A result of the altered landscape is a diminution of the World Health Assembly's influence on global decision making and WHO's role in technical assistance. At the same time, HIV/AIDS demonstrated WHO's unrivaled convening authority and the influence of its normative guidelines for global practice, such as for HIV/AIDS treatment. Major contributions from the Joint United Nations Programme on HIV/AIDS include global prioritization of HIV/AIDS and resource mobilization, epidemiologic monitoring, advocacy for treatment in low-income settings, and promotion of sound policies.

A problem with the early response in the United States as well as globally was an overemphasis on universal vulnerability, the concept that everyone is at risk. Predictions of widespread, generalized HIV/AIDS epidemics among heterosexual persons outside Africa, especially in Asia, were not borne out. The concept of "know your epidemic," highlighting the need to focus interventions where HIV transmission is most intense, came surprisingly late, along with acknowledgment of the fundamentally different nature of the epidemic in sub-Saharan Africa compared with elsewhere.

Many countries with concentrated epidemics have had difficulty accepting that communities of MSM and IDU existed in their midst, let alone mounting targeted responses. Vigilance is required to ensure that resources are deployed to the right places in a timely fashion, rather than to general population groups that are politically safer but at lower risk. Characteristically, it has taken AIDS to bring the existence of marginalized groups such as sexual minorities to attention in low- and middle-income countries and to highlight their vulnerability and needs.

AIDS and the Future

We should not expect a single leader or intervention to deliver an abrupt end to the HIV/AIDS pandemic, yet the tide can be turned with principled pragmatism, adequate resources, trust in communities, and science as our guide. At times the process is slow. For example, US government support for needle and syringe exchange to prevent HIV in IDU did not happen until Barack Obama became US President (2009), and scale-up of male circumcision has been inadequate. But a middle way has to be found between arguments for the magic bullet of the moment and calls for unrealistic social and behavioral change with regard to sex and drug use.

We (the authors) have 4 priorities: 1) defining the best ways to use existing interventions to interrupt HIV transmission, 2) continuing the focused search for new knowledge and interventions, 3) resolving how best to use HIV testing and antiretroviral drugs for prevention as well as treatment, and 4) ensuring sustainability and commitment for the global response. Aspirations for social justice, human rights, and decency must motivate the response while epidemiology and surveillance provide technical direction as well as evaluation. True country and community ownership of the response is essential because solutions wanted more by donors or governments than by affected communities themselves almost never succeed.

Further success in HIV/AIDS prevention and treatment is challenged by numerous threats including fatigue and shifting priorities on the part of donors, the global financial downturn, and diversion of attention to other health problems plaguing the developing world. Better integration of HIV/AIDS efforts and interventions with those addressing maternal and child health are needed, and the global health infrastructure supported by HIV/AIDS scale-up will have to face the looming pandemic of noncommunicable diseases. Regardless how global health evolves, the unfinished agenda of HIV/AIDS must remain central.

Conclusions

Although we continue to face many challenges while responding to HIV/AIDS, we must also acknowledge the enormous scientific, social, and human achievements of the past 3 decades. The epidemic has severely tested many countries, especially those with the most limited resources, yet these countries have generally responded with decency, compassion, and good judgment. Despite the human and financial costs, millions of infections have been prevented and millions of life-years saved. The response to AIDS will be a benchmark against which responses to future health threats will be compared.

Many themes of the HIV/AIDS epidemic were captured by Albert Camus in his classic novel The Plague, and the expectations expressed therein largely apply. Inevitably, the story of HIV/AIDS "could not be one of final victory. It could be only the record of what had to be done, and what assuredly would have to be done again in the never-ending fight against terror and its relentless onslaughts." An enduring frustration is that we will not know how the story of AIDS will finally end because the epidemic will outlast us. A perpetual challenge will be living up to the commitment and courage of those who went before—health workers, scientists, and affected persons—who faced the unknown and took risks. In general, 30 years of AIDS confirm that there is indeed "more to admire in men than to despise." And while the epidemic continues, the world of global health has changed for the better.

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Reassortant Pandemic (H1N1) 2009 Virus in Pigs, United Kingdom

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Surveillance for influenza virus in pigs in the United Kingdom during spring 2010 detected a novel reassortant influenza virus. This virus had genes encoding internal proteins from pandemic (H1N1) 2009 virus and hemagglutinin and neuraminidase genes from swine influenza virus (H1N2). Our results demonstrate processes contributing to influenza virus heterogeneity.

During the 1918 influenza pandemic, the virus likely passed from humans to pigs (*1*). Descendants of this virus (classical swine influenza viruses), first isolated in 1930 (*2*), have continued to circulate in pigs (*1*). Other influenza viruses have caused either sporadic or enzootic infections.

Until 2009, the predominant influenza virus subtypes in pigs in Europe were avian-like (H1N1), human-like (H3N2) (representing virus transmissions from birds and humans, respectively), and H1N2 (*3*). Subtype H1N2 viruses, first identified in the United Kingdom in 1994 and subsequently detected throughout Europe, arose by reassortment between human subtype H1N1 (hemagglutinin [HA] gene), human-like swine subtype H3N2 (neuraminidase [NA] gene), and avian-like swine subtype H1N1 viruses (internal gene segments; *4,5*).

Classical swine influenza viruses (H1N1) were dominant in North America (*6*). However, during the 1990s, infection of pigs with human subtype H3N2 virus resulted in viruses containing a triple-reassortant group of internal genes. These viruses contain genes derived from human, classical swine, and avian-origin viruses and can accept different HA and NA genes (*6*).

Pandemic (H1N1) 2009 virus is a reassortant virus with genes from recent North American triple reassortant (basic polymerase 2 [PB2], PB1, acidic polymerase, HA, nucleoprotein [NP], nonstructural gene) and European avian-like subtype H1N1 (NA, matrix [M]) viruses (*7*). Infections of domestic pigs with pandemic (H1N1) 2009 virus have been detected worldwide. In January 2010, a reassortant virus that contained a pandemic (H1N1) 2009 virus NA gene and an avian-like subtype H1N1 HA gene was detected in pigs in Hong Kong (*8*). This reassortant was efficiently transmitted between pigs (*8*). We report detection and characterization of a novel swine reassortant virus in the United Kingdom that has genes encoding internal proteins from pandemic (H1N1) 2009 virus and HA and NA genes from a swine subtype H1N2 virus.

The Study

In mid-April 2010, influenza-like illness was reported in pigs in a North Yorkshire gilt (female pig intended for breeding that has not farrowed) grower unit of ≈1,200 animals. Gilts were brought into the unit in batches of ≈100 animals at ≈5 months of age. The first batch of gilts arrived in mid-January 2010; previously, the unit did not contain animals for ≥4 months. Gilts were housed in stable groups of ≈20 in a naturally vented building with a straw yard and remained in the unit for ≈70 days. The nearest pig farm was ≈3 miles away.

A persistent moist cough and signs typical of epizootic swine influenza were observed in 40%–50% of a batch of pigs 2 weeks after their arrival. Seven days after the onset of clinical signs, nasal swabs and serum samples were obtained from 6 pigs, and serum samples were obtained from 4 acutely affected pigs. Convalescent-phase serum samples were obtained from 9 pigs in the same batch 21 days later. Clinical signs had subsided by early June 2010.

Total RNA was extracted from swab eluant and amplified by using an M gene real-time reverse transcription PCR (RT-PCR) capable of detecting pandemic (H1N1) 2009 virus (*9*); 4 of 6 swabs were positive. None of the samples were positive for pandemic (H1N1) 2009 virus with a modified real-time RT-PCR specific for the HA gene (*9*). Only the sample positive by real-time RT-PCR with the lowest cycle threshold value yielded virus when inoculated into embryonated fowl eggs (*10*). Egg-grown virus was identified as subtype H1N2 by using hemagglutinin inhibition (HI) and NA inhibition with standard methods (*10*) and designated A/swine/England/1382/10 (H1N2). The virus was reisolated from the original sample to exclude cross-contamination.

A/swine/England/1382/10 was characterized by using whole genome sequencing and phylogenetic analysis. Gene fragments were amplified by using a 1-step real-time RT-PCR (QIAGEN, Hilden, Germany), and HA and NA genes were sequenced by using subtype H1N2 virus–specific primers (*5*). Partial internal gene segment sequencing was initially performed by using primer pairs (*5*). Full sequencing of internal gene segments used universal (NP,

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Table 1. Genotypes of influenza virus subtype H1N2 and H1N1 isolates from pigs, United Kingdom, 2009–2010*

Virus	Date of sampling	Subtype	Gene segment							
			PB2	PB1	PA	HA	NP	NA	M	NS
A/swine/England/236/09	2009 Sep 28	H1N2	A	A	A	H	A	H	A	A
A/swine/England/1157/09	2009 Oct 5	H1N2	A	A	A	H	A	ND	A	A
A/swine/England/1428/09	2009 Dec 8	H1N2	A	A	A	H	A	H	A	A
A/swine/England/523/10	2010 Jan 4	H1N2	A	A	A	H	A	H	A	A
A/swine/England/1382/10†	2010 Apr 13	H1N2	P	P	P	H	P	H	P	P
A/swine/England/1389/10	2010 Apr 15	H1N1	A	A	A	A	A	A	A	A
A/swine/England/73690/10	2010 Jun 23	H1N1‡	P	P	P	P	P	P	P	P

*PB, basic polymerase; PA, acidic polymerase; HA, hemagglutinin; NP, nucleoprotein, NA, neuraminidase; M, matrix; NS, nonstructural; A, genes with closest homology to European avian-like swine influenza viruses (H1N1); H, genes with closest homology to European swine influenza viruses (H1N2); ND, gene not sequenced but typed as N2 by using standard NA inhibition assay; P, genes with closest homology to pandemic (H1N1) 2009 virus. Closest matching homologous genes were determined by analyzing nucleotide sequences (minimum of 180 bases) against the National Center for Biotechnology Information GenBank database (http://blast.ncbi.nlm.nih.gov). GenBank accession nos. JF290388–JF290395 and JF297995–JF298002.
†A/swine/England/1382/10 reassortant virus.
‡Pandemic (H1N1) 2009 virus.

M, and nonstructural genes) and pandemic (H1N1) 2009 virus–specific primers (PB2, PB1, acidic polymerase, and NP genes). Primer sequences are available upon request.

Analysis of sequence data by BLAST analysis (http://blast.ncbi.nlm.nih.gov) determined the closest similarity to influenza virus isolates in the GenBank database. A/swine/England/1382/10 had HA and NA genes closely related to UK swine subtype H1N2 viruses (Table 1). All genes encoding internal proteins showed the highest similarity to pandemic (H1N1) 2009 viruses (Table 1).

HA and NA genes of A/swine/England/1382/10 grouped within the European swine subtype H1N2 cluster, specifically, with contemporary subtype H1N2 isolates from England. The closest matching isolate for HA and NA was A/swine/England/1428/09, which is reported in this article (Figure).

The M gene of A/swine/England/1382/10 had the S31N amantadine-resistance mutation, typical of pandemic (H1N1) 2009 viruses. It also had 627E and 701D mutations in the PB2 gene and mutation 591R, a basic amino acid that reportedly compensates for lack of the 627K mammalian-adaptive mutation (*14*). The PB1-F2 open-reading frame encoded a truncated PB1-F2 protein of 11 aa, consistent with other pandemic (H1N1) 2009 viruses. The NA gene has mutations 119E and 292R, which are associated with susceptibility to oseltamivir in N2 subtypes.

Since the emergence of pandemic (H1N1) 2009 virus, 5 other subtype H1N2 viruses have been detected in pigs in the United Kingdom. Partial sequencing of internal genes of these viruses showed they were not reassortants (Table 1). Reassortment was not detected in a European avian-like

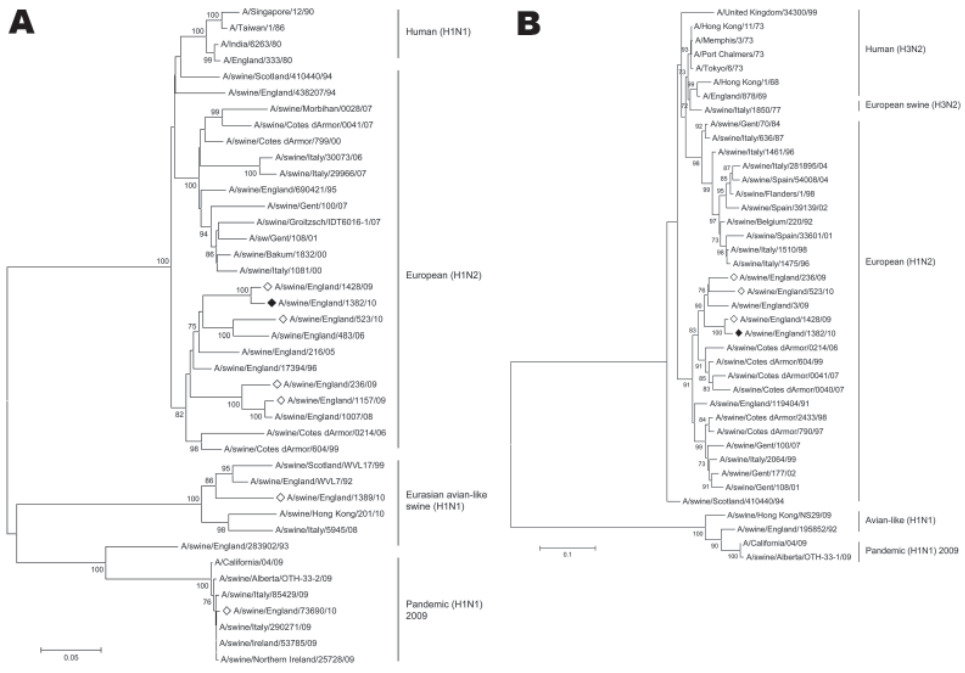

Figure. Phylogenetic analysis of influenza A virus hemagglutinin (A) and neuraminidase (B) genes. Trees were constructed by using the neighbor-joining method. Solid diamonds indicate A/swine/England/1382/10 genes from virus isolated in this study, and open diamonds indicate genes from other viruses reported in this study. Percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) is shown next to the branches for values >70% (*11*). Evolutionary distances were computed by using the Tamura-Nei method (*12*). Phylogenetic analyses were conducted by using MEGA4 (*13*). Scale bars indicate nucleotide substitutions per site.

swine subtype H1N1 virus isolated from another pig unit in the same region in April 2010, or in a pandemic (H1N1) 2009 virus isolated from another pig unit in the same region in June 2010 (Table 1).

The 10 acute-phase and 9 convalescent-phase serum samples were subjected to standard HI tests (*10*) with antigens from A/swine/England/195852/92 (avian-like subtype H1N1), A/swine/England/1353/09 pandemic (H1N1) 2009 virus, A/swine/England/438207/94 (subtype H1N2), and homologous A/swine/England/1382/10 (Table 2). Acute-phase serum samples were positive for antibodies against pandemic (H1N1) 2009 virus. Titers increased >10-fold in convalescent-phase serum samples. Antibody titers to endemic and reassortant subtype H1N2 viruses were negligible in acute-phase serum samples but increased 14-fold and 16-fold, respectively, in convalescent-phase serum samples.

Conclusions

We report detection of a novel reassortant virus between pandemic (H1N1) 2009 virus and a swine subtype H1N2 virus. In contrast to an earlier report of a reassortant virus that contained the NA gene of pandemic (H1N1) 2009 virus (*8*), in this study all genes encoding internal proteins of A/swine/England/1382/10 virus are derived from pandemic (H1N1) 2009 virus.

The source of A/swine/England/1382/10 could not be established. Appearance of clinical signs 2 weeks after arrival in the unit suggests that pigs were not previously infected with either a precursor or reassortant virus. However, detection of antibodies against pandemic (H1N1) 2009 virus in pigs coinciding with appearance of clinical signs suggests earlier subclinical infection with pandemic (H1N1) 2009 virus preceding co-circulation of subtype H1N2 or reassortant H1N2 viruses once pigs arrived at the unit. Earlier sampling of pigs in the unit may have detected subclinical precursor viruses.

We did not find evidence of similar reassortants in the United Kingdom. Therefore, it is unclear whether A/swine/England/1382/10 can be transmitted between pigs or has any selective advantage.

Our serologic results and those of others (*15*) indicate that antibodies against pandemic (H1N1) 2009 virus or subtype H1N2 virus produced during natural infection of pigs do not show cross-reactivity in HI tests. Therefore, pandemic (H1N1) virus and subtype H1N2 virus may continuously circulate in pigs in Europe, providing additional opportunities for reassortment.

Acknowledgments

We thank the pig owners/keepers; their veterinary surgeons; and colleagues at the Department for Environment, Food and

Table 2. Serologic cross-reactivity titers of acute-phase and convalescent-phase swine serum samples against subtypes of swine influenza viruses, United Kingdom*

Phase and pig no.	Virus			
	A/Swine/England/438207/94, subtype H1N2	A/Swine/England/195852/92, subtype H1N1	A/swine/England/1353/09, pandemic (H1N1) 2009	A/swine/England/1382/10, reassortant subtype H1N2
Acute phase				
1	<10	40	320	<10
2	<10	<10	160	<10
3	<10	<10	160	<10
4	<10	<10	320	<10
5	<10	<10	160	<10
6	<10	<10	320	<10
7	<10	<10	640	<10
8	<10	<10	320	<10
9	<10	<10	<10	<10
10	<10	<10	80	<10
Convalescent phase				
1	80	20	2,560	160
2	<10	<10	5,120	160
3	<10	<10	2,560	80
4	160	160	1,280	160
5	<10	<10	1,280	80
6	640	160	5,120	160
7	<10	<10	2,560	320
8	160	40	5,120	320
9	320	320	2,560	160

*Acute-phase serum samples were obtained from 10 pigs in the same batch during the time when clinical signs were apparent (≈2 wk after arrival at the unit). Convalescent-phase serum samples were obtained from 9 pigs in the same batch 21 d later. Standard hemagglutinin inhibition assays were conducted with subtype H1N1, pandemic (H1N1) 2009, and subtype H1N2 virus antigens derived from UK swine isolates. Homologous A/swine/England/1382/10 antigen was also used.

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Immunologic Changes during Pandemic (H1N1) 2009, China

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We analyzed changes in immunologic values over time for 28 hospitalized patients with pandemic (H1N1) 2009. Levels of interleukin-6, interferon-γ, and interleukin-10 increased 1 day after illness onset and then decreased to baseline levels. Levels of virus-specific antibody were undetectable 1 day after illness onset and peaked 36 days later.

Pandemic (H1N1) 2009 virus emerged in March 2009 and spread worldwide (*1*,*2*). Most patient laboratory data result from samples or information obtained on the day of hospitalization or when the patient was experiencing the acute phase of infection. Changes in laboratory data throughout the disease course have rarely been reported. We retrospectively analyzed immunologic changes for hospitalized patients through the entire course of pandemic (H1N1) 2009. These data may provide a better understanding of disease pathogenesis.

The Study

Twenty-eight patients admitted to Beijing 302 Hospital in Beijing, China, were enrolled in this study in September 2009. All patients had mild clinical courses and fulfilled the case definition for pandemic (H1N1) 2009 (*3*). Median interval from onset of illness to hospitalization was 1 day (range 0–3 days), and median time of hospitalization was 7 days (range 6–14 days). Serum samples were obtained from patients 1 day after illness onset and 4, 14, 36, and 48 days later for cytokine and antibody measurement. Serum samples from 22 healthy persons were used to determine cytokine and antibody baseline levels.

Serum cytokine concentrations were detected by using the BD Cytometric Bead Array Human Cytokine Kit (BD Biosciences, San Jose, CA, USA) and a BD FACSCalibur flow cytometer, according to the manufacturer's instructions. CD3 T-lymphocyte counts were <690 cells/mm^3 in 9 (32.1%) of 28 patients and represented <55% of total lymphocyte counts in 4 (14.3%) patients (Table). CD4 T-lymphocyte counts were <400 cells/mm^3 in 13 (46.4%) patients and represented <31% of total lymphocyte counts in 8 (28.6%) patients. CD8 T-lymphocyte counts were <190 cells/mm^3 in 3 (10.7%) patients. B-cell counts were <90 cells/mm^3 in 1 (3.6%) patient. Natural killer cells represented >27% of lymphocyte counts in 4 (14.3%) patients. Fourteen (50.0%) patients had a CD4:CD8 ratio less than the standard reference ratio of 1.4.

Flow cytometric results showing development of peripheral blood lymphocyte subsets during the disease course were divided into 3 groups on the basis of time of illness onset until date of blood sample collection: 1–3, 4–6, or 7–10 days. These groups were used because the longest time from onset of illness to hospitalization was 3 days for all patients, and the peak temperature for patients was observed 3 days after illness onset.

Mean counts and percentages of all T- and B-lymphocyte subsets increased after 3 days of illness compared with results obtained during the first 3 days of illness (Figure 1). However, the increase in CD8 and B cells was not significant. Another study showed a decrease in CD4, CD8, and B cells ≤2 days of symptom onset in patients with pandemic (H1N1) 2009 than in healthy persons (*4*). Our results show impaired adaptive immune responses and a gradual increase during recovery in mildly affected patients.

Table. Flow cytometric analysis of peripheral blood lymphocyte subsets for 28 patients with pandemic (H1N1) 2009 at hospitalization, China*

Lymphocyte subset	No. positive/no. tested (%)
CD45 <1,500 cells/mm^3†	21/28 (75.0)
CD3	
<690 cells/mm^3	9/28 (32.1)
<55%	4/28 (14.3)
CD4	
<400 cells/mm^3	13/28 (46.4)
<31%	8/28 (28.6)
CD8	
<190 cells/mm^3	3/28 (10.7)
<13%	0/28 (0)
B cells	
<90 cells/mm^3	1/28 (3.6)
<6%	0/28 (0)
Natural killer cells	
>590 cells/mm^3	1/28 (3.6)
>27%	4/28 (14.3)
Ratio of CD4:CD8 cells <1.4	14/28 (50.0)

*Lymphocyte subsets were defined as the following combinations: CD3+/CD45+ for CD3 T-lymphocytes; CD3+/CD4/+/CD45+ for CD4 T-lymphocytes; CD3+/CD8+/CD45+ for CD8 T-lymphocytes; CD19+/CD45+ for B-lymphocytes; and CD3+/CD(16+56)+/CD45+ for natural killer cells. †CD45 lymphocyte count was designated the total lymphocyte count.

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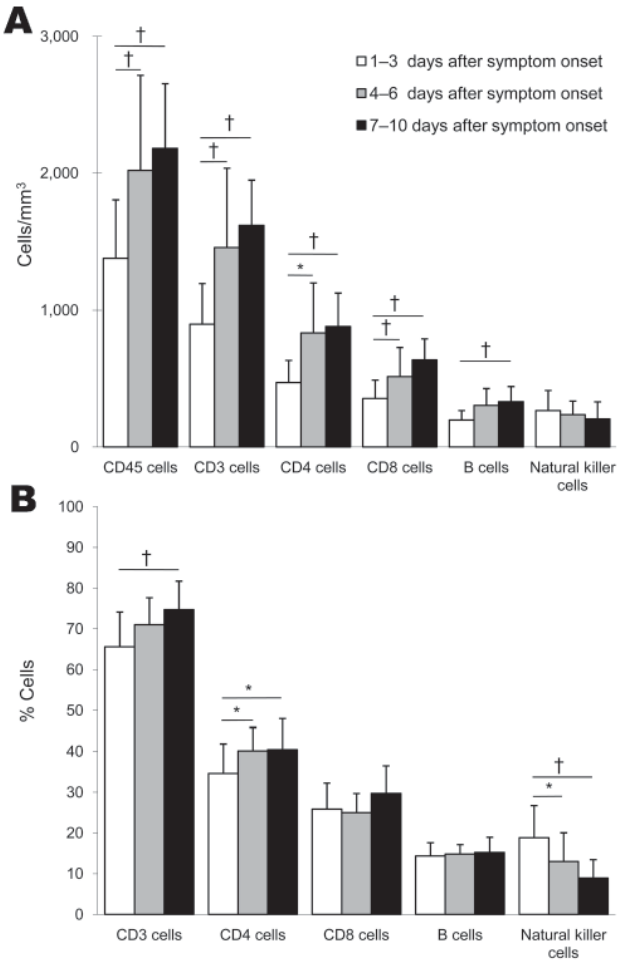

Figure 1. Flow cytometric analysis of peripheral blood lymphocyte subset counts of 28 patients with pandemic (H1N1) 2009, China. Counts and percentages are means. Error bars indicate SD. Each mean value was within the corresponding reference range. Lymphocyte subsets are as shown in the Table. A) Absolute count. B) Percentage of lymphocyte subset count compared with total lymphocyte count. *p<0.05; †p<0.01.

We measured serum cytokine concentrations and hemagglutination inhibition (HAI) antibody titers in patients during hospitalization and the follow-up period (Figure 2). We observed an increase in interleulin-6 (IL-6) levels 1 day after illness onset, which were 6.0-fold higher than the baseline level, and a 2.3-fold increase in interferon-γ (IFN-γ) levels. These levels decreased to baseline levels 5 days after illness onset, although the IL-6 level 5 days after illness onset was higher than levels 15 and 37 days after illness onset. The maximum IL-10 level 1 day after symptom onset was 3.2-fold higher than the baseline level. This level decreased to a value lower than the baseline level within 4 days, and then gradually increased to the baseline level 37 days after illness onset. Serum IL-6, IFN-γ, and IL-10 levels were not related to patient temperature 1 day after symptom onset, peak temperature during the disease, or period of fever. These levels showed minor differences that were not related to cough or sore throat in patients.

Only 1 patient had an HAI antibody titer ≥10 (titer 20) 1 day after illness onset. The HAI geometric mean titer increased 5 days after symptom onset compared with that 1 day after symptom onset and continued to increase until it reached a peak level of 137.9 at 37 days after symptom onset (25.5-fold increase). Peak HAI antibody titers ≥40 and ≥4-fold increases were observed in 27 (96.4%) patients.

Conclusions

Bermejo-Martin et al. reported increased serum levels of IL-6, IFN-γ, and tumor necrosis factor-α in patients with pandemic (H1N1) 2009 during the first 5 days after symptom onset; no difference in levels of these 3 cytokines was observed in patients with mild disease and controls (*5*). However, similar to another report (*4*), we detected increases of IL-6 and IFN-γ levels in patients with mild disease during the first 3 days after symptom onset. These different patterns may be caused by different intervals from time of symptom onset to date of sample collection (5 days vs. 3 days) because IL-6 and IFN-γ levels in our study quickly decreased to baseline levels ≤7 days after symptom onset. These results suggest that serum IL-6 and IFN-γ

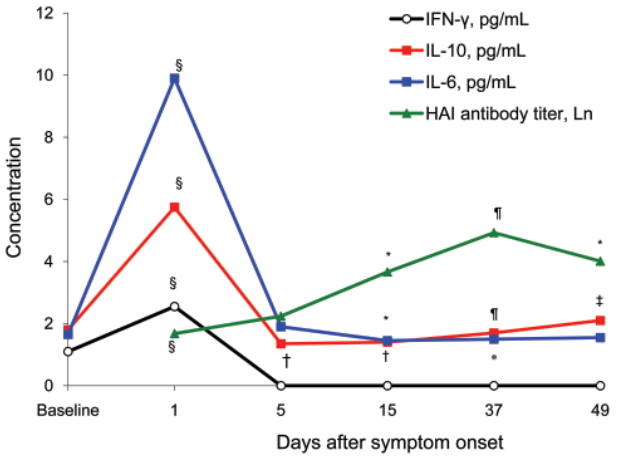

Figure 2. Serum cytokine concentrations and hemagglutination inhibition (HAI) antibody titers of 28 patients with pandemic (H1N1) 2009 during hospitalization and the follow-up period 15, 37, and 49 days after symptom onset, China. Serum concentrations of interferon-γ (IFN-γ), interleukin-10 (IL-10), and IL-6 are medians (pg/mL). Serum HAI antibody titers were transformed by using the natural logarithm and are shown as means. Baseline cytokine concentrations on the y-axis are values for healthy persons. *p<0.05 when IL-6 or HAI antibody levels were compared with those at day 5; †p<0.05 when IL-10 level was compared with those at baseline; ‡p<0.05 when IL-10 level was compared with those at days 5 or 15; §p<0.05 when value was compared with that at any other time point; ¶p<0.05 when value was compared with those at days 5, 15, or 49.

levels may be increased in patients with pandemic (H1N1) 2009 within the first 3 days after symptom onset, followed by a decrease to baseline levels ≤5 days after symptom onset in patients with mild disease or a continuous increase in severely affected patients.

IL-6 and IFN-γ are associated with antiviral immune responses during influenza infection (*6*–*8*). However, continuous, excessive release of IL-6 three days after illness onset likely contributed to serious pulmonary inflammation and tissue injury, as has been documented for severe acute respiratory syndrome and 1918 pandemic influenza, but this release could be tempered by production of IL-10 (*6*,*7*,*9*–*11*).

The proportion of persons 18–60 years of age with a ≥4-fold increase in HAI titer who received 1 dose (15 μg) of monovalent pandemic (H1N1) 2009 nonadjuvant vaccine was 96.2%, and the proportion with an increased HAI titer ≥40 was 97.1%, results similar to those of a recent study (*12*). However, the geometric mean titer in healthy vaccinated persons was 237.8, a 34.5-fold increase over the prevaccination titer, which was greater than that for patients naturally infected with pandemic (H1N1) 2009 virus (*12*). This finding may have resulted from impaired adaptive immune responses against pandemic (H1N1) 2009 virus in the initial phase, which included decreased numbers of CD4 and B lymphocytes and an increase in T regulatory cells (*4*).

In conclusion, our data indicated changes in cellular profiles during pandemic (H1N1) 2009 virus infection; showed that transient production of IL-6, IFN-γ, and IL-10 are main effectors of the early innate immune response against pandemic (H1N1) 2009 virus; and indicated that adaptive immune responses are impaired in the initial phase after infection. These factors may help clarify the pathogenesis of pandemic (H1N1) 2009 virus and provide new approaches in overcoming severe infections.

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Human Infection with Avian Influenza Virus, Pakistan, 2007

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Human infection with avian influenza (H5N1) virus raises concern for the possibility of a pandemic. We report 20 cases, which ranged from asymptomatic to fatal, in Pakistan in 2007. These cases indicate human-to-human-to-human transmission of this virus, and the number of cases may be higher than realized.

Evidence of human-to-human transmission of influenza A (H5N1) virus raises concern over a possible pandemic (*1*). Previous epidemiologic investigation of the outbreak of influenza (H5N1) among persons in the Northwest Frontier Province of Pakistan (Figure 1) in 2007 found 5 cases—3 confirmed, 1 asymptomatic, and 1 probable—as defined by the World Health Organization (WHO) (*2*). We report a larger set of 20 cases during this outbreak in Pakistan, supporting human-to-human-to-human transmission.

The Cases

Records were examined from all hospitals that treated patients with influenza (H5N1) virus in Northwest Frontier Province during 2007. Data were rendered anonymous and entered into a secure database with predetermined clinical and epidemiologic fields. Cases matching predefined criteria (Table 1) were classified as laboratory confirmed, likely, or possible. Cases not meeting classification criteria were excluded. We slightly modified WHO criteria to resemble criteria that clinicians might adopt during an actual outbreak, especially in a resource-poor setting (*3*).

We identified 20 cases—4 laboratory confirmed, 7 likely, and 9 possible—resulting in a ratio of 4 likely/possible cases for each laboratory-confirmed case. Median age was 29 years (range 7–60 years) for all patients and 30 years (range 23–35 years) for confirmed case-patients; 16 (80%) patients were male. The infecting exposure could not be established for all patients because multiple exposures, human and avian, were recorded for some. Of the 4 patients with laboratory-confirmed cases, 3 were treated with oseltamivir (2 [67%] of whom survived), and 1 had asymptomatic disease and received no antiviral treatment.

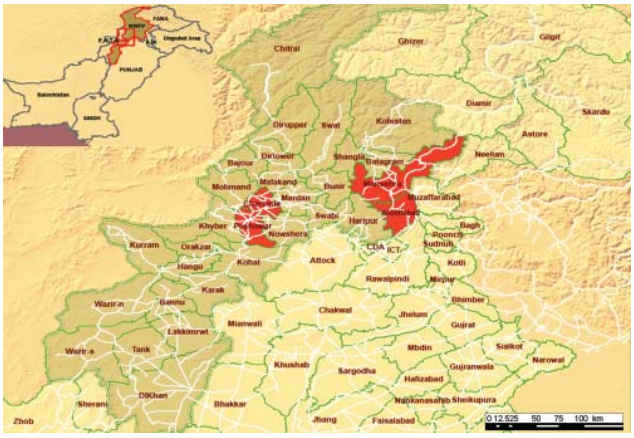

Figure 1. Areas of influenza (H5N1) cases in humans, Pakistan, 2007. Red shading indicates districts that reported suspected human cases of influenza (H5N1). Light brown shading indicates Northwest Frontier Province. Source: World Health Organization (WHO). Districts of avian influenza suspected cases in Northwest Frontier Province, Pakistan. WHO map no. WHO-PAK-002 (www.whopak.org/disaster).

Table 1. Case classification definitions used to diagnose influenza (H5N1) infection in humans, Pakistan, 2007*

Classification	Definition
Laboratory confirmed	Laboratory confirmation of influenza (H5N1) virus at local/regional or World Health Organization confirmatory laboratory
Likely	
Definition 1	Epidemiologically linked by time, place, and exposure to a likely or confirmed human or avian influenza (H5N1) case AND Equivocal test OR positive laboratory confirmation of an influenza A virus infection but insufficient laboratory evidence for influenza (H5N1) virus infection AND Clinical signs or symptoms consistent with disease (regardless of severity): fever or flu-like
Definition 2	Epidemiologically linked by time, place, and exposure to a likely or confirmed influenza (H5N1) case-patient AND Death due to unexplained acute respiratory illness AND Negative test/test not performed
Possible	Epidemiologically linked by time, place and exposure to a likely or confirmed human or avian influenza (H5N1) case AND Test not performed/negative test AND Clinical signs and symptoms consistent with disease (regardless of severity): fever or flu-like
Noncase	Confirmed positive for non-H5N1 influenza A virus subtype OR Patient condition determined to have etiology other than avian influenza

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Table 2. Clinical characteristics for persons with reported cases of influenza (H5N1), Pakistan, 2007

Clinical sign or symptom	Diagnostic certainty, no./total (%)		
	Laboratory confirmed	Likely	Possible
Respiratory			
Abnormal breath sounds (wheezing, rales, stridor, rhonchi)	1/2 (50)	1/5 (20)	2/5 (40)
Excessive sputum production	0/1 (0)	0/4 (0)	2/6 (33)
Rhinorrhea/nasal discharge	1/2 (50)	1/4 (25)	1/5 (20)
Unexplained respiratory illness with cough, shortness of breath, or difficulty breathing	1/3 (33)	4/7 (57)	7/9 (78)
Sore throat/pharyngitis	1/1 (100)	0/4 (0)	3/6 (50)
Tachypnea	0/2 (0)	1/5 (20)	1/5 (20)
Cyanosis	0/1 (0)	0/4 (0)	1/3 (33)
Chest pain	2/2 (100)	1/1 (100)	3/5 (60)
Pleural effusion	0	0	1/3 (33)
Hemoptysis	0	0	1/1 (100)
Orthopnea	0	0	0 (0)
Gastrointestinal			
Diarrhea	0/1 (0)	1/4 (25)	2/5 (40)
Abdominal pain	0	0	0
Vomiting	0/4 (0)	0/7 (0)	1/9 (11)
Rectal bleeding	0	0	0/1 (0)
Other			
Fever	3/4 (75)	7/7 (100)	6/9 (89)
Headache	2/3 (67)	2/4 (50)	2/5 (40)
Body aches	1/1 (100)	0	1/1 (100)
Backache	0	0	1/1 (100)
Pericardial effusion	0	0	0/1 (0)
Nonpitting pedal edema	0	0	0/1 (0)
Fatigue or malaise	0/4 (75)	0/7 (0)	1/9 (11)
Myalgia	0/1 (0)	1/3 (33)	2/4 (50)
Tachycardia	0	1/1 (100)	0

Signs and symptoms were mainly those of a febrile influenza-like illness (Table 2), although 1 patient with a laboratory-confirmed case was asymptomatic (microneutralization titer 320, Western blot positive, throat swab positive for H5 by reverse transcription–PCR); this case-patient was also described in a previous epidemiologic investigation (*2*). Gastrointestinal signs and symptoms were not prominent, and neurologic signs were not reported.

The first 8 cases constituted a cluster (Figure 2). The index case-patient (patient 1) had culled influenza (H5N1) virus–infected poultry. After becoming febrile (38°C) while in Abbottabad, he traveled by public transportation to his family home in Peshawar. His illness progressed and on November 5, 2007, he was admitted to Khyber Teaching Hospital, where the diagnosis of influenza (H5N1) infection was made. Infection appeared to spread initially from household family contacts (patients 2–6) to medical staff (patient 7, who had positive PCR but negative microneutralization test results) and to a frequent visitor to the intensive care unit (patient 8).

As previously noted (*2*), the extended period from the time persons were exposed to the index case-patient, during which family members became ill, points to human-to-human-to-human transmission; patient 2 probably accounted for intermediary or second-generation infection. The chain of infection illustrated in Figure 2 suggests that further human-to-human-to-human transmission might have occurred and suggests nosocomial transmission. Of note, patient 6 (a cousin of the index case-patient) had a microneutralization titer of 80 but a negative Western blot result. Although 4 contacts of patient 6 exhibited no signs or symptoms of influenza, they did have positive H5 microneutralization titers ranging from 80 to 160.

No evidence epidemiologically links the remaining 12 patients to the 8 patients in the cluster; each of the 12 either had direct contact with influenza (H5N1) virus–infected poultry or was near healthy or diseased poultry before symptom onset. Three patients worked on poultry farms: 1 had taken a sample from an influenza (H5N1) virus–infected chicken, 1 was directly involved in culling, and 1 was indirectly exposed to live poultry. Eight patients had negative test results for influenza (H5N1) virus, and 3 had positive results from the National Institute of Health Islamabad but negative confirmatory-testing results from WHO; 1 patient died before samples could be taken. Different laboratories reported conflicting results with respect to confirmation of infection, possibly because of the difficulties of complying with specimen-handling

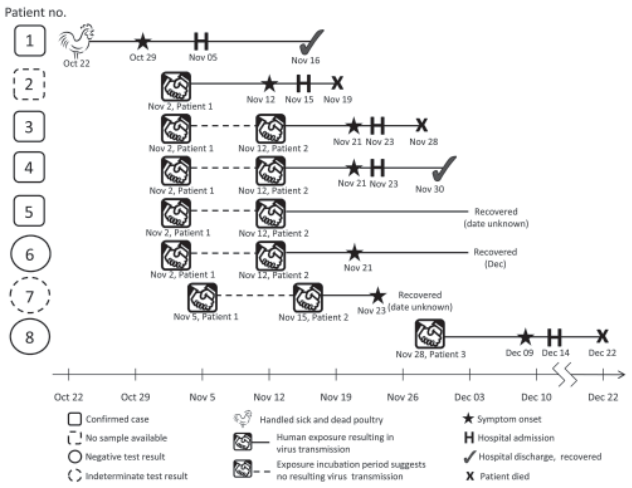

Figure 2. Path of infection of influenza (H5N1), Pakistan, 2007. During October 22–30, patient 1 worked culling infected chickens; on November 2, he moved home and had contact with 4 brothers (patients 2–5) and possibly a cousin (patient 6). He was hospitalized on November 5 and transferred to an intensive care unit the next day. His cousin cared for him and became patient 6; his attending doctor became patient 7. On November 23, patient 3 was hospitalized and on November 28 was transferred to an intensive care unit; during this time, patient 8 frequently visited his wife in the same intensive care unit.

requirements in resource-poor settings. Clinical details of these cases are shown in Table 2.

Conclusions

The preponderance of male patients is probably explained by sociocultural factors; the index case-patient was a poultry culler, a male-dominated task, and shared accommodation with male family members. Health care–seeking behavior may also account for this finding.

The human-to-human transmission from the index case-patient to at least some household contacts seems clear, and the extended period over which these contacts became ill supports subsequent human-to-human transmission. Figure 2 supports the conclusion that patient 2 initiated a chain of infection in which further human-to-human transmission to patients 7 and 8 occurred. Possible nosocomial transmission is of concern because full implementation of isolation procedures in resource-poor settings may be problematic.

Although virologically supported probable human-to-human transmission of influenza (H5N1) virus has been documented, it has been thought to occur only with prolonged and close contact (*4*). Household clustering and the difficulty of establishing exact virus exposures have encumbered efforts to investigate possible human-to-human transmission (*5*). Modeling has (*6*) suggested human-to-human transmission in Indonesia, but the utility of statistical modeling unsupported by field data has been questioned (*7*).

Although the index case-patient traveled by public transportation from Abbottabad, where he acquired his infection, no infections were reported for anyone other than household contacts, who were all related and exposed at his family home at Peshawar. In contrast, patients 2 and 6 might have spread infection through less intimate contact, which raises 2 questions. Might some persons shed virus more efficiently than others, possibly in greater quantity? And what role might host factors play in susceptibility to influenza (H5N1) virus infection and disease? A degree of virus adaptation to humans might also have occurred, although absence of sustained community transmission argues against this possibility.

Of concern is the 4:1 ratio of likely/possible to laboratory-confirmed cases, suggesting that official tallies understate true incidence of infection. Factors that may contribute to undercounting are the difficulty of obtaining virologic confirmation or of storing and transporting samples in resource-poor settings and reluctance by relatives to consent to autopsy. Another reason to believe that less fulminant cases may go unreported is the occurrence in Pakistan, and elsewhere, of clinically mild and asymptomatic cases (*5*,*8*–*14*), indicating that influenza (H5N1) virus may cause a spectrum of illness. The demonstration during the 1997 Hong Kong outbreak of influenza (H5N1) with seroconversion in apparently asymptomatic health care workers and social contacts suggests human-to-human transmission, although in Hanoi, no transmission to health care workers was detected (*8*,*13*,*15*). Also contributing to underreporting are the predominant clinical signs of undifferentiated influenza-like illness observed in Pakistan and elsewhere, which, unless clinical deterioration occurred, would be unremarkable in many tropical settings. Although the survival rate was greater for patients who received oseltamivir, the small number of patients and the inclusion of those with mild and asymptomatic illness prevent meaningful statistical comparison.

Several features of the outbreak are unusual or give cause for concern: human-to-human-to-human transmission, possible nosocomial transmission, occurrence of mild and asymptomatic cases, and difficulties of establishing laboratory confirmation of likely and possible cases (which also prevented genotypic matching of specimens from primary and putative secondary cases). Taken together, these features suggest that current surveillance might undercount the extent of human infection with influenza (H5N1) virus and that human-to-human transmission might possibly be associated with less severe disease.

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Novel Reassortant Highly Pathogenic Avian Influenza (H5N5) Viruses in Domestic Ducks, China

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In China, domestic ducks and wild birds often share the same water, in which influenza viruses replicate preferentially. Isolation of 2 novel reassortant highly pathogenic avian influenza (H5N5) viruses from apparently healthy domestic ducks highlights the role of these ducks as reassortment vessels. Such new subtypes of influenza viruses may pose a pandemic threat.

Aquatic birds are considered the natural reservoir for influenza A viruses of all known 16 hemagglutinin (HA) and 9 neuraminidase (NA) subtypes (*1*). Influenza viruses replicate preferentially in the cells lining the intestinal tracts of wild waterfowl, usually causing no clinical signs. Excretion of substantial amounts of virus in the feces can infect wild and domestic birds by waterborne transmission (*1*). In the People's Republic of China, domestic ducks raised in the traditional free-range system often share water with wild aquatic birds. Moreover, domestic ducks are often in close contact with poultry, livestock, and humans in the same village or farm. Therefore, domestic ducks play a major role in the ecology of influenza viruses (*2*) and can act as potential vessels for genetic reassortment (*3*). Systematic surveillance of influenza viruses in domestic ducks could provide timely and valuable epidemiologic information and should be continued.

The Study

As part of routine surveillance for avian influenza viruses from December 2008 through January 2009 in eastern China, tracheal and cloacal swab samples from apparently healthy domestic ducks in live poultry markets were collected for virus isolation and identification as described (*4*). From these samples, 2 influenza (H5N5) viruses—A/duck/eastern China/008/2008 (008 [H5N5]) and A/duck/eastern China/031/2009 (031 [H5N5])—were detected in mallard ducks (*Anas platyrhynchos*).

These 2 viruses grew efficiently in eggs and in MDCK cells, each with virus titers >8 $\log_{10}$ 50% egg infectious dose (EID_{50})/mL or 8 $\log_{10}$ 50% tissue culture infectious dose/mL (Table 1). The intravenous pathogenicity index for chickens and 50% lethal dose for mice were 2.6 and $10^{4.0}$ EID_{50} for 008 (H5N5), 2.5 and $10^{5.4}$ EID_{50} for 031 (H5N5), respectively (Table 1). Therefore, both novel influenza subtype H5N5 viruses were assumed to be highly pathogenic for chickens and moderately virulent for mice (*5*).

When mice were inoculated with a sublethal dose of $10^{3.5}$ EID_{50}, each influenza subtype H5N5 virus was able to replicate without prior adaptation. The highest virus titers were detected in the mouse lung. The viruses were able to spread to the brain and heart. Furthermore, influenza virus 008 (H5N5) was isolated from the spleen and liver, and influenza virus 031 (H5N5) was detected in the kidney (Table 1). Microscopic findings in infected mice were interstitial pneumonia with various amounts of erythrocytes in alveolar lumens, hyperemia and lymphocyte infiltration of meningeal veins and cardiac muscles, low numbers of lymphocytes in periarterial lymphatic sheaths and macrophage recruitment in the spleen, lymphocyte infiltration in the liver, and slight congestion in the renal cortex and glomerulus (data not shown).

Genomic analysis showed that the influenza viruses 008 (H5N5) and 031 (H5N5) were highly homologous with each other, sharing 99.2%–99.7% nt identities among the 8 gene segments except for the polymerase acidic protein (PA) gene (94.1%). Another 3 viruses isolated from the same surveillance study— A/duck/eastern China/108/2008 of H5N1 subtype (108 [H5N1]), A/duck/eastern China/909/2009 of H5N1 subtype (909 [H5N1]), and A/duck/Yangzhou/013/2008 of H6N5 subtype (013 [H6N5])—were more closely related to the novel influenza subtype H5N5 viruses than were those in GenBank (Table 2).

As outlined by the World Health Organization/World Organisation for Animal Health/Food and Agriculture Organization unified nomenclature system for subtype H5N1 highly pathogenic avian influenza (HPAI) viruses (*6*), the HA genes of the 2 subtype H5N5 viruses were classified into clade 2.3.4 (Figure 1, panel A), which has been the prevalent lineage in southern China since 2005 (*7*–*9*). In addition to the typical residues Q226 and G228 in HA, which confer receptor preference for SAα2,3Gal, influenza viruses 008 (H5N5) and 031 (H5N5) simultaneously carried an S227R mutation in the receptor-binding pocket. A recent report (*10*) indicated that the S227N substitution accompanied with deglycosylation at residue 158 could substantially increase the affinity of HA for SAα2,6Gal without reducing its binding affinity for SAα2,3Gal.

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Table 1. Characteristics of 2 novel avian influenza (H5N5) viruses isolated from domestic ducks, China, December 2008–January 2009*

Virus	Characteristics				Tissue	Virus replication in experimentally infected mice, no. virus-positive mice/no. tested mice (mean titer ± SD)†				
	IVPI	EID_{50}	$TCID_{50}$	MLD_{50}‡		dpi 2	dpi 4	dpi 6	dpi 8	dpi 10
008	2.6	$10^{8.5}$	$10^{8.6}$	$10^{4.0}$	Lung	1/2 (3.2 ± 0)	2/2 (3.8 ± 0.4)	2/2 (4.7 ± 0.4)	2/2 (4.4 ± 0.5)	0/2
					Brain	0/2	0/2	0/2	2/2 (3.8 ± 1.2)	1/2 (3.4 ± 0)
					Heart	1/2 (2.0 ± 0)	2/2 (2.6 ± 0.5)	2/2 (3.1 ± 0.1)	0/2	0/2
					Spleen	0/2	0/2	0/2	0/2	1/2 (2.3 ± 0)
					Liver	0/2	0/2	1/2 (2.2 ± 0)	0/2	0/2
					Kidney	0/2	0/2	0/2	0/2	0/2
031	2.5	$10^{8.3}$	$10^{8.5}$	$10^{5.4}$	Lung	1/2 (3.0 ± 0)	1/2 (3.3 ± 0)	2/2 (4.1 ± 0.2)	2/2 (4.5 ± 0.3)	1/2 (2.6 ± 0)
					Brain	0/2	0/2	0/2	1/2 (3.4 ± 0)	0/2
					Heart	0/2	1/2 (2.5 ± 0)	1/2 (3.0 ± 0)	0/2	0/2
					Spleen	0/2	0/2	0/2	0/2	0/2
					Liver	0/2	0/2	0/2	0/2	0/2
					Kidney	0/2	0/2	1/2 (2.1 ± 0)	0/2	0/2

*EID_{50}, 50% egg infectious dose; IVPI, intravenous pathogenicity index (determined in chickens); $TCID_{50}$, 50% tissue culture infectious dose (determined in MDCK cells); MLD_{50}, 50% lethal dose in mice (expressed as the EID_{50} value corresponding to 1 LD_{50}); dpi, day postinoculation; 008, A/duck/eastern China/008/2008; 031, A/duck/eastern China/031/2009.
†Mice were inoculated with $10^{3.5}$ EID_{50}; 2 infected mice were euthanized every other day for virus detection. Virus titers were expressed as $\log_{10}$ EID_{50}/g.
‡Influenza A viruses with MLD_{50} <$10^{3.0}$ EID_{50} were considered of high pathogenicity, MLD_{50} >$10^{6.5}$ EID_{50} were considered of low pathogenicity (*4*).

Whether this S227R variation with the changed residual polarity affects the receptor-binding property deserves further investigation.

To construct the NA tree, we retrieved 90 complete N5 sequences from GenBank, including the only 2 influenza subtype H5N5 viruses from the United States: A/mallard/MN/105/2000 and A/duck/Massachusetts/sg-00440/2005. The N5 viruses were grouped into 2 lineages—North American and Eurasian—in accordance with their geographic distribution (Figure 1, panel B). The 2 subtype H5N5 isolates from China belonged to the Eurasian lineage, whereas the 2 from the United States clustered within the North American lineage. In addition, 1 aa deletion at residue 42, located in the stalk region of NA, was identified in all Eurasian, but not in the North American, strains.

Although the PA genes of influenza viruses 008 (H5N5) and 031 (H5N5) diverged to assemble respectively with influenza viruses 013 (H6N5) and 108 (H5N1), the novel influenza subtype H5N5 viruses aggregated closely with recent Eurasian subtype H5N1 viruses, especially influenza viruses 108 (H5N1) and 909 (H5N1) in the trees of HA (Figure 1, panel A), polymerase basic protein (PB) 2, PB1, nucleocapsid protein, matrix protein, and nonstructural protein genes (online Technical Appendix, www.cdc.gov/EID/content/17/6/1060-Techapp.pdf). For NA, spatiotemporal correlation indicates that influenza

Table 2. Influenza viruses with highest nucleotide identity to each gene of 008 and 031*

Gene segment		Closest viruses in GenBank		Closest viruses isolated during surveillance, China, December 2008–January 2009	
		Strain	Nucleotide identity, %	Strain	Nucleotide identity, %
PB2	008	A/duck/Guangxi/xa/2001 (H5N1)	96.0	108	100
	031		95.9	909	99.8
PB1	008	A/duck/Hokkaido/Vac-1/04 (H5N1)	97.2	031	99.5
	031		97.1	909	99.7
PA	008	A/chicken/Hunan/8/2008 (H5N1)	98.1	013	99.7
	031	A/wild duck/Hunan/021/2005 (H5N1)	98.0	108	99.5
HA	008	A/wild duck/Hunan/211/2005 (H5N1)	97.7	108	99.9
	031		97.8	008 and108	99.4
NP	008	A/chicken/Jilin/hk/2004 (H5N1)	96.1	108	99.8
	031		96.5	108	99.6
NA	008	A/mallard/Switzerland/WV4060167/2006 (H3N5)	95.8	031	99.2
	031			008	99.2
M	008	A/China/GD01/2006 (H5N1)	99.1	031 and 909	99.8
	031			909	100
NS	008	A/wild duck/Hunan/021/2005 (H5N1)	97.9	108	99.5
	031		98.2	909	99.9

*PB, polybasic protein; 008, A/duck/eastern China/008/2008(H5N5); 108, A/duck/eastern China/108/2008(H5N1); 031, A/duck/eastern China/031/2009(H5N5); 909, A/duck/eastern China/909/2009(H5N1); 013, A/duck/Yangzhou/013/2008(H6N5); PA, polymerase acidic protein; HA, hemagglutinin; NP, nucelocapsid protein; NA, neuraminidase; M, matrix protein; NS, nonstructural protein.

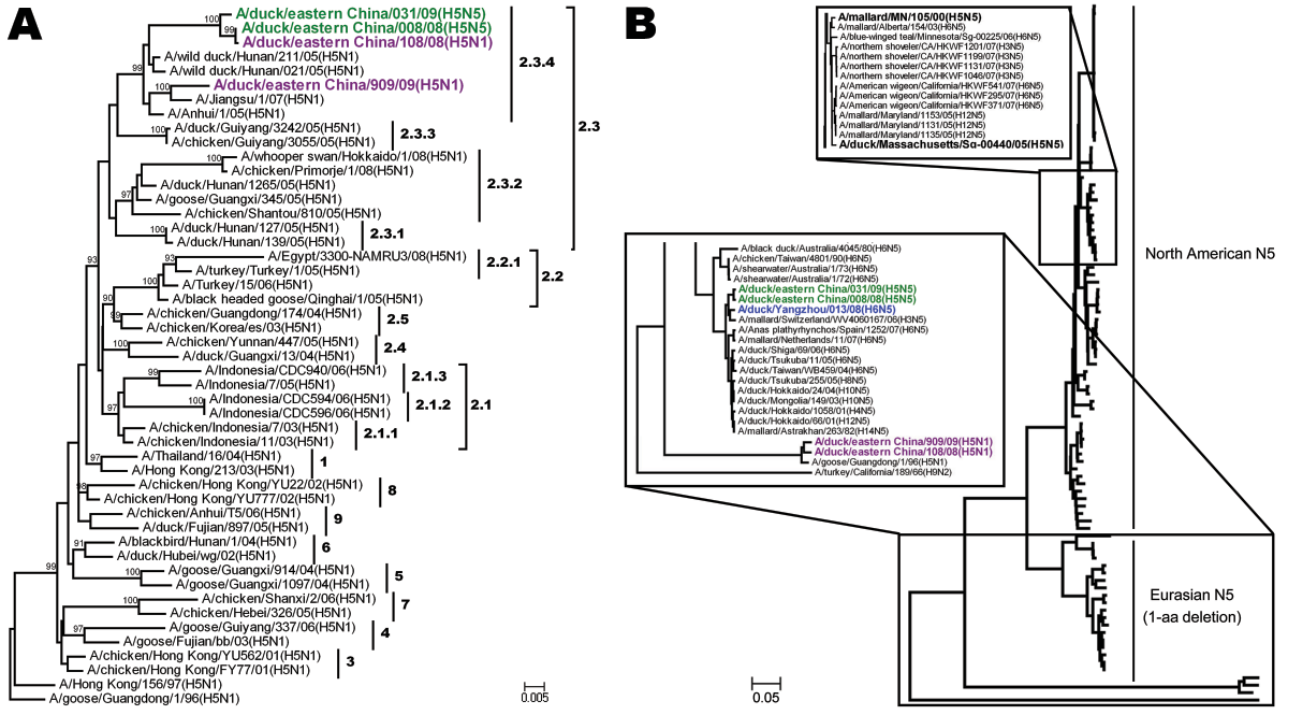

Figure 1. Phylogenetic trees of hemagglutinin (A) and neuraminidase (B) genes of novel avian influenza (H5N5) viruses isolated from domestic ducks in the People's Republic of China, December 2008–January 2009, with reference sequences. Green, A/duck/eastern China/008/2008 (H5N5) and A/duck/eastern China/031/2009 (H5N5); purple, A/duck/eastern China/108/2008 (H5N1) and A/duck/eastern China/909/2009 (H5N1); blue, A/duck/Yangzhou/013/2008 (H6N5); **boldface**, other H5N5 influenza viruses available from GenBank. Inset boxes in panel B indicate correspondence between thumbnail and panorama of related perspectives. Trees were generated by applying the neighbor-joining method in MEGA 4.0 (www.megasoftware.net) on the basis of full-length coding sequences. Numbers above or below the branch nodes indicate bootstrap values. Scale bars indicate branch length based on number of nucleotide substitutions per site.

virus 013 (H6N5), rather than its phylogenetically equidistant counterpart A/mallard/Switzerland/WV4060167/2006(H3N5), might be the N5 donor (Figure 1, panel B). However, because of the relatively low (95%) sequence similarity, it is also possible that Eurasian viruses of an unidentified HA subtype (H?N5), not detected in our epidemiologic survey, could provide the NA genes. Therefore, we speculate that influenza viruses 008 (H5N5) and 031 (H5N5) may be reassortants between contemporary Eurasian subtype H5N1 and some subtype H?N5 and/or H6N5 avian influenza viruses with distant evolutionary relationship with the 2 subtype H5N5 viruses from the United States (Figure 1; online Technical Appendix). In addition, regarding the especially high sequence identities of PB1, HA, nucleocapside protein, NA, and matrix protein genes exclusively between influenza viruses 008 (H5N5) and 031 (H5N5), the possibility that the 2 subtype H5N5 viruses donated some gene segments to each other cannot be excluded (Figure 2).

Conclusions

The 2 novel HPAI (H5N5) viruses isolated and characterized in this study are most likely reassortants of recent Eurasian viruses sharing approximate spatiotemporal distribution. It is less likely that they were introduced through intercontinental transmission of subtype H5N5 strains from North America. Considering the endemicity that clade 2.3.4 subtype H5N1 viruses have gained in China since 2005 (*7*–*9*), it is plausible that subtype H5N1 viruses have provided the backbone for generating the novel subtype H5N5 viruses instead of the opposite gene flow.

Ducks have been considered "Trojan horses" for influenza (H5N1) because of their pivotal role in virus propagation and evolution (*11*–*13*). In our study, the 2 reassortant influenza viruses (008 [H5N5] and 031 [H5N5]) and their 3 possible parent viruses (108 [H5N1], 909 [H5N1], and 013 [H6N5]) were all isolated from apparently healthy domestic ducks. We speculate that domestic ducks may serve as reassortant vessels for creating new subtypes of influenza viruses. In view of the practice of raising ducks in a free-range system, these novel strains could be transmitted to other domestic poultry and even humans. There is evidence that these subtype H5N5 viruses have been transmitted to terrestrial poultry (Zhao et al., unpub. data). Thus, the role of domestic ducks in the influenza virus ecosystem should not be neglected. Systematic

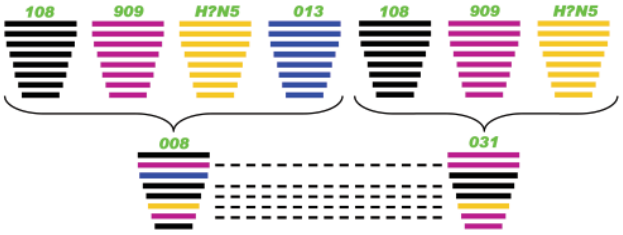

Figure 2. Putative genomic compositions of the novel avian influenza (H5N5) viruses isolated from domestic ducks in the People's Republic of China, December 2008–January 2009, with their possible donors. The 8 gene segments (from top to bottom) in each virus are polymerase basic protein 2, polymerase basic protein 1, polymerase acidic protein, hemagglutinin (HA), nucleocapsid protein, neuraminidase, matrix protein, and nonstructural protein. Each color indicates a separate virus background. Dashed lines indicate high sequence identity and suggest a second possibility that the 2 influenza (H5N5) viruses could be donors of some gene segments for each other. 108, A/duck/eastern China/108/2008 (H5N1); 909, A/duck/eastern China/909/2009 (H5N1); 008, A/duck/eastern China/008/2008 (H5N5); 031, A/duck/eastern China/031/2009 (H5N5); 013, A/duck/Yangzhou/013/2008 (H6N5). H?N5 denotes possible parental viruses of unidentified HA subtype but N5 subtype. The simplified schematic illustration is based on nucleotide-distance comparison and phylogenetic analysis.

surveillance should be instituted to identify emerging HPAI (H5N5) viruses and to reduce their potential threat to animal and human health.

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Multidrug-Resistant *Acinetobacter baumannii* Harboring OXA-24 Carbapenemase, Spain

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In February 2006, a patient colonized with a multidrug-resistant sequence type 56 *Acinetobacter baumannii* strain was admitted to a hospital in Madrid, Spain. This strain spread rapidly and caused a large outbreak in the hospital. Clinicians should be alert for this strain because its spread would have serious health consequences.

The increasing resistance of *Acinetobacter baumannii* to antimicrobial drugs, including carbapenems (*1*–*3*), and resistance to desiccation and disinfectants (*4*) contribute to its persistence in hospital environments and propensity to cause outbreaks (*5*,*6*). In February 2006, a patient colonized with a multidrug-resistant *A. baumannii* strain was admitted to the medical–surgical intensive care unit (ICU) of a hospital in Madrid, Spain. This strain then spread rapidly, persisted for ≥30 months, and caused a large outbreak in the hospital. We report details of this outbreak.

The Study

We conducted a retrospective longitudinal study at 12 de Octubre University Hospital, Madrid, Spain, of patients colonized/infected with *A. baumannii* during January 2006–May 2008. We also conducted a cohort study of patients with *A. baumannii* bacteremia during January 2002–May 2008.

MICs of drugs were confirmed by using Etest strips (AB Biodisk, Solna, Sweden) according to the manufacturer's criteria. Multidrug-resistant (MDR) phenotypes were defined as resistance to 5 classes of drugs: antipseudomonal cephalosporins (ceftazidime, cefepime), carbapenems (imipenem, meropenem), piperacillin/tazobactam, fluoroquinolones, and aminoglycosides (gentamicin, tobramycin, amikacin). Isolates were classified on the basis of antimicrobial susceptibility patterns: antibiotype 1, MDR isolates; antibiotype 2, isolates resistant to carbapenems but not MDR; and antibiotype 3, isolates susceptible to carbapenems. Colonization was defined as isolation of *A. baumannii* from ≥1 clinical specimen in the absence of clinical symptoms consistent with infection. Bacteremia was determined by application of criteria proposed by the Centers for Disease Control and Prevention (Atlanta, GA, USA) (*7*).

Clonal relatedness between clinical isolates was determined by using pulsed-field gel electrophoresis (PFGE) and the CHEF DRIII system (Bio-Rad Laboratories, Hercules, CA, USA) according to reported techniques (*8*). Migration of DNA fragments was normalized, and computer-assisted analysis of PFGE patterns was conducted by using Bionumerics software (Applied Maths, Sint-Martens-Latem, Belgium). Multilocus sequence typing (MLST) was performed according to published protocols (*9*). Isolates were assigned to a sequence type according to the allelic profiles database (http://pubmlst.org/abaumannii/). Univariate analysis was performed by using the *t* test for continuous variables and the χ^2 or Fisher exact tests for categorical variables. Adjusted odds ratios (ORs) were calculated by using logistic regression analysis. Data were analyzed by using SPSS software (SPSS Inc., Chicago, IL, USA). A p value <0.05 was considered significant.

During January 2006–May 2008, a total of 377 patients were colonized/infected with *A. baumannii.* Mean age of the patients was 57 years and 63.4% were men. Patients were hospitalized mostly in ICUs (184, 48.8%), and in surgical (100, 26.5%), medical (85, 22.5%), and pediatric (8, 2.1%) wards. A total of 76.9% (290/377) of the isolates were antibiotype 1, 9.0% (34/377) were antibiotype 2, and 14.1% (53/377) were antibiotype 3. Temporal distribution of cases is shown in Figure 1, panel A. Bacterial isolates of antibiotype 1 were assigned to the major clonal type (clone AbH12O-A2) by PFGE. Of 290 patients with *A. baumannii* antibiotype 1 isolates (clone AbH12O-A2), 165 patients were infected (57%) and 125 (43%) were colonized.

MLST analysis of 3 isolates belonging to clone AbH12O-A2 was performed to determine the relationship between these isolates and other described strains. The 3 isolates showed the same allelic profile of 7 housekeeping genes (allele no. in brackets; *gltA* [1], *gyrB* [18], *gdhB* [18], *recA* [10], *cpn60* [14], *gpi* [29], and *rpoD* [18]) and were identified as sequence type 56 according to the MLST database (http://pubmlst.org/abaumannii/).

A. baumannii clone AbH12O-A2, which showed a broad antimicrobial drug-resistance profile, resistance to carbapenems, and susceptibility only to tigecycline and colistin, was present throughout the entire 30-month study

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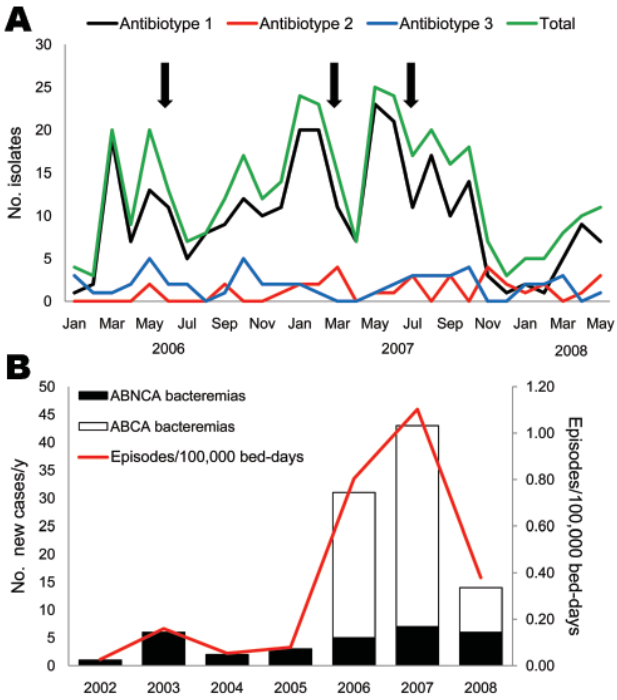

Figure 1. Temporal distribution of patients with *Acinetobacter baumannii* infections, Spain. A) Patients colonized/infected with *A. baumannii* classified by antibiotype. Arrows indicate times of intensification of infection control measures. The medical–surgical intensive care unit at Octubre University Hospital, Madrid, Spain, was refurbished in July 2007. B) Annual incidence of *A. baumannii* bacteremia. ABCA, *A. baumannii* clone A or AbH12O-A2; ABNCA: *A. baumannii* nonclone A.

and peaked several times until the medical–surgical ICU was refurbished in July 2007. The number of new case-patients with clone AbH12O-A2 then decreased; ≤3 cases/month were observed during October 2007–February 2008 (Figure 1, panel A).

Annual incidence of *A. baumannii* bacteremia increased from 0.03 episodes/100,000 bed days in 2002 to 1.1/100,000 bed days in 2007 (Figure 1, panel B), which coincided with the outbreak peak caused by clone AbH12O-A2. Clinical features of patients with *A. baumannii* bacteremia are shown in Table 1. Multivariate analysis of bacteremia caused by clone AbH12O-A2 and nonclone AbH12O-A2 showed that variables independently associated with AbH12O-A2 bacteremia were hospitalization in ICUs (OR 3.48, 95% confidence interval [CI] 1.23–9.54), exposure to ≥3 antimicrobial drugs (OR 3.13, 95% CI 1.12–8.76), and ventilator-associated pneumonia as the source of bacteremia (OR 8.35, 95% CI 1.12–8.76).

Plasmid pMMA2 (GenBank accession no. GQ377752), which was isolated from the clone causing the outbreak (AbH12O-A2), harbored a bla_{OXA-24} gene (*10*) coding for carbapenemase OXA-24 (also called OXA-40) as described (*11*). Four additional clones were detected during the outbreak (AbH12O-D, AbH12O-CU1, AbH12O-CU2, and AbH12O -CU3), which harbored plasmids pMMD, pMMCU1, pMMCU2, and pMMCU3, respectively (GenBank accession nos. GQ904226, GQ342610, GQ476987, and GQ904227). Carbapenem resistance in all clones was linked to a plasmid harboring the bla_{OXA-24} gene flanked by XerC/XerD-like recombination sites (*11*). Comparative analysis among plasmid sequences showed different patterns and coding regions. All plasmids, including pMMA2, harbored the bla_{OXA-24} gene as part of a DNA module flanked by XerC/XerD-like sites, which suggested that these sites are involved in mobilization of DNA containing the bla_{OXA-24} gene by site-specific recombination (*11*).

Two genes with a putative role in virulence were detected in plasmids from clones AbH12O-A2 and AbH12O-CU3 upstream of bla_{OXA-24}: a septicolysin-like gene coding for a pore-forming toxin (*12*), and a TonB-dependent receptor gene coding for an outer membrane protein involved in iron uptake and virulence (*13–15*). Insertion sequence 4, which provided an additional promoter sequence, was detected upstream from the septicolysin gene in plasmid pMMA2;

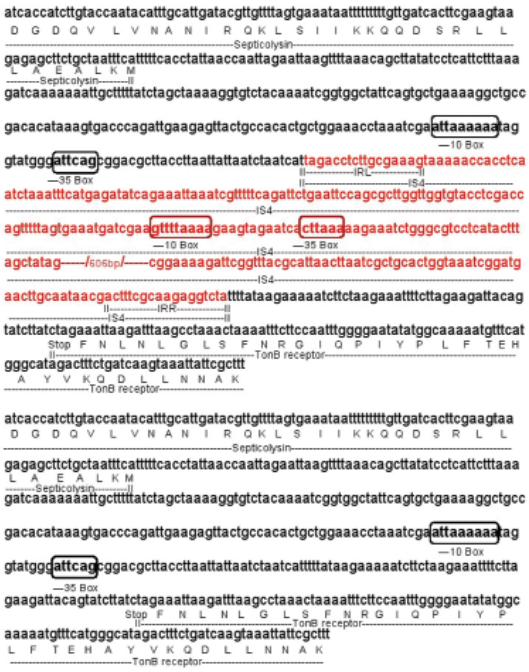

Figure 2. Nucleotide sequence of the region between the septicolysin and Ton-B dependent receptor genes of *Acinetobacter baumannii* in plasmids pMMA2 and pMMCU3 from clone AbH12O-A2 (upper panel) and AbH12O-CU3 (lower panel), respectively. Integrated insertion sequence 4 (IS4) (red letters) provided a new promoter sequence for septicolysin in plasmid pMMA2 from clone AbH12O-A2. Upper case letters indicate amino acids. IRL, inverted repeated left sequence; IRR, inverted repeated right sequence from IS4; Stop, stop (termination) codon.

Table 1. Clinical characteristic of patients with *Acinetobacter baumannii* bacteremia, Spain*

Characteristic	Clone ABCA, n = 65	Clone ABNCA, n = 29	p value	OR (95% CI)
Age, y	57.5 ± 14.2	58.7 ± 19.6	0.730	NA
Male sex	50 (76.9)	21 (72.4)	0.639	1.27 (0.47–3.45)
Concurrent conditions				
Immunosuppression	12 (18.5)	6 (20.7)	0.800	0.87 (0.29–2.60)
Solid tumor	16 (24.6)	6 (20.7)	0.678	1.25 (0.43–3.62)
Hematologic malignancy	1 (1.5)	1 (3.4)	0.553	0.44 (0.03–7.25)
Diabetes mellitus	9 (13.8)	9 (31.0)	0.050	0.36 (0.12–1.03)
Liver cirrhosis	11 (16.9)	3 (10.3)	0.408	1.76 (0.45–6.88)
Heart failure	4 (6.2)	3 (10.3)	0.475	0.57 (0.12–2.72)
Chronic obstructive pulmonary disease	7 (10.8)	3 (10.3)	0.951	1.05 (0.25–4.37)
Liver transplant	15 (23.1)	7 (24.1)	0.911	0.94 (0.34–2.64)
Duration of hospitalization before *A. baumannii* bacteremia, d	34.8 ± 36.1	23.9 ± 27.5	0.150	NA
Hospital location				
Intensive care unit	41 (63.1)	9 (31.0)	0.004	3.80 (1.50–9.66)
Medical ward	6 (9.2)	12 (41.4)	0.001	0.14 (0.05–0.44)
Surgical ward	18 (27.7)	8 (27.6)	0.992	1.00 (0.38–2.68)
Source of bacteremia				
Catheter-related infection	25 (38.5)	9 (31.0)	0.489	1.39 (0.54–3.52)
Pneumonia associated with mechanical ventilation	18 (27.7)	1 (3.4)	0.006	10.72 (1.36–84.8)
None (primary bacteremia)	12 (18.5)	14 (48.3)	0.003	0.24 (0.09–0.63)
Intraabdominal infection	7 (10.8)	2 (6.9)	0.716	1.62 (0.32–8.37)
Urinary tract infection	3 (4.6)	2 (6.9)	0.642	0.65 (0.10–4.13)
Other	0	1 (3.4)	0.309	3.32 (2.43–4.52)
Carbapenem resistance	65 (100.0)	7 (24.1)	0.001	0.09 (0.50–0.20)
Prior colonization with *A. baumannii*	43/62 (69.4)	1/17 (5.9)	0.001	36.21(4.47–293.1)
Antimicrobial drugs used				
Cephalosporin	7/62 (11.3)	3/29 (10.3)	0.893	1.10 (0.26–4.61)
Piperacillin/tazobactam	21/62 (33.9)	4/29 (13.8)	0.046	3.20 (0.98–10.41)
Fluorquinolone	24/62 (38.7)	9/29 (31.0)	0.478	1.40 (0.54–3.59)
Glycopeptide	44/62 (71.0)	12/29 (41.4)	0.007	3.46 (1.38–8.69)
Aminoglycoside	17/62 (27.4)	8/29 (27.6)	0.987	0.99 (0.37–2.66)
Carbapenem	41/62 (66.1)	11/29 (37.9)	0.011	3.20 (1.28–7.99)
≥3 drugs	36/62 (58.1)	8/29 (27.6)	0.007	3.63 (1.40–9.47)
Invasive procedure or device				
Central venous catheter†	51/64 (79.7)	15/29 (51.7)	0.006	3.66 (1.42–9.46)
Surgical procedure‡	33/64 (51.6)	11/29 (37.9)	0.223	1.74 (0.71–4.27)
Mechanical ventilation†	49/64 (76.6)	14/29 (48.3)	0.007	3.50 (1.38–8.87)
Duration of hospitalization after *A. baumannii* bacteremia, d	46.6 ± 72.9	20.5 ± 21.2	0.050	NA
Died during hospitalization	35 (53.8)	9 (31.0)	0.041	2.59 (1.03–6.54)

*Values are mean ± SD or no. (%) except as indicated. Clone ABCA, *A. baumannii* clone A (AbH12O-A2); ABNCA, *A. baumannii* nonclone A; OR, odds ratio; CI, confidence interval; NA, not applicable.
†Week before bacteremia.
‡Month before bacteremia.

this sequence was absent in plasmid pMMCU3 (Figure 2). Two nucleotide changes detected in promoter regions provided an additional promoter region for the TonB-dependent receptor gene in plasmid pMMA2.

Real-time PCR (Table 2) was performed to analyze expression of septicolysin and TonB-dependent receptor genes in clones AbH12O-A2 and AbH12O-CU3. Expression of septicolysin in clone AbH12O-A2 was 2.1× times higher than that of clone AbH12O-CU3. Conversely, the TonB-dependent receptor was also overexpressed in clone AbH12O-A2 (1.8× higher than in clone AbH12O-CU3).

Conclusions

Outbreaks of MDR *A. baumannii* have been demonstrated in many studies (*1*,*2*,*5*). We report a large outbreak during 2006–2008 that persisted for ≥30 months. The AbH12O-A2 strain was pathogenic and caused 65 cases of bacteremia.

Clone AbH12O-A2 had unique characteristics. First, it was an MDR (including carbapenems) clone (ST56), susceptible only to tigecycline and colistin. Second, it harbored a carbapenemase $bla_{\text{OXA-24}}$ gene, flanked by XerC/XerD binding sites located on a plasmid, which probably spread to other *Acinetobacter* clones by a Xer

Table 2. Oligonucleotides used in real-time reverse transcription PCRs for *Acinetobacter baumannii*, Spain*

Primer	Gene	Sequence, 5′ → 3′
TonB-Forw	TonB-dependent receptor	GGACTGGTGATAAAGCACTAT
TonB-Rev	TonB-dependent receptor	GCCGCATAGAGTTATCACATC
Septicolysin-Forw	Septicolysin	CACCATCTTGTACCAATACATTT
Septicolysin-Rev	Septicolysin	GAAATTAGCAGAAGCTCTCTTAC
rpoB-Forw	RNA polymerase subunit B	CAGCCGCGAYCAGGTTGACTACA
rpoB-Rev	RNA polymerase subunit B	GACGCACCGCAGGATACCACCTG
gyrB-Forw	DNA gyrase subunit B	AAGTGAGGTAAAACCAGCGGTA
gyrB-Rev	DNA gyrase subunit B	AATCTTGCCTGCAATTGATTTT

*Forw, forward; rev, reverse.

recombination system (*11*). Third, this clone overexpressed 2 putative virulence factors, septicolysin and TonB-dependent receptor.

The septicolysin gene showed 2× overexpression caused by insertion of IS4, which provided an additional promoter. Although the exact role of septicolysin is unknown, it has been designated a cholesterol-dependent cytolysin, which has been reported to be produced by pathogenic bacteria such as *Clostridium perfringens*, *Bacillus anthracis*, and *Streptococcus pneumoniae* to aid invasion of tissues or cells (*12*).

The protein produced by the TonB-dependent receptor gene has been associated with virulence and iron uptake in *A. baumannii* (*13*) and may be involved in survival of bacteria in the lungs and blood. This characteristic may explain the large rate of bacteremia caused by clone AbH12O-A2. Thus, clinicians should be alert for the MDR ST56 *A. baumannii* clone because its spread would have serious health consequences.

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Internet Queries and Methicillin-Resistant *Staphylococcus aureus* Surveillance

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The Internet is a common source of medical information and has created novel surveillance opportunities. We assessed the potential for Internet-based surveillance of methicillin-resistant *Staphylococcus aureus* and examined the extent to which it reflects trends in hospitalizations and news coverage. Google queries were a useful predictor of hospitalizations for methicillin-resistant *S. aureus* infections.

Staphylococcus aureus is the most common bacterial pathogen isolated from human infections (*1*). Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates are strains constitutively resistant to β-lactam antimicrobial drugs. MRSA was initially largely confined to patients with health care exposures (*2*), but in the late 1990s, genetically distinct strains emerged and spread rapidly among healthy persons in the United States. These new strains, known as community-associated MRSA (CA-MRSA), differ epidemiologically and genetically from older strains (*2,3*). CA-MRSA strains have become the most common cause of skin infections in US emergency departments (*4*).

There is no systematic surveillance system in the United States for MRSA. The Centers for Disease Control and Prevention (CDC) tracks a limited group of infections defined as invasive through the Active Bacterial Core (ABC) surveillance system reported from 9 regions. These include MRSA infections at normally sterile sites. In a 2007 report, CDC used ABC surveillance to estimate that there were 94,000 cases and 18,650 deaths caused by invasive MRSA disease in the United States in 2005 (*5*). This report received extensive media coverage and increased public awareness of MRSA (*6*).

Recent efforts to overcome surveillance limitations, in particular delay and limited geographic coverage, have included Internet protocol (IP) surveillance. IP surveillance monitors Internet search terms related to a specific disease, assuming that greater disease activity correlates with more searches. The best known IP surveillance is Google Flu Trends (*7*), although other researchers have created additional models (*8,9*). Given the lack of comprehensive surveillance, we examined whether Google search data might productively supplement existing systems to track the changing epidemiology of MRSA infections. Because MRSA, unlike influenza, is unfamiliar to many persons, we hypothesized that Internet search activity might reflect curiosity inspired by news reports and information-seeking related to actual infections or symptoms.

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The Study

We used the Google Trends database to obtain the proportion of all Google searches that contained the words "MRSA" or "staph." "Staph" was included because many news stories refer to MRSA as "antibiotic resistant staph." "Methicillin-resistant *Staphylococcus aureus*" was too infrequently searched to be useful. Google Trends reports search activity relative to the average number of similar queries in February 2004. We only included US searches determined from IP addresses.

We extracted counts of US newspaper, wire service, and radio and television stories mentioning "MRSA" or "staph" from the LexisNexis Academic database. We spot-checked stories with the word "staph" to confirm they were about MRSA. One event or medical publication could generate multiple news stories. We hypothesized that the volume of news coverage captured the relative effect of the story on search behavior.

We used quarterly hospital discharge data from the University HealthSystems Consortium Clinical Database, which includes >90% of US academic medical centers, to calculate the proportion of hospitalizations including an MRSA diagnosis. These data were a proxy for true MRSA incidence. We used the diagnostic code for MRSA from the International Classification of Disease, 9th Revision (V09.0). MRSA hospitalizations include CA-MRSA infections that led to hospitalization and infections that developed during a hospitalization. This database includes ≤99 codes per discharge, more than other national hospital discharge databases. The likelihood of recording an MRSA diagnosis increases with longer lists of codes because of the many concurrent conditions in complex hospitalizations. Some medical centers systematically used <99 diagnoses fields. We adjusted hospitalization rates for the maximum number of codes submitted by each medical center each year. Data after the 3rd quarter of 2008 were not included because of implementation of a nationwide coding change for MRSA.

We related quarterly variation in MRSA hospitalizations to quarterly variations in search queries and news stories in a linear regression model. Because of

the effect of the 2007 CDC report on MRSA awareness, we tested 2 indicator variables: 1 to capture the spike in search activity during the 4th quarter of 2007, and 1 to account for higher levels of search activity in subsequent quarters (*10*). These 2 indicators enable the model baseline to differ during the quarters before, during, and after the 4th quarter of 2007, while keeping the relationship between hospitalization rates and Internet searches and news counts the same during the 3 periods. All statistical analyses were performed in Stata version 10.0 (StataCorp LP, College Station, TX, USA).

Details of the model and statistical methods are available in the online Technical Appendix (www.cdc.gov/EID/content/17/6/1068-Techapp.pdf). Weekly news counts are shown in Figure 1. They range from 4 to 130 before the October 2007 peak of 719, related to the CDC report, the effect of which appears to linger. The prior peak of 130 in April 2005 was related to articles in the New England Journal of Medicine describing necrotizing fasciitis associated with MRSA and the emergence of CA-MRSA in 2001–2002 (*11,12*).

Quarterly variation in Google searches for "MRSA" and "staph" are shown in Figure 2. Search behavior changed markedly after the October 2007 publication. In addition to the spike, there was a subsequent change in the relative frequency of search term "MRSA" compared with "staph." Note that the news count peak in 2005 is not seen in the Google searches, and the peak in the Google searches in the 3rd quarter of 2006 is not apparent in the news counts.

Google queries were a useful predictor of MRSA hospitalizations and explained 33% of quarterly variation when used alone. Adding news counts to the model resulted in increasing the percentage of explained variation only modestly to 41%. The news counts were not a significant addition to the model ($p = 0.18$).

Our final model, which includes search queries and the 2 temporal indicator variables, but not the news counts, is shown in the Table. The correlation between model predictions and observed hospitalization rates was 0.93 ($p<0.001$). Although data after 2007 are insufficient for definitive comparison, a better prediction before than after the 4th quarter of 2007 is suggested (Figure 2).

Conclusions

We report an IP surveillance model for MRSA incidence. We hypothesized that news coverage for such an unfamiliar disease would strongly influence search activity. However, news coverage did not affect the relationship between search queries and hospitalization rates before the 2007 CDC report. The congruence of the Internet search activity and the hospital discharge data suggest that their temporal pattern represents the actual trend in MRSA: an increasing incidence during 2004–2007, with a suggestion

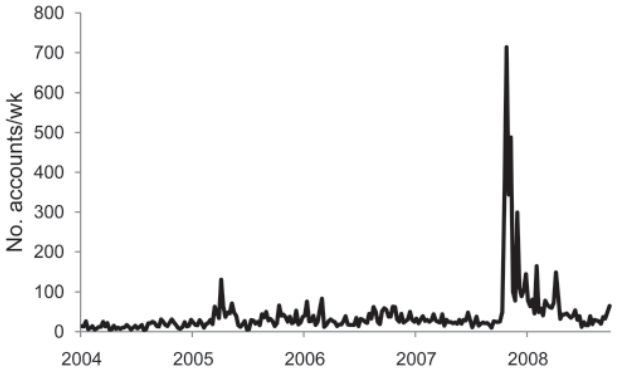

Figure 1. Weekly counts of news coverage (newspaper stories, wire service stories, and television and radio news transcripts) that mention "MRSA" (methicillin-resistant *Staphylococcus aureus*) or "staph," 2004–2008. Extracted from the LexisNexis Academic Database.

of seasonal variation, and no increase in 2008. This pattern is not the same pattern documented by the ABC surveillance data for invasive MRSA infections (*13*).

The unfamiliarity of the public with MRSA poses a challenge to using Google Trends. Searches using the phonetic misspelling "mersa" show a parallel trend to searches using "MRSA," although they are less frequent, and the correctly spelled "methicillin" is too rare to track.

Hospitalized MRSA infections include hospital-associated MRSA infections and the more serious CA-MRSA infections. Because evidence has shown that invasive hospital-associated MRSA infections decreased during the study period (*13*), the generally upward secular

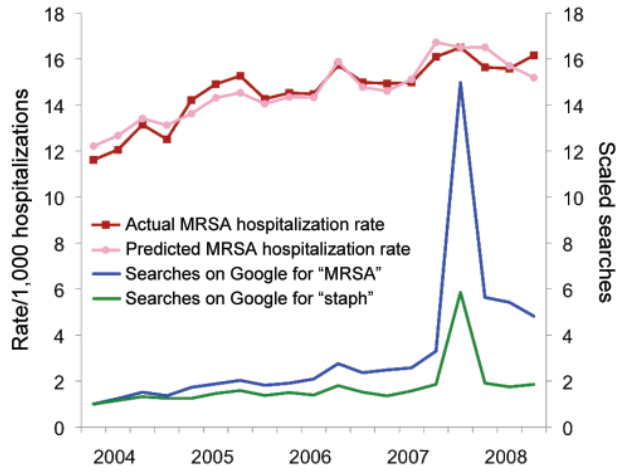

Figure 2. Actual and predicted hospitalization rates per 1,000 hospitalizations with an International Classification of Disease, 10th Revision, diagnostic code for methicillin-resistant *Staphylococcus aureus* (MRSA) and the fraction of Google search queries for "MRSA" or "Staph" (relative to the fraction of February 2004), 2004–2008.

Table. Multiple regression results for model relating UHC MRSA hospitalization rates per 1,000 hospitalizations to Google searches for "MRSA" or "staph" (normalized and scaled)*

Characteristic	Coefficient	95% CI	SE	t value	p>t
Intercept	9.03	7.56 to 10.50	0.69	13.07	<0.001
Google searches	0.25	0.18 to 0.32	0.032	7.73	<0.001
2007 4th quarter indicator	−21.45	−28.10 to −14.80	3.12	−6.87	0.001
2008 indicator	−3.06	−4.55 to −1.57	0.70	−4.37	<0.001

*UHC, University HealthSystems Consortium; MRSA, methicillin-resistant *Staphylococcus aureus*; CI, confidence interval. The overall model F(3,15) was 29.69 (p<0.0001), R^2 0.8559, and adjusted R^2 0.8270. Correlation coefficient between predicted values of this model and observed rates was 0.9251.

trend in MRSA hospitalizations is more likely to represent the trend in CA-MRSA, especially because we now know that most MRSA infections have onset in the community (*3*). The inability to distinguish community and health care infections is nonetheless a limitation of the Google and the hospitalization data. Although some hospital databases include more hospitals, they include fewer diagnostic codes. Therefore, there are no additional comprehensive data available for MRSA incidence. The lack of any true standard for MRSA incidence is why IP surveillance is potentially useful.

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Porcine Reproductive and Respiratory Syndrome in Hybrid Wild Boars, China

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We conducted a serologic investigation of porcine reproductive and respiratory syndrome virus (PRRSV) in hybrid wild boar herds in China during 2008–2009. PRRSV isolates with novel genetic markers were recovered. Experimental infection of pigs indicated that hybrid wild boars are involved in the epidemiology of PRRSV.

Hybrid wild boars, also known as special wild pigs in China, are a hybrid animal with 75% wild boar lineage and 25% domestic Duroc pig lineage. Hybrid wild boars are genetically stable and retain the appearance of wild boars. In addition, they have less body fat, are strongly adaptable to various environments, and have an estrus cycle in all 4 seasons.

We observed an increased incidence of high fever and respiratory disorders in hybrid wild boars. This illness resulted in increased economic losses in Shandong Province, China. We conducted a serologic study and identified highly pathogenic porcine reproductive and respiratory syndrome virus (PRRSV) in affected hybrid wild boars.

The Study

During April 2008–December 2009 in Shandong Province, 613 blood samples were obtained from hybrid wild boars that had not been vaccinated against PRRSV (Table). The Herd Check PRRS commercial ELISA kit (IDEXX Laboratories, Norcross, GA, USA) was used for serologic analysis; 167 (27.2%) of 613 samples were seropositive for PRRSV, and 2 PRRSV isolates (TAYZ and ZCYZ) were obtained. The viruses were plaque-purified once in MARC-145 cells and amplified for sequencing and infection of other pigs.

Because open reading frame 5 (ORF5) and the nonstructural protein 2 (NSP2) gene are 2 of the most variable genes in PRRSV (*1*–*3*), these genes were sequenced to identify genetic variation in the 2 isolates. Primers and reverse transcription PCR were used as described (*4*). Sequences for NSP2 and ORF5 genes were submitted to GenBank (accession nos. HM854221 and HM854224 for TAYZ and HQ388394 and HQ384170 for ZCYZ). Nucleotide sequences were analyzed by using MEGA 4.0 (www.megasoftware.net).

TAYZ and ZCYZ had higher nucleotide homologies in ORF5 with highly pathogenic PRRSV (prototype virus JXA1) than North American-type PRRSV (prototype virus VR2332), and European-type PRRSV (prototype virus LV). For NSP2 of TAYZ, 2 deletions were identified: a 1-aa deletion at position 482, and a 29-aa deletion at positions 533–561. These deletions were similar to those in JXA1, HUB1, and SD-JN isolates associated with porcine high fever disease reported in China (*4*–*7*). For NSP2 of ZCYZ, 2 deletions were identified: a novel 25-aa deletion at positions 476–500, and a 29-aa deletion at positions 533–561 (Figure 1).

To determine the virulence and pathogenesis of the PRRSV isolated, we performed experimental infection with the ZCYZ isolate in ten 6-week-old domestic Duroc crossbred pigs and ten 6-week-old hybrid wild boars. The animal infection protocol was reviewed and approved by the Shandong Province Animal Ethics Committee. Domestic pigs and hybrid wild boars were randomly divided into 2 groups, each consisting of 5 pigs. These pigs were shown by serologic analysis to be negative for antibodies against PRRSV, swine influenza virus, and mycoplasmas.

In hybrid wild boars injected intramuscularly with 1 mL of ZCYZ isolate (10^3 50% tissue culture infectious

Table. Serologic prevalence of PRRSV in hybrid wild boars, China, April 2008–December 2009*

No. pigs/ herd	Farm environment	No. farms tested	No. positive samples/ no. tested (%)
70–99	Hill	4	19/81 (23.5)
	Slope	6	33/122 (27.0)
	Forest	3	17/60 (28.3)
100	Hill	6	37/135 (27.4)
	Slope	4	29/97 (29.9)
	Forest	5	32/118 (27.1)
Total		28	167/613 (27.2)

*PRRSV, porcine reproductive and respiratory syndrome virus.

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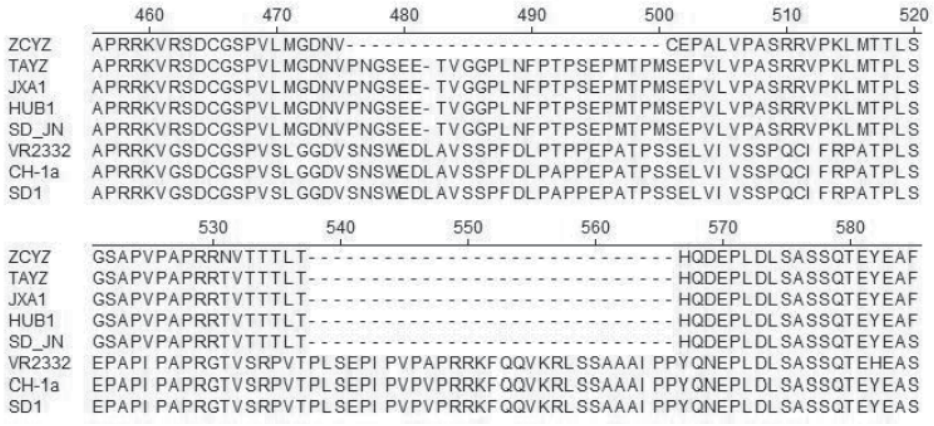

Figure 1. Amino acid sequence alignments of partial nonstructural protein 2 genes of porcine reproductive and respiratory syndrome virus isolates, China. Dashes indicate deletions.

doses/mL), high fever >40.8°C and respiratory disorders were observed at 5–6 days postinfection (dpi). Two boars died at 8 dpi and 1 boar died at 9 dpi. Two pigs were killed at 21 dpi and autopsies were then performed.

Pulmonary hyperplasia and consolidation (Figure 2, panel E) and cardiac hemorrhage and edema (Figure 2, panel H) were observed in the dead hybrid wild boars. PRRSV-specific antibodies were detectable by 7 dpi, had increased by 14 dpi, and remained high until autopsy at 21 dpi. For the infected domestic Duroc crossbred pigs, high fever >41°C and respiratory problems were observed at 4–5 dpi, and red discoloration was observed in the ears (Figure 2, panel C). Two pigs in this group died at 6 dpi; the other 3 pigs in this group died at 7 dpi. The dead pigs all had multiple lesions in various organs, such as edema and hemorrhage in the lung (Figure 2, panel F) and hemorrhagic spots in the heart (Figure 2, panel I). Duroc crossbred pigs and hybrid wild boars in the control group injected with Dulbecco minimal essential medium had a normal appearance (Figure 2, panels A, B), appetite, and rectal temperature during the experiment, and no obvious pathologic changes were observed in the lungs (Figure 2, panel D) and heart (Figure 2, panel G).

Conclusions

PRRSV was identified in hybrid wild boars during our serologic study. Prevalence of antibodies against PRRSV was similar for different types of farm environments (Table). Two PRRSV isolates were obtained from hybrid wild boars; 1 of the isolates (ZYCZ) has a novel genetic marker in the NSP2 gene. The ZCYZ isolate showed high virulence in domestic Duroc crossbred pigs and hybrid

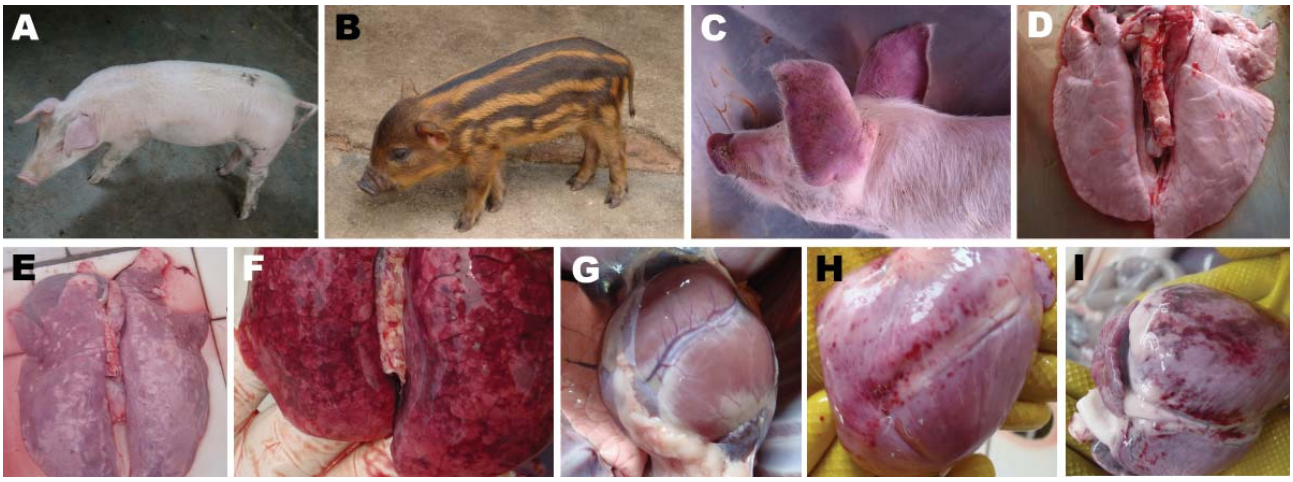

Figure 2. Experimental infection of domestic Duroc crossbred pigs and hybrid wild boars with porcine reproductive and respiratory syndrome virus isolate ZCYZ. A) Domestic Duroc crossbred pig (control) injected with Dulbecco minimal essential medium (DMEM), showing normal skin color. B) Hybrid wild boar (control) injected with DMEM, showing normal skin color. C) Red discoloration in ears of a domestic Duroc crossbred pig infected with ZCYZ). D) Normal pathologic appearance of lungs in a control domestic Duroc crossbred pig. E) Pulmonary hyperplasia and consolidation in a hybrid wild boar infected with ZCYZ. F) Edema and hemorrhage in the lungs of domestic Duroc crossbred pig infected with ZCYZ). G) Normal pathologic appearance of heart in a control hybrid wild boar. H) Cardiac hemorrhage and edema in a hybrid wild boar infected with ZCYZ. I) Hemorrhagic spots in heart of domestic Duroc crossbred pig infected with ZCYZ.

wild boars. The virulence of the TAYZ isolate has not yet been determined.

Shandong Province is one of the major pig-producing areas in China; annual production is 60 million domestic pigs and 500,000 hybrid wild boars. It has been reported that 793 (59.5%) of 1,332 serum samples from domestic pigs were seropositive for PRRSV, and seropositivity was >80% in some large-scale commercial production farms in the Shandong area (*8*). As a transitional group between domestic pigs and wild boars, hybrid wild boars were sensitive to highly pathogenic PRRSV. However, 167 (only 27.2%) of 613 samples from hybrid wild boars were positive for PRRSV, indicating that the prevalence of PRRSV differed between domestic pigs and hybrid wild boars in this area. Hybrid wild boars are usually raised on hills or slopes or in forests, characterized by a relatively low-density pig population and separation from commercial domestic pigs. These features may have helped to limit transmission of PRRSV to hybrid wild boar herds.

When PRRSV enters the wild pig population, its subsequent spread has been found to be limited, probably because the virus is not easily transmitted within a low-density or medium-density population (*9*). Some genetic traits in local wild boars may have been inherited over decades or even for hundreds of years. Whether these traits confer resistance to PRRSV remains unknown and warrants further study.

PRRSV is currently divided into 2 distinct genotypes, European and North American, which show ≈60% nt sequence similarity (*10*). In this study, the 2 PRRSV isolates from hybrid wild boars belonged to the North American genotype on the basis of sequence analysis and were similar to highly pathogenic PRRSV isolated during 2006–2009 in China. This finding suggests that infection of hybrid wild boars with PRRSV may be caused by the spread of highly pathogenic PRRSV in domestic pigs in recent years.

Experimental infection of pigs showed that the ZCYZ isolate was highly pathogenic in domestic pigs and hybrid wild boars, indicating that hybrid wild boars likely play a role in the epidemiology of PRRSV and may be an alternative model that can be used to study transmission of PRRSV among the wild boar population. However, how PRRSV has evolved and varies in hybrid wild boars is not clear, and further studies, including extensive genomic sequence analyses, should be conducted.

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Hepatitis E Virus Seroprevalence and Chronic Infections in Patients with HIV, Switzerland

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We screened 735 HIV-infected patients in Switzerland with unexplained alanine aminotransferase elevation for hepatitis E virus (HEV) immunoglobulin G. Although HEV seroprevalence in this population is low (2.6%), HEV RNA can persist in patients with low CD4 cell counts. Findings suggest chronic HEV infection should be considered as a cause of persistent alanine aminotransferase elevation.

Unexplained liver enzyme elevation is frequently encountered in HIV-infected persons (*1*). Hepatitis E virus (HEV) can cause deranged liver function. Chronic infection with HEV has been reported in patients with organ transplants, treated malignancies, and HIV infection (*2*–*7*).

The Study

The aim of this study was to investigate the prevalence and role of HEV infection among participants of the Swiss HIV Cohort Study enrolled up to December 2008 who had persistent elevated alanine aminotransferase (ALT) levels. Patients were eligible if they fulfilled the following criteria: 1) ≥18 years of age; 2) history of ≥2 consecutively elevated ALT values (>60 IU/L); and 3) negative for both hepatitis B antigen and hepatitis C virus antibody. Serum HEV immunoglobulin (Ig) G was detected by an enzyme immunoassay (EIA), the MP Diagnostics HEV ELISA Kit (MP Biomedicals, formerly Genelabs Diagnostics, Singapore). Positive samples were retested, and only those with a repeated positive result were considered positive. HEV RNA was determined in plasma by real-time PCR of a 189-bp product located in the open reading frame 2 region. Strains were sequenced and compared with reference HEV strains (GenBank) as described earlier (*8*).

For all patients meeting inclusion criteria, HEV serology was performed on the most recently stored blood sample. When available, for patients with positive serologic test results, HEV serology and blood HEV real-time PCR were performed on plasma samples stored 3 months before the first documented elevated ALT value. In these patients, HEV PCR was also performed on samples stored at the time of, and 3 months after, the first elevated ALT level.

To exclude occult infection, HEV PCR analysis was also performed on all available samples from IgG-negative patients with <150 CD4 cells/mm^3 at the time of initial ALT elevation. To characterize more fully the patients with positive PCR, additional serologic testing was performed by using EIAgen HEV IgG and EIAgen HEV IgM kits (Adaltis Ingen, Paris, France).

Of 15,713 patients in the database, 2,000 patients had persistently elevated ALT values. Of these, 1,256 patients who were co-infected with hepatitis B virus or hepatitis C virus and 9 with missing data were excluded; 735 patients met the inclusion criteria (Table 1). IgG serologic tests were performed at a mean of 2.1 years after the first ALT elevation, and results were positive for 19 patients (2.6%). HEV-seropositive patients were more often female ($p = 0.059$) and of Asian origin ($p = 0.007$). In the univariate analysis, presence of HEV IgG was not associated with age, route of infection, lowest CD4 count, viral load, body mass index, or ALT values (Table 1). However, age- and gender-adjusted multivariate logistic regression analysis, with HEV IgG status as the outcome variable, yielded significant odds ratios for patients with low CD4 counts compared with patients with higher CD4 counts. Patients whose lowest CD4 counts were 100–350 cells/mm^3 were 4.7 times more likely to be HEV positive compared with those with lowest CD4 counts <100 cells/mm^3 (Table 2).

In 16 of the 19 HEV seropositive patients, additional samples were available a median of 6.6 months (interquartile

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range [IQR] 4.5–6.0) before the first ALT elevation. Seroconversion from a prior negative to a positive result was found in 5 of these 16 patients (31.3%). There were no significant differences in ALT values (median, interval between and during high ALT periods), CD4 count, or HIV viral load of those who exhibited HEV seroconversion compared to those who did not.

Real-time PCR for HEV RNA was positive in 1 of the 19 HEV IgG–positive patients (genotype 3b), with presence of HEV RNA over a 24-month period, while HEV IgG remained negative for the first 12 months. This 46-year-old white man, who had sex with men (MSM) and received a diagnosis of HIV infection in June 2001, started highly active antiretroviral therapy (HAART) with a CD4

Table 1. Population characteristics in a study of prevalence and role of HEV infection among participants in the Swiss HIV Cohort Study, Switzerland, 2008*

Characteristic	No. (%) all participants, N = 735	No. (%) HEV negative, n = 716	No. (%) HEV positive, n = 19	p value
Sex				0.0587
M	618 (84.1)	605 (84.5)	13 (68.4)	
F	117 (15.9)	111 (15.5)	6 (31.6)	
Ethnic group				<0.0001
White	607 (82.6)	594 (83.0)	14 (73.7)	
Black	70 (9.5)	69 (9.6)	1 (5.3)	
Hispanic	26 (3.5)	25 (3.5)	1 (5.3)	
Asian	29 (3.9)	26 (3.6)	3 (15.8)	
Other	3 (0.4)	2 (0.28)	1 (0.14)	
Probable route of HIV infection				NS
Heterosexual	291 (39.6)	283 (39.5)	8 (42.1)	
MSM	411 (55.9)	400 (55.9)	11 (57.9)	
IDU	4 (0.6)	4 (0.6)	0	
Blood	5 (0.7)	5 (0.7)	0	
Unknown/other	24 (3.2)	24 (3.4)	0	
Current or past IDU				NS
Yes	14 (2.3)	14 (2.0)	0	
No	721 (99.7)	702 (98.0)	19 (100.0)	
Prison history				NS
Yes	45 (6.1)	44 (6.1)	1 (5.3)	
No	690 (93.9)	672 (93.9)	18 (94.7)	
Alcohol consumption				NS
Yes	261 (35.5)	256 (35.8)	5 (26.3)	
No	474 (64.5)	460 (64.2)	14 (73.7)	
BMI group, kg/m^2				NS
≤25	408 (55.5)	398 (55.6)	10 (52.6)	
25.1–30	231 (31.4)	225 (31.4)	6 (31.6)	
>30	96 (13.1)	93 (12.9)	3 (15.8)	
ALT peak value, IU/L				NS
≤180	606 (82.4)	591 (82.5)	15 (78.95)	
>180	129 (17.6)	125 (17.5)	4 (21.05)	
Lowest CD4 count, cells/mm^3				0.0685
<100	276 (37.6)	273 (38.1)	3 (15.8)	
100–350	288 (39.2)	276 (38.6)	12 (63.2)	
>350	171 (23.2)	167 (23.3)	4 (21.1)	
HAART history				NS
Yes	610 (83.0)	595 (75.4)	15 (78.9)	
No	125 (17.0)	121(16.9)	4 (21.6)	
Cancer occurrence				NS
Yes	25 (3.4)	24 (3.4)	1 (5.3)	
No	710 (96.6)	692 (94.6)	18 (94.7)	
Outcome				NS
Dead	29 (3.9)	28 (3.9)	1 (5.3)	
Alive	652 (88.7)	638 (89.1)	14 (73.7)	
Lost to follow-up	54 (7.4)	50 (7.0)	4 (21.1)	

*HEV, hepatitis E virus; MSM, men who have sex with men; IDU, intravenous drug use; BMI, body mass index; ALT, alanine aminotransferase; HAART, highly active antiretroviral therapy; NS, not significant.

Table 2. Logistic regression derived odds ratios/estimates for positive HEV serology in study of prevalence and role of HEV infection among participants in the Swiss HIV Cohort Study, Switzerland, 2008*

Variable	Odds ratio (95% CI)	p value
Male vs. female	0.207 (0.041–1.016)	0.0523
CD4 100–350 vs. CD4 <100 per mm^3	4.683 (1.268–17.295)	0.0206
CD4 ≥350 vs. CD4 <100 per mm^3	2.448 (0.522–11.468)	0.256
Other ethnicity vs. Asian ethnicity	0.295 (0.073–1.191)	0.0864
Alcohol history, no vs. yes	1.802 (0.582–5.581)	0.3071
Risk group, other vs. MSM	0.422 (0.100–1.774)	0.2392
Age at ALT elevation	1.017 (0.966–1.070)	0.5257
Duration of ALT elevation	1.001 (1.000–1.002)	0.0207

*HEV, hepatitis E virus; MSM, men who have sex with men; ALT, alanine aminotransferase.

count of 34 cells/mm^3 (5%) and a normal ALT level. ALT level increased 1 month later, but HAART was continued because the patient was asymptomatic. The patient refused a liver biopsy. Real-time PCR for HEV RNA was performed between August 2001 and December 2004. HEV PCR became negative for the virus when the CD4 count reached 83 cells/mm^3 (17.5%), and ALT levels became normal 6 months later, when the CD4 count exceeded 100 cells/mm^3. Figure 1 shows the course of ALT values, CD4 counts, and HIV and HEV plasma viral load.

Given the prolonged viremia and late seroconversion (IgG) observed in patients with low CD4 counts, we performed real-time PCR for HEV RNA on 135 samples obtained from 54 HEV IgG–negative patients with low CD4 counts (<150 cells/mm^3) at the time of initial ALT elevation. Real-time PCR for HEV was positive in 1 HEV IgG–negative patient over a 5-month period (Figure 2). This 59-year-old MSM started HAART in October 1996 with a CD4 count of 140 cells/mm^3. CD4 counts remained <250 cells/mm^3 over the subsequent 12 years. Real-time PCR for HEV RNA was performed between March 2002 and April 2008. HEV-positive samples (genotype 3c) were identified between October 2005 and March 2006. Additional serologic analysis revealed the presence of IgM over a 7-month period following first positive real-time PCR but absence of IgG seroconversion.

Conclusions

This study of participants of the Swiss HIV Cohort Study shows a 2.6% (19/735) seroprevalence of HEV in HIV-infected patients without HBV or HCV infection with a history of persistently elevated ALT level. We did not find higher HEV prevalence in subgroups previously considered at higher risk of HEV infection such as MSM, injection drug users, and prisoners. The prevalence in Switzerland is lower than in other European countries and the United States (*9*). It is now well established that pigs and other animal species constitute reservoirs for HEV and that transmission in industrialized countries occurs mainly through contaminated meat. We hypothesize that strict regulation of animal imports in Switzerland (*10*) may reduce HEV prevalence among farm animals. Food preferences and possible regional factors may also contribute to the differences between countries and merit further study.

We observed anti-HEV seroconversion in 5 patients, including one with prolonged HEV RNA (>24 months). In this single patient, who had a very low CD4 count, seroconversion (IgG) was delayed until immune reconstitution occurred. As the CD4 count exceeded 100 cells/mm^3, HEV RNA cleared, and ALT levels became normal (Figure 1). According to a literature search, 7 real-time PCR documented cases of HEV have been reported (*3*,*4*,*11*–*14*). Of these, 5 case-patients with CD4 counts

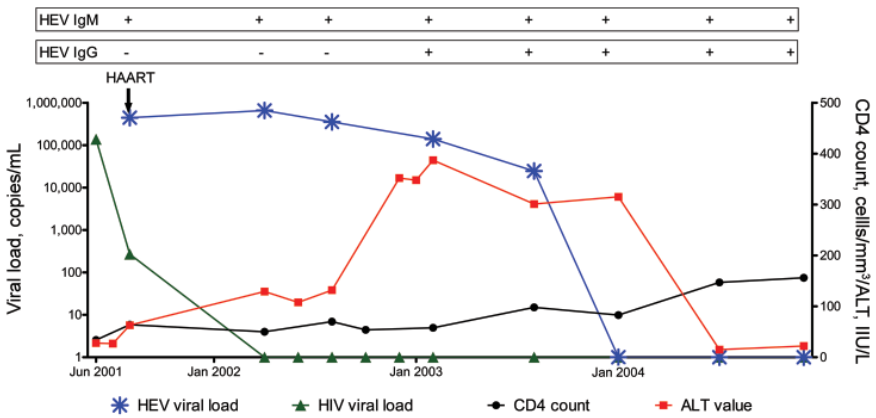

Figure 1. Longitudinal description of blood hepatitis E virus (HEV) serology, HEV RNA, alanine aminotransferase (ALT) levels, HIV RNA, and CD4 count in patient with chronic HEV infection, positive results by real-time PCR for HEV RNA, and seroconversion to immunoglobulin (Ig) G. HAART, highly active antiretroviral therapy.

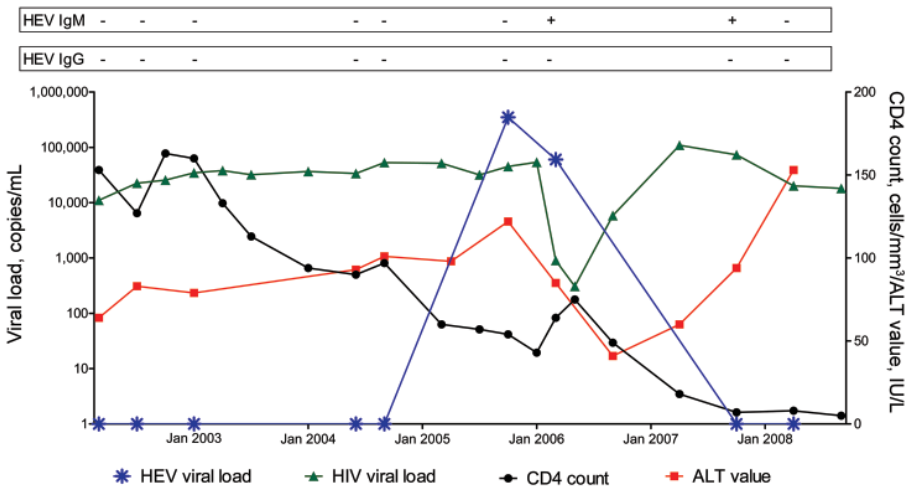

Figure 2. Longitudinal description of blood hepatitis E virus (HEV) serology, HEV RNA, alanine aminotransferase (ALT) levels, HIV RNA, and CD4 count in patient with positive real-time PCR results for HEV infection but without serologic seroconversion to immunoglobulin (Ig) G.

>200 cells/mm^3 sought treatment with acute infection and the virus cleared, whereas in 2 patients with <200 CD4 cells/mm^3 persistent hepatitis developed (as we observed in our patient).

This study faced 2 common limitations regarding HEV diagnosis and comparability of seroprevalence: lack of an approved algorithm and variability of serologic tests in terms of sensitivity and specificity. According to a recent comparative study, the test used in our study may underestimate seroprevalence because of its inherent lower sensitivity (*15*). Moreover, the sensitivity of EIA tests among immunosuppressed patients is unknown and may be limited. We therefore screened all HEV IgG–negative patients (using HEV real-time PCR) with low CD4 counts at the time of unexplained, elevated ALT level and identified 1 patient who had 2 positive samples over a 5-month period. Unfortunately, later samples were not available to determine the precise duration of HEV viremia.

Taken together, our results suggest that serologic screening alone may be insufficient to diagnose HEV infection in HIV-infected patients with very low CD4 counts because seroconversion (IgG) may be delayed or not occur. When investigating unexplained, elevated ALT level in HIV-infected patients, we propose that HEV infection should be considered.

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Macrolide Resistance in *Mycoplasma pneumoniae*, Israel, 2010

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Macrolide resistance in *Mycoplasma pneumoniae* is often found in Asia but is rare elsewhere. We report the emergence of macrolide-resistant *M. pneumoniae* in Israel and the in vivo evolution of such resistance during the treatment of a 6-year-old boy with pneumonia.

Mycoplasma pneumoniae is a leading respiratory pathogen in both pediatric (*1*,*2*) and adult (*1*,*3*) populations. Macrolides are considered the first line of therapy and are almost the only treatment for children. In recent years, alarming rates of *M. pneumoniae* with macrolide resistance (≤90%) have occurred in eastern Asia, including the People's Republic of China, Japan, and Korea (*2*,*4*–*7*). This was initially reported in children; however, a surge of resistance in adults was recently reported (*2*,*4*,*7*). Macrolide-resistant *M. pneumoniae* has also been suggested to be associated with a longer course of disease (*2*,*4*).

In the Western Hemisphere, lower rates of macrolide resistance have been reported (≤10%), however, several epidemics with notable complications have occurred (*8*–*11*). We report the detection of macrolide resistance in *M. pneumoniae* in Israel.

The Study

A previously healthy 6-year-old boy was hospitalized after 2 weeks with fever up to 40°C. At onset of illness, a diagnosis of pharyngitis was made. *Streptococcus pyogenes* was isolated from his throat, and amoxicillin was prescribed without any clinical response. Later, a clinical diagnosis of sinusitis was made, and amoxicillin-clavulanate was prescribed. A chest radiograph done at that time reportedly showed no abnormalities. Laboratory investigation before admission showed leukocytosis of 19,600 cells/mm^3 with 2,200 monocytes/mm^3 and 7,600 neutrophils/mm^3; L-lactate dehydrogenase (LDH) was 1,854 U/L (reference value up to 600 U/L).

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Ten days after the beginning of his illness, his fever decreased for 2 days and then reappeared, together with cough, resulting in hospitalization. At admission, pneumonia of the right middle and lower lobe was confirmed by chest radiograph. Laboratory tests showed leukocytes within normal ranges, erythrocyte sedimentation rate (ESR) 80/h, and C-reactive protein (CRP) 15 mg/L (reference range up to 0.5 mg/L). Treatment with penicillin was started without clinical improvement. Azithromycin (10 mg/kg/d) was added on the third day. After receiving this treatment, his leukocytes increased to 20,000 cells/mm^3 with ESR 97/h and CRP 22.5 mg/L. The β-lactam coverage was switched to cefuroxime and later to ceftriaxone because no response was observed. Chest ultrasound showed a small pleural effusion. Bronchoscopy showed thick mucus secretions; respiratory specimens tested were negative for respiratory syncytial virus, influenza viruses A and B, parainfluenza virus, human metapneumovirus, and adenovirus, as were results of urine tests for *Legionella* spp. and blood tests for pneumococcal antigen and cryptococcal antigen.

Throat swab specimens were collected and DNA extracted by boiling. Samples were positive for *M. pneumoniae* by real-time PCR based on the detection of a 188-nt amplicon from the 3′ region of the repeat RepMP4 (primers: MpP1–1217_F-GTTGAAGAACG CCCAAGTGAA; MpP1–1292_R-CCGGTGGTTTGGAG CAAA). The target DNA was amplified by using Polymerase (SYBR Green) master mix (KapaTM SYBR Fast qPCR; Kapa Biosystems, Cape Town, South Africa), followed by a melt curve analysis (Rotor-Gene 6000; Corbett Research, Mortlake, NSW, Australia) with 1:30 polymerase activation at 95°C, followed by 42 cycles of 95°C for 10 s, 60°C for 18 s, 72°C for 8 s. The assay has an analytical sensitivity of 10 fg DNA or 1 to 3.3 genome copies.

Despite the *M. pneumoniae*–positive throat swab at admission, no clinical response was observed for treatment with azithromycin for 8 days. This led to the suspicion of macrolide-resistant *M. pneumoniae*. Samples were further analyzed for possible resistance-associated mutations (A2063G/C, A2064G, and A2067G) that constitute 98% of macrolide resistance–associated mutations in *M. pneumoniae* (*2*). Real-time PCR of domain V region of the 23S rRNA gene with a high-resolution melt curve analysis utilizing the SYBR Green amplification primers was used (*10*). The emergence of resistant mutants during treatment was confirmed by sequence analysis of the whole region with additional PCR of a larger amplicon size (*10*) (Figure 1). This confirmed the presence of the resistant A2063G mutation on the later samples.

Accordingly, on hospital day 21, treatment was switched to doxycycline, 4 mg/kg 2×/d for 5 days, with a

[1]These authors contributed equally to this article.

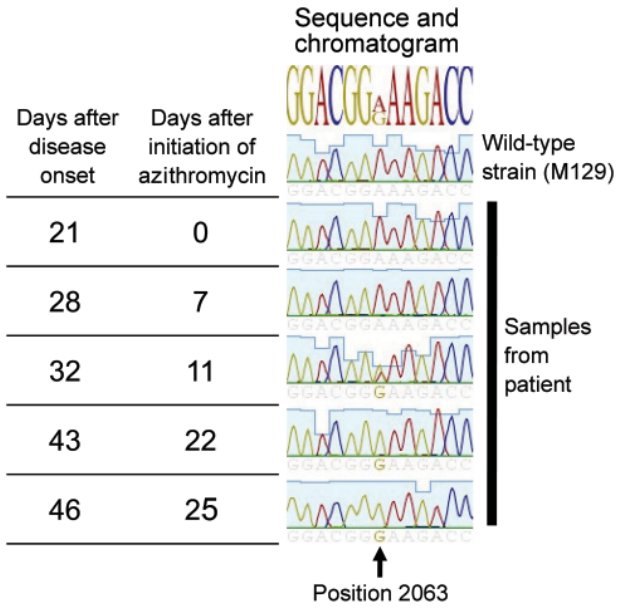

Figure 1. Time elapsed and chromatogram of wild-type *Mycoplasma pneumonia* strain M129 (ATCC 29342) compared with results from 5 samples from a 6-year-old boy in Israel. The A2063G mutation is shown to be evolving during treatment and predominates at the end.

prompt response; the fever subsided, the patient felt well, and the child was discharged. However, on day 33 from his first admission, he was readmitted with fever, cough, and hypoxia (oxygen saturation 85%). Chest radiograph showed atelectasis in the right middle lobe and lingula. PCR of a throat swab specimen for *M. pneumoniae* was again positive. Treatment with ciprofloxacin was started with prompt response; hypoxia subsided gradually and fever normalized. The child was treated with ciprofloxacin for 4 weeks and fully recovered.

During January 2010–August 2010, we observed a surge in *M. pneumoniae*–associated respiratory disease (30 cases during 8 months compared with 2 cases during 2009) at the Hadassah–Hebrew University Medical Centers. The Centers comprise 2 hospitals with a total of 1,000 beds in the city of Jerusalem. The Ein-Kerem campus is a tertiary center serving west Jerusalem, while the Mount Scopus campus is a secondary hospital providing primary care services to east Jerusalem. Samples from both hospitals are processed at Ein-Kerem's clinical microbiology laboratory. During the survey period, 274 samples were submitted, of which 42 samples from 30 patients were positive for *M. pneumoniae*. Following this case, we screened all *M. pneumoniae*–positive samples for macrolide resistance. In 9 of 30 patients tested, we found the A2063G mutation, which was confirmed by sequencing. In 2 pediatric patients, a mixed population of both resistant (A2063G) and sensitive (A2063A) organisms was identified (Figure 2). Notably, a 31-year-old woman was found to carry the A2063G mutation as well. No significant difference was found in clinical parameters between the 9 patients with resistant and 19 patients with sensitive *M. pneumoniae* species (Table). Other than our propositus case, we did not observe in our sample a longer course of disease in patients with macrolide-resistant *M. pneumoniae*, as suggested in previous studies (*6*,*12*).

Conclusions

The observed rate of resistance in our hospital-based patients in the current surge of *M. pneumoniae*–associated

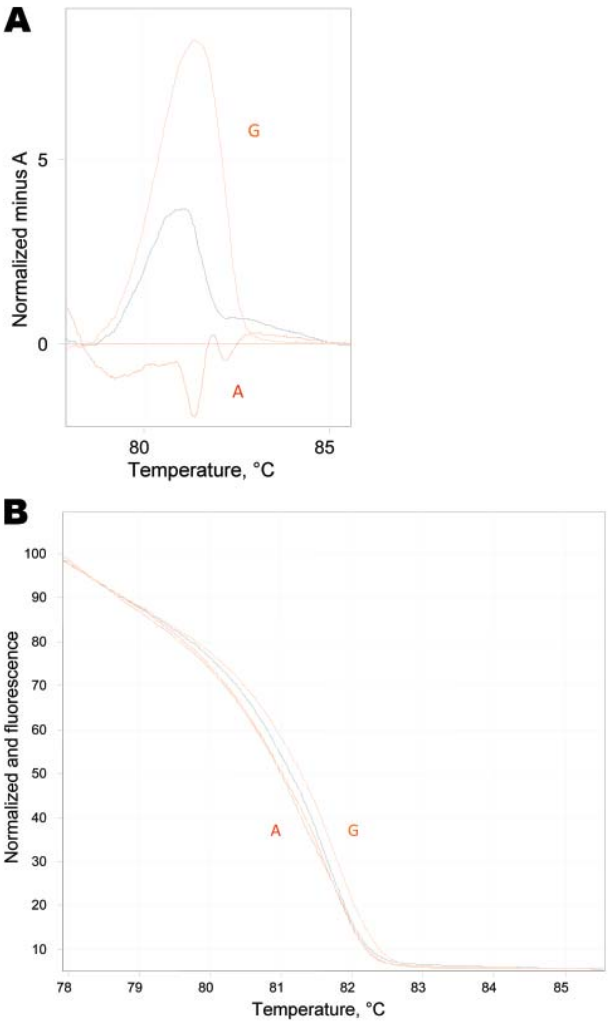

Figure 2. Real-time PCR high-resolution melting assay. A) High-resolution melt profiles are shown for wild-type (WT) *Mycoplasma pneumoniae* A (A2063A), macrolide resistance mutation G (A2063G) sample, and the mixed genotype sample in the normalized graph mode. B) Temperature-shifted difference graph demonstrates the deviations between WT, resistant, and mixed samples. The WT isolate has been selected to normalize the temperature shift graph and displays the deviation of samples from it.

Table. Clinical information of patients positive for *Mycoplasma pneumoniae*, according to macrolide resistance genotype, Israel, 2010

Characteristic	Macrolide-sensitive *M. pneumoniae*, n = 19*	Macrolide-resistant *M. pneumoniae*, n = 9
Mean age, y, ± SD	7 ± 14.98	9 ± 8.39
>21	3	1
Admission, mean d, ± SD	4 ± 6.6	3 ± 8.2
Pneumonia confirmed by chest radiograph	16	9
Male/female	6/13	4/5
Ethnicity (Arab/Jewish)	5/14	1/8
Complications†	3	0
Concurrent conditions‡	4	2
Prior antibiotic use	9	6
Prior macrolide use	3	2

*Values are no. patients except as indicated. Clinical data were not available for 2 patients.
†Two patients had Stevens-Johnson syndrome, and Still's disease developed in 1 patient.
‡Four patients had asthma, 1 had metabolic syndrome with cardiomyopathy, and 1 had carcinoma of the pancreas.

cases is 30%. A2063G was the only resistance-associated mutation we found out of 4 possible mutations the assay we used can find. This is not surprising because this mutation is reportedly responsible for 90.5% of resistant *M. pneumoniae* (*2*). As recently reported from Japan, China, and Germany (*2*,*4*,*7*), we also found resistance in an adult patient. The simultaneous finding of both the macrolide-resistant (A2063G) and the macrolide-sensitive (A2063A) *M. pneumoniae* genotype in at least 1 sample from our propositus patient while he was receiving treatment suggests that the A2063G mutation may have evolved de novo during therapy with azithromycin. A similar phenomenon was observed recently in the closely related pathogen *M. genitalium* (*13*). Additionally, it was shown previously that exposing *M. pneumoniae* to sublethal concentrations of macrolides such as azithromycin can lead to de novo occurrence of macrolide resistance with A2063G mutations (*14*). This finding might suggest that lower concentrations of azithromycin on mucosal surfaces could lead to the induction of macrolide resistance in mycoplasmas. The phenomenon of a mixed population is unusual in *M. pneumoniae*–infected patients; analysis of the P1 gene of 102 *M. pneumoniae* isolates, including those obtained during epidemics (*15*), did not show such a phenomenon. Still, the infecting *M. pneumoniae* population may have been a mixture of both A2063A and A2063G species, which was later dominated by the resistant species. The occurrence of this phenomenon should be studied further in the community to evaluate its distribution and clarify whether it is related to the occurrence of de novo mutation or mixed infections.

Our study emphasizes again the therapeutic challenge in pediatric patients with *M. pneumoniae*–associated infections whose illlnesses do not respond to macrolide treatment. In such cases, we suggest that quinolones be considered as alternative therapy, although they are currently not approved for this indication in the pediatric populations.

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Dr Averbuch is a specialist in infectious diseases and pediatrics at the Hadassah–Hebrew University Medical Center. Her research interests include common pediatric infections and the immunocompromised host and HIV care in the pediatric population.

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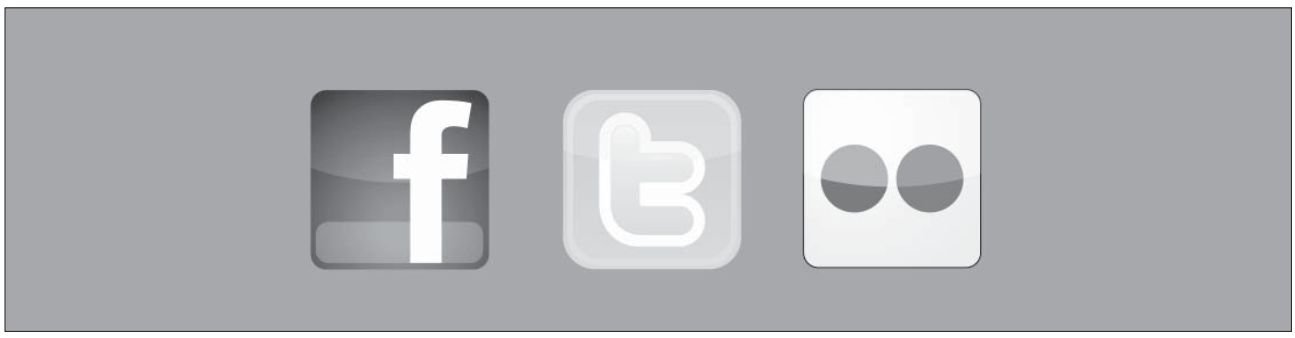
etymologia

Yaws
[yôz]

From either the Carib *yaya*, for sore or lesion, or *yaw*, an African word for berry. The term yaws was in common use by the 17th Century, when Dutch physician Willem Piso provided one of the earliest recorded descriptions of yaws in South America in De medicina Brasiliense in 1648. Because lesions associated with the disease resemble berries, another common name for yaws is frambesia tropica, from the French *framboise*, meaning raspberry.

Sources: Harper D. Online etymology dictionary. Yaws [cited 2011 Apr 7]. http://www.etymonline.com/index.php?search=yaws&searchmode=none; Book notice. Opuscula selecta Neerlandicorum de arte medica [in Dutch]. JAMA. 1937;109:1225 [cited 2011 Apr 7]. http://jama.ama-assn.org/content/109/15/1224.4.full.pdf+html



Outcome Predictors in Treatment of Yaws

Oriol Mitjà, Russell Hays, Anthony Ipai, David Gubaila, Francis Lelngei, Martin Kirara, Raymond Paru, and Quique Bassat

To estimate failure rates after treatment with benzathine penicillin and to identify determinants of failure that affected outcomes for yaws, we conducted a cohort study of 138 patients; treatment failed in 24 (17.4%). Having low initial titers on Venereal Disease Research Laboratory test and living in a village where yaws baseline incidence was high were associated with increased likelihood of treatment failure.

Yaws is a tropical infection of the skin and bones caused by *Treponema pallidum* subsp. *pertenue* and is transmitted by direct, nonsexual contact with infectious lesions (*1,2*). Although a multinational mass eradication campaign in the 1950s greatly reduced the incidence of this disease (*3–5*), a resurgence of yaws has occurred in west and central Africa, Southeast Asia, and the Pacific Islands (*6–9*). The currently recommended drug therapy for yaws is penicillin G benzathine, administered intramuscularly as a single dose of 1.2 million units (*3,5*). *T. pallidum* is a primary human pathogen that has eluded in vitro cultivation (*10*). Hence, although penicillin treatment failure has been reported for yaws (*11*), to date penicillin resistance has not been proven. Most serologically defined treatment failures are thought to be caused by either reinfection after treatment or patient-to-patient variation in the rate of decline in nontreponemal test titers after treatment (i.e., ≥4-fold decrease), rather than by relapse (*10*). The aim of this study was to estimate failure rates after treatment with benzathine penicillin and to identify determinants of failure that affected the outcome.

The Study

We conducted a retrospective cohort study involving patients diagnosed with yaws at Lihir Medical Centre from January through September 2009. Ethics approval was obtained from the Papua New Guinea Ministry of Health Medical Research Advisory Committee. Diagnosis of yaws was based on correlation of the clinical findings, positive serologic results, and epidemiologic history. Patients <15 years of age whose mothers had negative treponemal test results at antenatal screening, with clinical evidence of early yaws (primary or secondary stage), and whose Venereal Disease Research Laboratory (VDRL) and *T. pallidum* hemagglutination test results were positive, were eligible to participate in the study. We included only case-patients with clear documentation of the village of residence, contact history, yaws clinical stage, clinical outcome, pretreatment titer, and at least 1 follow-up titer 12–15 months after treatment. We also estimated the minimum incidence rate for each of the 27 villages served by our hospital; a high incidence rate was defined as >1.5%. This percentage was calculated by dividing the number of new cases diagnosed at Lihir Medical Centre within the study period by the estimated population from each village, according to the local annual census. Treatment outcome was measured at a follow-up visit 12–15 months after treatment. Treatment failure was defined as the lack of a 4-fold decrease in VDRL titers at least 365 days after treatment.

A total of 138 patients were identified during enrollment. Table 1 summarizes patient demographic characteristics, clinical signs and symptoms, laboratory results, and outcomes. Eighty-one (58.7%) persons displayed active primary cutaneous yaws lesions (Figure 1), and 63 (45.7%) exhibited signs of secondary stage yaws (hyperkeratotic skin papules or bone involvement). All patients were administered 3 doses of intramuscular benzathine penicillin 1×/wk, and only 6 (4.4%) children

Table 1. Demographic data, clinical signs/symptoms, laboratory results, and outcome after treatment of yaws in 138 case-patients, Papua New Guinea, January–September, 2009*

Characteristic	Total no. (%) patients, N = 138
Mean age, y (SD)	9.6 (4.4)
Male sex	81 (58.7)
VDRL titer	
16	54 (39.1)
32	33 (23.9)
64	42 (30.4)
128	9 (6.5)
Primary skin lesion	81 (58.7)
Secondary stage	63 (45.7)†
Family history	36 (26.1)
Treatment with IM penicillin G benzathine	138 (100.0)
Clinical healing	132 (95.7)
Concurrent disease	7 (5.1)‡
Seroconversion	63 (45.7)
Serologically defined treatment failure	24 (17.4)

*VDRL, Venereal Disease Research Laboratory; IM, intramuscular.
†Includes 7 cases of early yaws osteoperiostitis, among whom were 3 patients with dactylilitis.
‡Includes 3 cases of *Plasmodium falciparum* malaria, 1 case of *P. vivax* malaria, 1 case of acute diarrhea diagnosed at the 12-month follow-up visit, and 2 case-patients with a chronic underlying disease (1 case of congenital heart disease and 1 case of chronic asthma).

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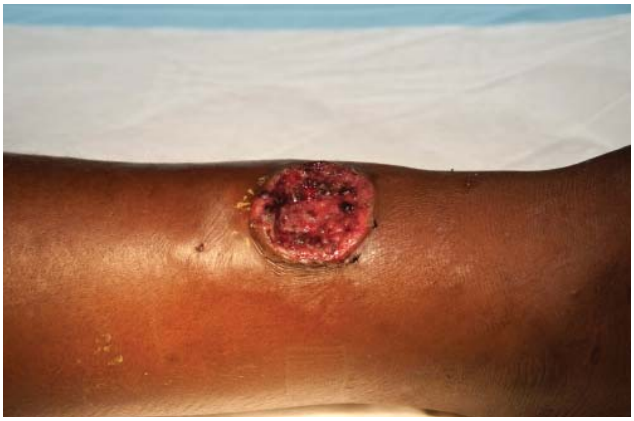

Figure 1. Painless ulcer with raised edges corresponding to a primary yaws skin lesion on an infant case-patient's leg, Papua New Guinea, 2009. Source of photograph: Lihir Medical Centre, Dr Oriol Mitjá.

required 1 or 2 additional doses before initial symptoms disappeared. According to the estimated minimum incidence, in 9 villages the disease was classified as highly endemic, and in 15 villages, the disease was considered of low endemicity (Figure 2). Of the 138 analyzed case-patients, 90 (65.2%) persons came from a high minimum incidence village (HMIV) and 48 (34.8%) came from a low minimum incidence village (LMIV). Secondary stage lesions were found in 47 (52.2%) of 90 case-patients among the HMIV group and only in 16 (33.3%) of 40 case-patients in the LMIV group ($p = 0.035$). VDRL titers were significantly lower in case-patients in the HMIV group than in those in the LMIV group; 70% of case-patients in the HMIV group had a titer ≤32 ($p = 0.026$), as did 50% of those in the LMIV group. A positive association between a low initial VDRL titer and secondary stage disease was also found (79% of case-patients with secondary yaws had a low initial titer, compared with 51% who had primary yaws [$p<0.01$]). Overall, 24 (17.4%) case-patients experienced serologically defined treatment failure during follow-up, including 21 (23.3%) and 3 (6.3%) from the HMIV and LMIV groups, respectively. Multivariate analysis (Table 2) showed that only residence in a high incidence village (odds ratio 3.75, 95% confidence interval 1.02–13.76) and an initial VDRL titer ≤32 (odds ratio 4.05, 95% confidence interval 1.06–15.38) proved to be independent predictors for treatment failure.

Conclusions

Serologically defined treatment failures occurred in ≈17% of case-patients in our series. Treatment failure could have been influenced by the capacity of the infecting agent to develop resistance to the antimicrobial drug used, or the failure could have been caused by other factors related to the human host. Our findings show that in Lihir, the factors predicting treatment failure after 12 months of drug therapy were the following: residence in a village where incidence of infection was high and initial VDRL titer was low. False-positive VDRL reactions, classically associated with viral and autoimmune diseases (*12*), are unlikely to be the cause of failure in our series, because no chronic underlying disease or concurrent febrile illnesses were registered in 23 (96%) of the case-patients who did not achieve a cure. Moreover, the strict epidemiologic criteria required (obtained through patient history) for inclusion in the study aimed to reduce the likelihood of false-positive results for syphilis. A VDRL titer of <32 dilutions proved to be a robust predictor of failure.

In our experience, this low titer is also associated with longer lasting infections and is more commonly found in high incidence villages (as are longer lasting infections). Even after multivariate analysis, clarifying the role of these confounding factors is difficult. We suspect that the true factor at work here rests upon the assumption that a chronic infection is more difficult to resolve. The tissue-to-plasma ratios for bone penetration are usually between 0.1 and 0.3 for penicillins and are even lower for cortical bone than for cancellous bone (*13*). On the basis of these ratios, treponemes that invade the bone would encounter subtherapeutic levels of penicillin, which could simply lead to persistent infection or even provide selective pressure for mutations for penicillin resistance. On the other hand, the risk for reinfection caused by repeated contact with infected children seems to be a pivotal factor in predicting

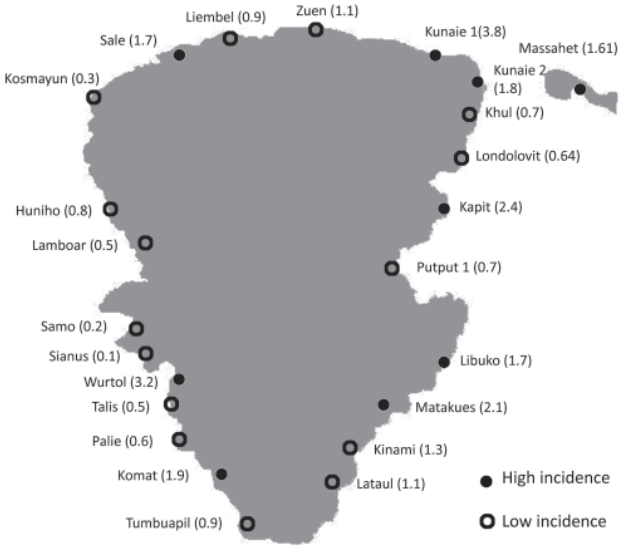

Figure 2. Map of Lihir Island, Papua New Guinea, showing incidence of infection in the 24 villages where cases of yaws were diagnosed, 2009. Lihir Medical Center is located in Londolovit village. Incidence proportions are shown within parentheses.

Table 2. Association between characteristics of case-patients and *Treponema pertenue* infection treatment failure, Papua New Guinea, January–September 2009*

Characteristic	No. (%) patients treated		Univariate analysis		Multivariate analysis	
	Success, n = 114	Failure, n = 24	OR (95% CI)	p value	OR (95% CI)	p value
Mean age, y (SD)	9.54 (4.69)	10.13 (2.96)	0.58 (–1.39 to 2.56)	0.56	NA	NA
Male sex	69 (60.5)	12 (50.0)	0.65 (0.27–1.58)	0.34	NA	NA
Lived in a high-incidence village	69 (60.5)	21 (87.5)	4.57 (1.29–16.20)	0.02	3.75 (1.02–13.76)	0.04
Secondary yaws	49 (43.0)	14 (58.3)	1.86 (0.76–4.53)	0.17	1.01 (0.37–2.75)	0.99
Clinical healing	108 (94.7)	24 (100.0)	NA	0.59	NA	NA
Positive family history	27 (23.7)	9 (37.5)	1.93 (0.76–4.91)	0.17	1.91 (0.70–5.28)	0.20
VDRL titer ≤32	67 (58.8)	21 (87.5)	4.91 (1.39–17.41)	0.01	4.05 (1.06–15.38)	0.04

*p<0.05 was considered significant. OR, odds ratio; CI, confidence interval; NA, not applicable; VDRL, Venereal Disease Research Laboratory test.

treatment failure. The high number of asymptomatic persons or persons with few symptoms in high prevalence areas is the main reservoir of the infection and a known obstacle to achieving the eradication of yaws. A limitation of our study is the use of incidence rates derived from hospital-based detected cases, which likely underestimated the real incidence of infection. Also, the calculation of this proportion might be less precise because we did not take into account factors such as the distance of the village from the health center or the proportion of children to adults in a particular village.

On the basis of our findings, we anticipate that a community-based strategy will be required to effectively control yaws on Lihir Island. The current strategy for eradication of yaws in areas where the disease is moderately endemic (prevalence <5%) is to treat patients with active cases and their contacts. In our experience, most children did not have a family history of yaws. Thus, the disease likely is not clustered in households but, rather, transmission is more likely to occur among children in the community, in schools, and in other public places. Future eradication programs will need to take into account all epidemiologic, biological, and pharmacologic factors, along with the practical considerations of a mass campain to deliver and administer drugs in isolated and underresourced communities. In this context, the potential treating yaws with oral, single-dose therapy, for example, with azithromycin, should be explored.

Acknowledgments

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Dr Mitjà is a physician at the Department of Internal Medicine, Lihir Medical Centre, Papua New Guinea. His specialty is infectious and tropical diseases, and his primary research interests are yaws, Bancroftian filariasis, and *Plasmodium vivax* malaria.

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Increasing Ceftriaxone Resistance in Salmonellae, Taiwan

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In Taiwan, despite a substantial decline of *Salmonella enterica* serotype Choleraesuis infections, strains resistant to ciprofloxacin and ceftriaxone persist. A self-transferable bla_{CMY-2}-harboring IncI1 plasmid was identified in *S. enterica* serotypes Choleraesuis, Typhimurium, Agona, and Enteritidis and contributed to the overall increase of ceftriaxone resistance in salmonellae.

Salmonella enterica serotype Choleraesuis usually causes invasive infection (*1*). When resistant *Salmonella* infection is encountered, fluoroquinolones or extended-spectrum cephalosporins are frequently used (*2*). Fluoroquinolone resistance has been common in this invasive serotype (*3*). Isolation of SC-B67, a strain of *S. enterica* ser. Choleraesuis that was resistant to ciprofloxacin and ceftriaxone (CIP^r/CRO^r), has exacerbated the problem (*4*). Ceftriaxone resistance in SC-B67 was attributed to a plasmid-mediated bla_{CMY-2}, located on a specific IS*Ecp1*-bla_{CMY-2}-*blc*-*sugE* structure (*4*). This conserved DNA fragment, subsequently named Tn*6092* (*5*), has been reported from different geographic areas and is widely distributed among various *Salmonella* serotypes and other Enterobacteriaceae (*6*).

The Study

Since 1999, computerized records of bacterial culture results have been stored at Chang Gung Memorial Hospital, a 3,500-bed medical center in northern Taiwan. Periodic review of these records indicated a reverse trend in the prevalence of serogroups D (increase) and B (decrease) isolates during the past decade (Figure 1, panel A). A significant decrease in the prevalence of *S. enterica* ser. Choleraesuis was also evident (Figure 1, panel A). Nevertheless, in recent years, ceftriaxone resistance has increased from <5% to >10% in *S. enterica* ser. Choleraesuis and in serogroup B salmonellae (Figure 1, panel B).

Since isolation of SC-B67 in 2002 (*4*), 10 CIP^r/CRO^r *S. enterica* ser. Choleraesuis isolates have been recovered. All CIP^r/CRO^r isolates were resistant to nalidixic acid and ciprofloxacin, but SC-B134 remained susceptible to ciprofloxacin (Table 1). PCR and sequencing with specific primers (online Technical Appendix Table 1, www.cdc.gov/EID/content/17/6/1086-Techapp.pdf) revealed 3 identical amino acid changes in GyrA and ParC among all CIP^r/CRO^r isolates except SC-B134 (Table 1). Reduced fluoroquinolone susceptibility of SC-B134 could be explained by the single amino acid change at codon 87 of GyrA. Amino acid changes were not found in GyrB or ParE.

In terms of clinical features (online Technical Appendix Table 2), most patients with these infections were adults who had a wide spectrum of underlying diseases. Antimicrobial agents were prescribed for all patients, and extended-spectrum cephalosporins were used most frequently. Two patients died. Seven blood isolates from the 10 patients with CIP^r/CRO^r *S. enterica* ser. Choleraesuis infections, together with the ceftriaxone-resistant isolates noted below, were investigated further. SC-B67 was used as a reference.

During the first 6 months of 2010, a total of 6 cases of ceftriaxone-resistant *Salmonella* infection were found: serogroup B in 5 patients (*S. enterica* ser. Agona, n = 1; *S.*

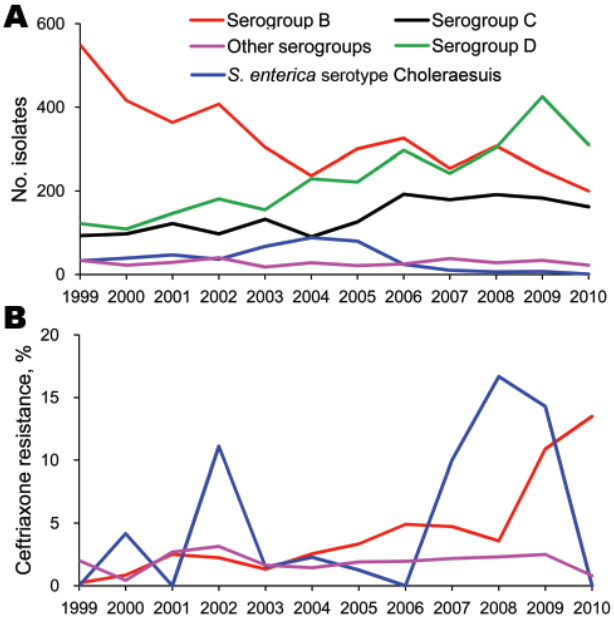

Figure 1. Secular trends in annual numbers (A) and rates (B) of ceftriaxone resistance among various serogroups or serotype of nontyphoidal *Salmonella enterica* isolates in Chang Gung Memorial Hospital, 1999–2010.

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Table 1. Characteristics of the resistant *Salmonella enterica* isolates from 17 patients at Chang Gung Memorial Hospital, Taiwan*

Serotype and isolate (no. patients)	Year	Type of specimen	Susceptibility profile†	Puls‡	Plasmid profile, kb§	DNA–DNA hybridization¶			PCR sequencing#		
							rep		*gyrA*		*parC*
						spvC	FIIA/FIB	Rep_3	Ser(83)	D(87)	Ser(80)
Choleraesuis											
SC-B67	2002	Blood	R/R/R	C-1-a	50, **138**	50	50	138	F	N	I
SC-B104 (1)	2003	Blood	R/R/R	C-1-b	50, **150**	50	50	150	F	N	I
SC-B93 (2)	2003	Blood	R/R/R	C-1-b	50, **115**	50	50	50,115	F	N	I
SC-B98 (3)	2004	Blood	R/R/R	C-1-c	50, **138**	50	50	138	F	N	I
NA (4)	2004	Pus	R/R/R	NA	NA	NA	NA	NA	NA	NA	NA
SC-B131 (5)	2004	Blood	R/R/R	C-1-d	50, **138**	50	50	138	F	N	I
SC-B132 (6)	2004	Blood	R/R/R	C-1-b	50, **150**	50	50	150	F	N	I
NA (7)	2005	Pus	R/S/R	NA	NA	NA	NA	NA	NA	NA	NA
SC-B134 (8)	2007	Blood	R/S/R†	C-1-e	40, 65, **105**	65	65	40	Ser	N	Ser
SC-B136 (9)	2008	Blood	R/R/R	C-2	115, **138**	115	115	115	F	N	I
NA (10)	2009	Pus	R/S/R	NA	NA	NA	NA	NA	NA	NA	NA
Typhimurium var. Copenhagen SB-5	2010	Feces	R/R/R	B-1-a	7, **125**, 180, 260	Neg	Neg	Neg	Ser	D	Ser
Typhimurium											
SB-28	2010	Urine	R/R/S	B-2	**115**, 210	Neg	Neg	Neg	Ser	D	Ser
SB-151	2010	Feces	S/S/S	B-1-b	**85**	Neg	Neg	Neg	Ser	D	Ser
SB-193	2010	Feces	R/R/S	B-2	**105**, 210	Neg	Neg	Neg	Ser	D	Ser
Agona SB-105	2010	Feces	R/S/S	B-3	**95**	Neg	Neg	Neg	Ser	D	Ser
Enteritidis SD-166	2010	Feces	R/S/S	D-1	45, 60, **95**	60	Neg	Neg	Ser	D	Ser

*Puls, pulsotype; R, resistant; F, phenylalanine; N, asparagine; I, isoleucine; NA, not available; S, susceptible; ser, serine; neg, negative reaction; var., variant; D, aspartic acid.
†Antimicrobial drug susceptibility to chloramphenicol, trimethoprim/sulfamethoxazole, and quinolones. Results were the same for the 2 quinolones (nalidixic acid and ciprofloxacin) tested, except that SC-B134 was resistant to nalidixic acid and susceptible to ciprofloxacin. All isolates were resistant to ampicillin and ceftriaxone.
‡Pulsed-field gel electrophoresis; pulsotypes are expressed as serogroup-major type-subtype.
§Plasmids harboring the bla_{CMY-2}-carrying Tn*6092* element are shown in **boldface**. IncI1 plasmids are underlined. Both are evidenced by DNA–DNA hybridization.
¶The size (kb) of the plasmid showing positive results in the respective DNA–DNA hybridization experiments is indicated. Rep_3, replicon of pSC138, the Tn*6092*-containing resistant plasmid of strain SC-B67.
#Amino acid changes compared with the quinolone resistance–determining regions of *gyrA* (codons 83 and 87) and *parC* (codon 80) in *S. enterica* serotype Typhimurium LT2. No mutation was found in *gyrB* and *parE*.

enterica ser. Typhimurium, n = 4, including 1 Copenhagen variant) and serogroup D (*S. enterica* ser. Enteritidis) in 1 patient (Table 1). All isolates were derived from fecal specimens of patients <3 years of age, except *S. enterica* ser. Typhimurium SB-28, which was isolated from the urine of a 77-year-old patient. In contrast to *S. enterica* ser. Choleraesuis, these ceftriaxone-resistant isolates generally remained susceptible to fluoroquinolones (Table 1).

Pulsed-field gel electrophoresis performed as described showed close association among all *S. enterica* ser. Choleraesuis isolates, including SC-B67 (Table 1; Figure 2, panel A) (*7*). Only strain SC-B136, recovered in 2008, demonstrated a relatively different pattern. Two pulsotypes, with minor differences, were found among the 4 *S. enterica* ser. Typhimurium isolates (Table 1).

Ceftriaxone resistance was investigated by using PCR and sequencing as described (*6*). The specific bla_{CMY-2}-carrying Tn*6092* was present in all isolates tested (Table 1). Tn*6092* was located within a *finQ* gene at a position identical to that in SC-B67. The only difference was in SC-B134; a 1,338-bp insertion sequence, IS*10*, was inserted 262 bp upstream of the bla_{CMY-2}. No CTX-M and SHV genes were found in these isolates.

Using an alkaline lysis method (*8*), we found various numbers of plasmids among the isolates studied (Table 1). DNA–DNA hybridization indicated that Tn*6092* was located on large (85-kb to 150-kb) plasmids (Table 1) (*9*). An identical Rep_3 replicon was found in all CIP^r/CRO^r *S. enterica* ser. Choleraesuis isolates studied (*5*). Similar to SC-B67, the Rep_3 replicon was located on the Tn*6092*-harboring resistance plasmid in the 5 resistant isolates recovered before 2004 (Table 1). However, in SC-B134, the Rep_3 replicon was found on the smaller 40-kb plasmid, and in SC-B136, the Rep_3 replicon was on the 115-kb large virulence plasmid that contained the *spvC* gene (Table 1; Figure 2, panels B, C). Replicons FIIA and FIB were simultaneously present in all the *spvC*-containing virulence plasmids among the *S. enterica* ser. Choleraesuis isolates studied (Table 1). Virulence plasmids in the 2 recent isolates, SC-B134 and SC-B136, appeared larger than those in earlier isolates, including SC-B67 (Table 1; Figure 2, panels B, C). The 2 bands

demonstrated by the *spvC* probe in SC-B134 were from the same single virulence plasmid, as proven by hybridization experiments on *Bam*HI-digested plasmid DNA of SC-B134 (data not shown).

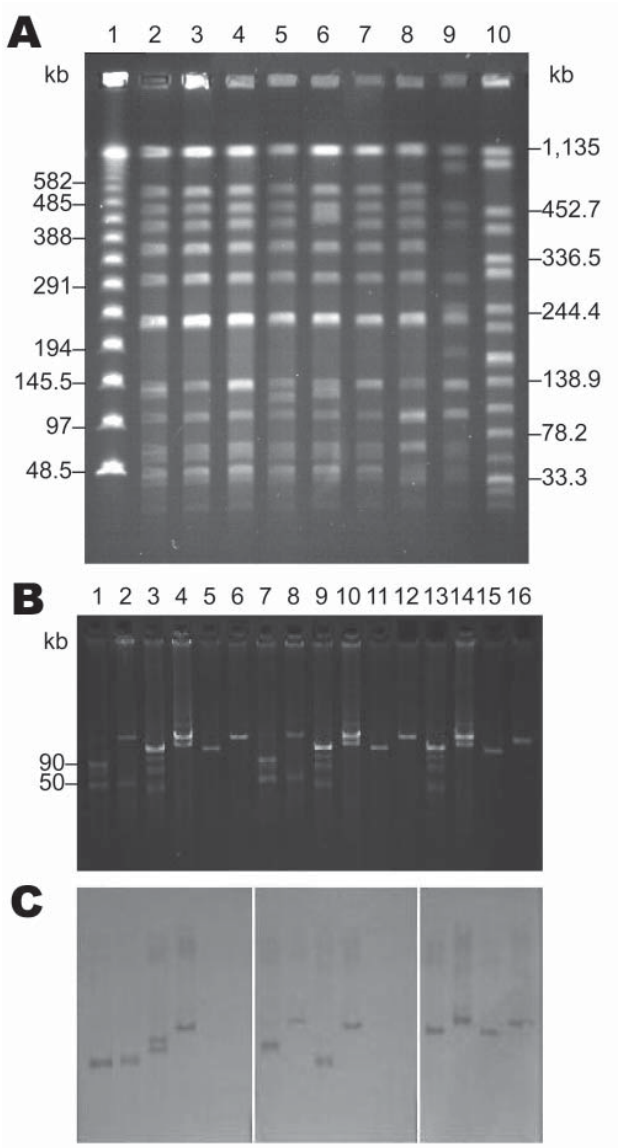

Figure 2. Analyses of *Salmonella enterica* serotype Choleraesuis isolates from Chang Gung Memorial Hospital, 1999–2010. A) Pulsed-field gel electrophoresis patterns. Lanes 1 and 10, DNA size markers demonstrated by a λ DNA concatemer standard and *S. enterica* ser. Braenderup H9812, respectively; lanes 2 to 9, *S. enterica* ser. Choleraesuis SC-B67, SC-B104, SC-B93, SC-B98, SC-B131, SC-B132, SC-B134, and SC-B136. B) Plasmid analysis and C) DNA–DNA hybridization. Probes for DNA–DNA hybridization of lanes 1–6, 7–12, and 13–16 were prepared from amplicons of *spvC*, Rep_3 replicon of pSC138 in SC-B67, and repI1, respectively; lanes 1 and 7, *S. enterica* ser. Choleraesuis OU7529 containing 2 plasmids of known sizes, 50 kb and 90 kb, was used as the size marker; lanes 2 and 8, SC-B67; lanes 3, 9, and 13, SC-B134; lanes 4, 10, and 14, SC-B136; lanes 5, 11, and 15, *Escherichia coli* J53/pSC-B134; lanes 6, 12, and 16, *E. coli* J53/pSC-B136.

Replicon typing through a published multiplex PCR system revealed a replicon I1 from the Tn*6092*-harboring resistance plasmids in SC-B134 and SC-B136 (Table 1; Figure 2, panel C) (*10*). Similarly, Tn*6092*-carrying plasmids among the other ceftriaxone-resistant salmonellae isolates all belonged to the IncI1 group (Table 1). Conjugation experiments using a filter mating method showed that all IncI1 resistant plasmids were self-transferrable (*11*). With azide-resistant *Escherichia coli* J53 and *S. enterica* ser. Typhimurium LBNP4417 as the recipients, the IncI1-resistant plasmids were confirmed to be self-transferrable.

Subtyping of the 8 conjugated IncI1 plasmids was achieved by using a recently described plasmid multilocus sequence typing (pMLST) method specifically set up for IncI1 plasmids (*12*). Six combinations of allele variants were obtained (Table 2). Because these pMLST patterns differed from those reported elsewhere, 6 new sequence types (STs) were designated (Table 2). Two major groups were further derived: ST54 (pSB28, pSB193) and ST52 (pSC-B136) that differed only in *trbA*, and ST56 (pSD166, PSB105) and ST53 (pSB5) that only differed in *pilL* (Table 2). pMLST patterns of representative bla_{CMY-2}-carrying IncI1 plasmids published in recent years (Table 2) were derived from *E. coli* or various *Salmonella* serotypes in Europe or North America (*12–14*). Nine STs and 2 major clonal complexes, CC-2 and CC-12, were observed. pMLST patterns found in the present study differed from these STs by at least 3 alleles (Table 2).

Conclusions

Resistance to ciprofloxacin and ceftriaxone remains high, indicating persistence of antimicrobial drug–resistant traits in *S. enterica* ser. Choleraesuis. The conserved genotypes found in the clinical isolates suggest a mode of clonal dissemination. However, plasmid analysis indicates that the location of the Tn*6092*-containing resistance element had shifted from the nonconjugative Rep_3 plasmids in early isolates to the self-transferable IncI1 plasmids in recent isolates. The emergence of such self-transferable resistance plasmids seems to provide an efficient way for *S. enterica* ser. Choleraesuis to spread its ceftriaxone resistance trait.

Because infections with nontyphoid salmonellae are rampant in Asia, emergence of a conjugative IncI1 resistance plasmid in ceftriaxone-resistant salmonellae from an Asian country is of public health concern. Presence of bla_{CMY-2}-carrying IncI1 plasmids in a variety of *Salmonella* serotypes has been reported, but to our knowledge, not in *S. enterica* serotypes Enteritidis or Choleraesuis (*12–14*). IncI1 plasmids of the same or similar STs have been found in isolates of different bacterial species; with different resistance genes; or from different countries or sources, including human,

Table 2. Characteristics of conjugative *bla*$_{CMY-2}$-harboring IncI1 plasmids derived in this study and comparison of pMLST patterns with similar plasmids published previously*

IncI1 plasmid	*Salmonella enterica* serotype or *Escherichia coli*	Susceptibility profile†	Country	Year of isolation	pMLST‡ repI1	*ardA*	*trbA*	*sogS*	*pilL*	ST§	Clonal complex	Reference
pSC-B134	Choleraesuis	R/S/S	Taiwan	2007	1	1	15	9	3	51	NA	This study
pSC-B136	Choleraesuis	R/R/S	Taiwan	2008	1	4	15	11	2	52	NA	This study
pSB-5	Typhimurium variant Copenhagen	R/R/R	Taiwan	2010	2	1	15	11	2	53	NA	This study
pSB28	Typhimurium	S/S/S	Taiwan	2010	1	4	5	11	2	54	NA	This study
pSB151	Typhimurium	S/S/S	Taiwan	2010	4	5	15	11	3	55	NA	This study
pSB193	Typhimurium	S/S/S	Taiwan	2010	1	4	5	11	2	54	NA	This study
pSB105	Agona	R/S/S	Taiwan	2010	2	1	15	11	3	56	NA	This study
pSD166	Enteritidis	R/S/S	Taiwan	2010	2	1	15	11	3	56	NA	This study
398T	*E. coli*	NA	Italy	2006	1	2	3	2	1	2	CC-2	(*12*)
05–1909	Heidelberg	NA	Canada	2005	1	2	3	2	1	2	CC-2	(*13*)
1358T	Thompson	NA	USA	1996	1	3	3	4	1	4	CC-12	(*12*)
DH-20406	Heidelberg	NA	USA	2004	1	4	3	4	1	12	CC-12	(*14*)
06–3048	4,5,12:i:–	NA	Canada	2006	1	4	3	4	1	12	CC-12	(*13*)
06–3539	Agona	NA	Canada	2006	1	4	3	4	1	12	CC-12	(*13*)
N07–0084	*E. coli*	NA	Canada	2005	1	4	3	4	1	12	CC-12	(*13*)
05–2835	Heidelberg	NA	Canada	2005	1	4	3	4	1	12	CC-12	(*13*)
06–3985	Litchfield	NA	Canada	2006	1	4	3	4	1	12	CC-12	(*13*)
05–5567	Typhimurium	NA	Canada	2005	1	4	3	4	1	12	CC-12	(*13*)
N06–523	*E. coli*	NA	Canada	2006	1	4	3	2	1	18	CC-2/ CC-12	(*13*)
N06–0537	*E. coli*	NA	Canada	2006	1	3	3	4	3	19	CC-12	(*13*)
05–6117	4,5,12:1:–	NA	Canada	2006	1	1	3	9	1	20	NA	(*13*)
N07–0093	*E. coli*	NA	Canada	2005	1	1	3	9	1	20	NA	(*13*)
06–0753	Heidelberg	NA	Canada	2006	1	2	11	3	3	21	CC-5	(*13*)
N07–0079	*E. coli*	NA	Canada	2005	1	6	3	4	1	22	CC-12	(*13*)
N07–0081	*E. coli*	NA	Canada	2005	1	2	3	1	1	23	CC-2	(*13*)

*pMLST, plasmid multilocus sequence typing; ST, sequence type; R, resistant; S, susceptible; NA, not applicable.
†Antimicrobial drug susceptibility to chloramphenicol, trimethoprim/sulfamethoxazole, and quinolones (nalidixic acid and ciprofloxacin). The transconjugants were also resistant to ampicillin and ceftriaxone.
‡pMLST results were compared to those published in the plasmid MLST website (http://pubmlst.org/plasmid).
§STs were designated according to the different combinations of allele variants observed among the IncI1 plasmids.

animals, and the environment (*12–15*). Emergence of the IncI1 plasmid in Taiwan represents a need for continuous efforts to monitor and control its further spread.

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Salmonella enterica Serotype Typhi with Nonclassical Quinolone Resistance Phenotype

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We report *Salmonella enterica* serotype Typhi strains with a nonclassical quinolone resistance phenotype (i.e., decreased susceptibility to ciprofloxacin but with susceptibility to nalidixic acid) associated with a nonsynonymous mutation at codon 464 of the *gyrB* gene. These strains, not detected by the nalidixic acid disk screening test, can result in fluoroquinolone treatment failure.

Typhoid fever caused by *Salmonella enterica* serotype Typhi (hereafter referred to as *Salmonella* Typhi) remains a major health problem in the developing world (*1*). Treatment with appropriate antimicrobial drugs has become hampered by gradual plasmid-mediated resistance to ampicillin, chloramphenicol, and cotrimoxazole, particularly in southern and Southeast Asia (*2*). Consequently, since the early 1990s, fluoroquinolones (such as ofloxacin and ciprofloxacin [Cip]) have been widely used. However, multidrug-resistant *Salmonella* Typhi isolates that are also resistant to nalidixic acid (Nal^R) (MIC >256 μg/mL) and show decreased susceptibility to Cip (Cip^{DS}) (MIC range, 0.125 μg/mL–1 μg/mL) have emerged and become endemic on the Indian subcontinent and in Southeast Asia (*3*–*5*). This resistance to quinolones was caused by amino acid substitutions in the quinolone resistance–determining region (QRDR) of the DNA gyrase subunit *gyrA*, a key target of quinolones. Because these Nal^R–Cip^{DS} *Salmonella* Typhi strains have been associated with slower clinical responses to fluoroquinolones and treatment failures, clinical laboratories should attempt to identify these isolates (*3,6,7*). However, despite the accumulation of clinical, microbiologic, and pharmacokinetic–pharmacodynamic studies suggesting a resistance breakpoint of ≥0.125 μg/mL for ciprofloxacin, the clinical breakpoints published by the Clinical and Laboratory Standards Institute (CLSI) (susceptibility ≤1 μg/mL, resistance ≥4 μg/mL) and those from the antibiogram committee of the French Society for Microbiology (susceptibility ≤0.5 μg/mL, resistance ≥1 μg/mL) (www.sfm.asso.fr/nouv/general.php?pa=2) have not been reevaluated (*6*–*9*). Use of these standard breakpoints has probably resulted in the underreporting of Cip^{DS} *Salmonella* Typhi strains. The Nal^R screening test has been proposed as an alternative since the mid–1990s and recommended since 2004 by CLSI and 2010 by the French Society for Microbiology (*3,7*). This screening test is based on the fact that Cip^{DS} *Salmonella* Typhi isolates with nonsynonymous (NS) mutations in codons 83 or 87 of *gyrA* are uniformly Nal^R. However, recent reports have indicated that this approach cannot identify the newly described *Salmonella* Typhi isolates that are Nal susceptible (Nal^S)–Cip^{DS} for which mechanisms of resistance are not linked to mutations in *gyrA* (*7,10,11*). Recently, NS mutations in codons 464 (Ser to Phe) and 466 (Glu to Asp) of *gyrB* were found in 7 Nal^S–Cip^{DS} *Salmonella* Typhi isolates (*12*). We present data on the occurrence and characterization of the resistance mechanisms of Nal^S–Cip^{DS} isolates in 685 *Salmonella* Typhi isolates of the French National Reference Center for *Salmonella* (FNRC-Salm).

The Study

In France, laboratory surveillance of typhoid fever infections is performed by the FNRC-Salm through its network of ≈1,500 hospital and private clinical laboratories. Almost all *Salmonella* Typhi isolates in France are referred to the FNRC-Salm, and almost all are acquired abroad, mainly in Africa and Asia. Until 2009, Cip^{DS} *Salmonella* Typhi was monitored with the 30-μg Nal screening test. A total of 685 *Salmonella* Typhi isolates collected during 1997–2009 were reanalyzed to identify Nal^S–Cip^{DS} *Salmonella* Typhi isolates. The scattergram correlating the zone diameters around the 5-μg ciprofloxacin disk with those of the 30-μg Nal disk showed 4 subpopulations, which were labeled A (554 isolates), B (11 isolates), C (119 isolates), and D (1 isolate) (Figure 1). The characteristics of these populations are shown in Tables 1 and 2. The QRDRs of *gyrA*, *gyrB*, *parC,* and *parE* genes were studied on 133 isolates selected to represent diversity in terms of year of isolation, geographic origin, and MICs. To analyze the isolate characteristics, we used the following approaches: sequencing (*5*), denaturing high performance liquid chromatography (*4*), and Luminex-based genotyping assays (*12*). QRDR DNA sequences were compared with those of *Salmonella* Typhi strain Ty2

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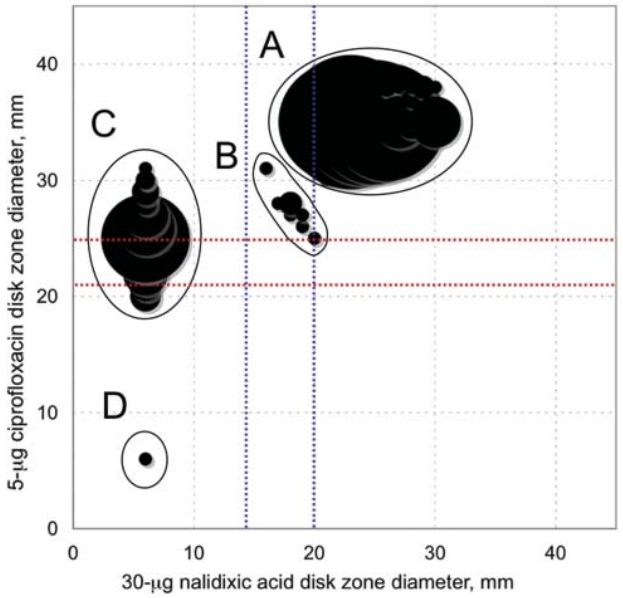

Figure 1. Scattergrams for 685 *Salmonella enterica* serotype Typhi isolates correlating the zone diameters around the 5-µg ciprofloxacin disk with those of the 30-µg nalidixic acid disk. Circle sizes are proportional to the number of isolates. Red lines indicate the respective antibiogram committee of the French Society for Microbiology breakpoints for ciprofloxacin (susceptible [S] ≥25; resistant [R] <22 mm). Blue lines indicate the respective French Society for Microbiology breakpoints for nalidixic acid (S ≥20 and R <15 mm). Letters indicate 4 subpopulations.

(GenBank accession no. AE014613). In subpopulation A, 75 isolates had wild-type QRDR sequences, whereas 2 isolates had a *gyrB* mutation at codon 465 leading to amino acid substitution Gln to Leu. Their Nal MICs were 2 and 4 µg/mL, respectively, and those of Cip were 0.04 µg/mL and 0.08 µg/mL, respectively. Notably, both isolates were acquired in Mexico during 1998 and 2009, respectively. In subpopulation C, the lowest MIC values for Cip (0.06 µg/mL) were associated with a mutation at codon 87 of the *gyrA* gene, whereas MICs did not increase with the additional mutation in the *parE* gene. Subpopulation D consisted of 1 isolate, highly resistant to ciprofloxacin, which was acquired by a traveler in India in 2004. This isolate contained 2 NS mutations in the *gyrA* gene and 1 in the *parC* gene.

Eleven isolates of subpopulation B were categorized as susceptible to Nal by determining MICs and by using CLSI breakpoints (susceptibility, ≤16 µg/mL; resistance, ≥32 µg/mL). Of the 11 isolates, 8 (from 7 patients) had a ciprofloxacin MIC ≥0.125 µg/mL and were thus classified as Cip^{DS} isolates. We were able to review the medical records of 2 patients infected with a Nal^{S}–Cip^{DS} isolate. One patient (isolates 08-7675 and 09-1986) relapsed 15 days after completion of the treatment (oral ofloxacin, 200 mg 2×/d for 8 days) (*13*). The second patient (isolate 05-2556) was treated with extended-spectrum cephalosporins, and no fluoroquinolones. Regarding the resistance mechanisms the plasmid-mediated quinolone resistance–conferring genes *qnr (qnrA, B, S, D), qepA,* and *aac(6')-Ib-cr* were not detected by PCR (*5,14*). The QRDRs of *gyrA*, *parC*, and *parE* genes were of a wild type, whereas an NS mutation was found in *gyrB* for all but 1 isolate. However, only the 8 isolates with mutations at codon 464 were Nal^{S}–Cip^{DS}. To assess whether these isolates were genetically related, haplotyping (*4*) and *Xba*I-pulsed-field gel electrophoresis (PFGE) subtyping (*5*) were performed. On the strength of the results, we concluded that the *gyrB* mutation was acquired independently by strains belonging to different PFGE types (Figure 2). According to a newly developed single nucleotide polymorphism assay (Y.S.), 2 of these strains belong to the current emerging H58 Asian population (*4*), whereas the others do not (Table 2). In our study, the Nal^{S}–Cip^{DS} isolates with *gyrB* mutations at codon 464 were most often non–multidrug-resistant and acquired mainly in India. Our first Nal^{S}–Cip^{DS} isolate was isolated 13 years ago, and since is rare (prevalence ≈1%.). Although Cooke et al. (*10*) did not characterize isolates for their resistance mechanisms, they reported that Nal^{S}–Cip^{DS} represented 11.6% (49/421) of *Salmonella* Typhi isolated in England, Scotland, and Wales during 1999–2003, while Lynch et al. (*11*) reported that such isolates were 4.6% (36/770) of *Salmonella* Typhi isolates identified in the United States

Table 1. Characteristics of the 674 *Salmonella enterica* serovar Typhi isolates belonging to subpopulations A, C, and D, France, 2007–2009*

Subpopulation	No. isolates	Nal MICs,† µg/mL			Cip MICs,† µg/mL			QRDR mutation (no./no. tested)
		MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	
A	554	4	4	1–8	0.008	0.025	0.002–0.08	WT (75/77)
								gyrB Leu465 (2/77)
C	119	>256	>256	128–>256	0.25	0.5	0.06–0.5	*gyrA* Phe83 (24/44)
								gyrA Tyr83 (12/44)
								gyrA Asn87 (4/44)
								gyrA Gly87 (2/44)
								gyrA Phe83 and *parE* Asn420 (2/44)
D	1			>256			8	*gyrA* Phe83, *gyrA* Asn87, and *parC* Ile80

*Nal, nalidixic acid; Cip, ciprofloxacin; QRDR, quinolone resistance–determining region of the *gyrA*, *gyrB*, *parC,* and *parE* genes; WT, wild type.
†MICs of Nal and Cip were determined by Etest strips. MIC_{50}, 50% below; MIC_{90}, 90% below.

Table 2. Characteristics of the 11 *Salmonella enterica* serovar Typhi isolates belonging to subpopulation B, France, 2007–2009*

Isolate	Year	Geographic origin	Antimicrobial drug resistance type	Disk diffusion, mm		MIC, µg/mL		*gyrB*	Haplotype	PFGE
				Nal	Cip	Nal	Cip			
97-5123	1997	Unknown	Cip^{DS}	18 [I]	28 [S]	8 [S/S]	0.125 [S/S]	Tyr464	Non-H58	X8
02-2759	2002	India	Cip^{DS}	19 [I]	26 [S]	4 [S/S]	0.125 [S/S]	Phe464	H58	X2
05-1578	2005	India	Pansusceptible	18 [I]	28 [S]	8 [S/S]	0.047 [S/S]	Asp466	Non-H58	X6
05-2556	2005	India	Cip^{DS}	17 [I]	31 [S]	16 [I/S]	0.19 [S/S]	Phe464	Non-H58	X7
05-9141	2005	India	Cip^{DS}	17 [I]	28 [S]	12 [I/S]	0.125 [S/S]	Tyr464	Non-H58	X3
06-426	2006	India	Cip^{DS}	20 [S]	25 [S]	8 [S/S]	0.125 [S/S]	Tyr464	Non-H58	X3
07-6086	2007	Tunisia	Pansusceptible	16 [I]	31 [S]	16 [I/S]	0.047 [S/S]	WT	ND	ND
08-7675†	2008	India	$ASCSulTmpSXTCip^{DS}$	18 [I]	28 [S]	8 [S/S]	0.125 [S/S]	Phe464	H58	X1
09-1986†	2008	India	$ASCSulTmpSXTCip^{DS}$	18 [I]	27 [S]	8 [S/S]	0.125 [S/S]	Phe464	ND	X1
09-0350	2009	Unknown	Cip^{DS}	19 [I]	27 [S]	8 [S/S]	0.125 [S/S]	Phe464	Non-H58	X5
09-2317	2009	French Guyana	Pansusceptible	19 [I]	32 [S]	8 [S/S]	0.032 [S/S]	Glu468	Non-H58	X4

*PFGE, pulsed-field gel electrophoresis; Nal, nalidixic acid; Cip, ciprofloxacin; WT, wild type; ND, not determined; A, ampicillin; S, streptomycin, C, chloramphenicol; Su, sulfamethoxazole; Tmp, trimethoprim; SXT, cotrimoxazole; Cip^{DS}, decreased susceptibility to ciprofloxacin. Disk diffusion test was performed and interpreted ([S], susceptible; [I], intermediate) following recommendations of antibiogram committee of the French Society for Microbiology. MICs were determined by Etest strips, and categorization was made according to French Society for Microbiology and Clinical and Laboratory Standards Institute values.
†Previously described same patient (*13*).

during 1999–2006. Epidemiologic data were available for 39 isolates in the British study, 18 of which were acquired in India, 8 in Pakistan, and 4 in Bangladesh (*10*). The 10-fold difference in the prevalence observed between our study and that of Cooke et al. are probably related to the historical links and the subsequent population flow between the United Kingdom and the Indian subcontinent.

Conclusions

Nal^S–Cip^{DS} *Salmonella* Typhi isolates originating from Asia comprise ≈1% of *Salmonella* Typhi isolates in France but are more prevalent in the United States and the United Kingdom. The NS *gyrB* mutation at codon 464 was found exclusively in Nal^S–Cip^{DS} isolates; however, the effects of this mutation need to be formally demonstrated by site-directed mutagenesis. Furthermore, the involvement of an efflux system, such as AcrAB-TolC and OqxA, or the *qnrC* gene, have not been investigated and cannot be excluded.

Whatever the molecular mechanism of resistance of such strains, the main concern is detection of such isolates in clinical practice to prevent fluoroquinolone treatment failures. Consequently, the Nal^R screening test should no longer be recommended and ciprofloxacin drug MICs should be determined for all *Salmonella* Typhi isolates instead. There is also a clear need to reevaluate the clinical breakpoints for this pathogen.

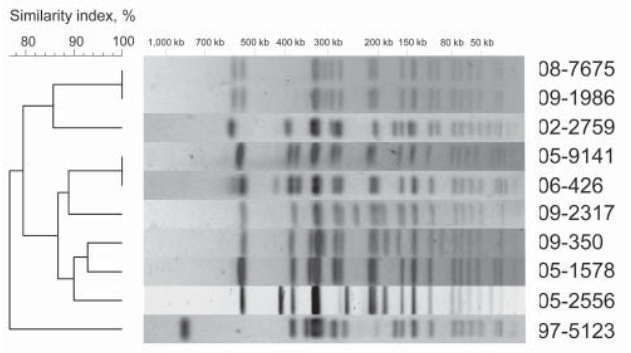

Figure 2. *Xba*I pulsed-field gel electrophoresis (PFGE) profiles obtained from 10 *Salmonella enterica* serotype Typhi isolates belonging to subpopulation B. The dendrograms generated by BioNumerics version 3.5 software (Applied Maths, Sint-Martens-Latem, Belgium) show the results of cluster analysis on the basis of PFGE fingerprinting. Similarity analysis was performed by using the Dice coefficient, and clustering was done by using the unweighted pair-group method with arithmetic averages.

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Ciprofloxacin-Resistant *Salmonella enterica* Serotype Typhi, United States, 1999–2008

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We report 9 ciprofloxacin-resistant *Salmonella enterica* serotype Typhi isolates submitted to the US National Antimicrobial Resistance Monitoring System during 1999–2008. The first 2 had indistinguishable pulsed-field gel electrophoresis patterns and identical *gyrA* and *parC* mutations. Eight of the 9 patients had traveled to India within 30 days before illness onset.

Typhoid fever, caused by *Salmonella enterica* serotype Typhi, is a systemic bacterial illness that has been rare in the United States in the era of modern sanitation (*1*,*2*). However, typhoid fever remains common in many developing countries. In the United States, 72%–81% of patients with typhoid fever report international travel in the month before illness onset (*1*,*3*–*5*). Highest risk has been associated with travel to southern Asia (*1*–*5*).

Fluoroquinolones (e.g., ciprofloxacin) are frequently used to treat typhoid fever in adults (*4*,*6*). Ciprofloxacin resistance is rare; however, resistance to the quinolone nalidixic acid in the US National Antimicrobial Resistance Monitoring System (NARMS) increased from 19% of isolates tested in 1999 to 59% in 2008 (*7*). Nalidixic acid resistance in *S. enterica* serotype Typhi, which has been associated with overseas travel, particularly to southern Asia, correlates with decreased susceptibility to ciprofloxacin (MIC ≥0.12 μg/mL) (*4*–*6*,*8*). Increased risk for fluoroquinolone treatment failure has been demonstrated in *Salmonella* infections from strains with decreased susceptibility to ciprofloxacin (*6*,*8*,*9*). Chromosomal point mutations in the *gyrA* and *parC* topoisomerase genes are mechanisms of quinolone resistance in *Salmonella* spp. Other resistance mechanisms include efflux pumps, reduced outer membrane permeability, and plasmid-borne genes (e.g., *qnr*, *aac-6'-Ib-cr* genes) (*6*,*8*,*10*–*12*). We report 9 ciprofloxacin-resistant (MIC ≥4 μg/mL) *S. enterica* serotype Typhi isolates detected in the United States during 1999–2008.

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The Cases

State public health laboratories receive *Salmonella* isolates from clinical diagnostic laboratories as part of routine surveillance. State and local health department officials report demographic, clinical, and travel information about laboratory-confirmed typhoid fever on a standard form to the Centers for Disease Control and Prevention (CDC, Atlanta, GA, USA). Participating states began submitting all *S. enterica* serotype Typhi isolates to NARMS in 1999; since 2003, all state public health laboratories have participated. Isolates were tested for susceptibility by using broth microdilution (Sensititre: Trek Diagnostics, Westlake, OH, USA). MICs were determined for 15 antimicrobial agents and interpreted by using Clinical and Laboratory Standards Institute (CLSI) criteria when available (Table 1) (*7*,*13*). For ciprofloxacin-resistant isolates, subtyping by pulsed-field gel electrophoresis (PFGE) was performed by using the protocol established by the National Molecular Subtyping Network for Foodborne Disease Surveillance (PulseNet) (*14*). PFGE pattern similarity was assessed by cluster analysis (Dice, UPGMA [unweighted pair group method using arithmetic averages]) and band-matching applications of BioNumerics software (Applied Maths, Sint-Martens-Latem, Belgium) and confirmed by visual comparison (Figure). For ciprofloxacin-resistant isolates detected for 1999–2005, sequencing of the quinolone resistance–determining region (QRDR; defined as amino acids 67–106 for *gyrA*) was performed according to the methods described by Crump et al. (*6*), and additional patient information (e.g., antimicrobial drug treatment) was requested by using a questionnaire with institutional review board approval.

During 1999–2005, we detected 2 (0.1%) cases of ciprofloxacin resistance among 1,690 *S. enterica* serotype Typhi isolates. Case reports follow.

In 2003, a 1-year-old girl had onset of fever 1 day before arriving in the United States from India. A blood specimen collected 3 days after fever onset yielded *S. enterica* serotype Typhi. Diarrhea or vomiting at time of specimen collection was not reported. Information about

Table 1. MICs of antimicrobial agents tested for 9 ciprofloxacin-resistant *Salmonella enterica* serotype Typhi isolates detected in the National Antimicrobial Resistance Monitoring System, United States, 1999–2008

	MIC, μg/mL,* by patient no. (isolate)								
Antimicrobial class and agent*	Patient 1 (MA-03)	Patient 2† (CA-05)	Patient 3 (CA-06)	Patient 4 (TX-06)	Patient 5 (AZ-06)	Patient 6 (NY-07)	Patient 7 (CA-07)	Patient 8 (NJ-07)	Patient 9 (LAC-07)
Quinolones									
Ciprofloxacin	>4	>4	>4	>4	>4	>4	>4	>4	>4
Nalidixic acid	>32	>32	>32	>32	>32	>32	>32	>32	>32
Aminoglycosides									
Amikacin	≤0.5	1	1	1	1	1	1	1	1
Gentamicin	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25
Kanamycin	≤8	≤8	≤8	≤8	≤8	≤8	≤8	≤8	≤8
Streptomycin	≤32	≤32	≤32	>64	≤32	≤32	≤32	≤32	≤32
β-lactam–β-lactamase inhibitor									
Amoxicillin-clavulanic acid	≤1/0.5	≤1/0.5	≤1/0.5	8/4	≤1/0.5	≤1/0.5	≤1/0.5	≤1/0.5	≤1/0.5
Cephems									
Cefoxitin	4	4	4	4	2	4	4	4	4
Ceftiofur	0.5	0.5	0.5	0.5	0.25	0.5	0.5	0.5	0.5
Ceftriaxone	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25	≤0.25
Folate pathway inhibitors									
Sulfonamide‡	>512	>256	≤16	>256	>256	≤16	>256	≤16	≤16
Trimethoprim-sulfamethoxazole	>4/76	>4/76	≤0.12/2.38	>4/76	>4/76	≤0.12/2.38	>4/76	≤0.12/2.38	≤0.12/2.38
Penicillins									
Ampicillin	2	≤1	≤1	>32	≤1	≤1	≤1	≤1	≤1
Phenicols									
Chloramphenicol	4	4	4	>32	4	4	4	4	4
Tetracyclines									
Tetracycline	>32	>32	≤4	≤4	>32	≤4	>32	≤4	≤4

*Classes of antimicrobial agents defined by the Clinical and Laboratory Standards Institute (CLSI) were used to categorize agents (*7,13*). MICs were interpreted by using CLSI criteria when available (*7,13*): ciprofloxacin (resistance breakpoint, ≥4 μg/mL); nalidixic acid (≥32); amikacin (≥64); gentamicin (≥16); kanamycin (≥64); amoxicillin-clavulanic acid (≥32/16); cefoxitin (≥32); ceftiofur (≥8); ceftriaxone (≥4); sulfamethoxazole/sulfisoxazole (≥512); trimethoprim-sulfamethoxazole (≥4/76); ampicillin (≥32); chloramphenicol (≥32); and tetracycline (≥16). For streptomycin, resistance was defined as MIC ≥64 μg/mL (*7*). If growth was not inhibited by the highest concentration of the agent in the panel, the MIC was reported as above the highest concentration.
†Isolate was cultured from a blood specimen. Another isolate was cultured from fecal samples, which had MIC ≤0.5 μg/mL for amikacin and same MICs for other agents tested.
‡Sulfamethoxazole was used during 1999–2003 and sulfisoxazole since 2004 to represent sulfonamides.

antimicrobial drug treatment was not available. The child was hospitalized for 14 days.

In 2005, a 2-year-old girl had onset of diarrhea, which was treated with ofloxacin, 2 days before she arrived in the United States from India. Seven days later, she continued to have diarrhea, and fever, vomiting, and abdominal cramps developed. She was hospitalized and treated with antimicrobial agents, including ciprofloxacin. Blood and fecal specimens collected 3 weeks after illness onset yielded *S. enterica* serotype Typhi. The patient was discharged after 14 days of hospitalization. She had lived in India for 6 months before traveling to the United States.

The *S. enterica* serotype Typhi isolates were resistant to ciprofloxacin (Tables 1, 2) and had indistinguishable PFGE patterns when restriction enzymes *Xba*I and *Bln*I were used: PulseNet-designated *Xba*I pattern JPPX01.0026 and *Bln*I pattern JPPA26.0110 (Table 2; Figure). QRDR sequencing showed *gyrA* mutations resulting in a serine to tyrosine substitution at codon 83 and an aspartic acid to asparagine substitution at codon 87, and a *parC* mutation conferring a serine to isoleucine substitution at codon 80.

Seven (0.6%) ciprofloxacin-resistant infections were detected among patients from whom 1,131 *S. enterica* serotype Typhi isolates were submitted during 2006–2008 (Table 2). The 7 cases occurred in 2006 and 2007. Patients were a median of 22 years of age (range 5–48 years); 5 (71%) were male. All 6 patients with known travel histories reported travel to India in the 30 days before illness onset. In addition to *Xba*I JPPX01.0026 and *Bln*I JPPA26.0110, 3 different *Xba*I and *Bln*I pattern combinations were detected in the 7 isolates (Table 2; Figure).

Conclusions

We describe ciprofloxacin-resistant *S. enterica* serotype Typhi isolates from 9 patients in the United States. The first 5 cases were reported previously in aggregated form, without molecular characterization of the isolates (*5*). The first 2 patients were young children apparently infected in India in 2003 and 2005.

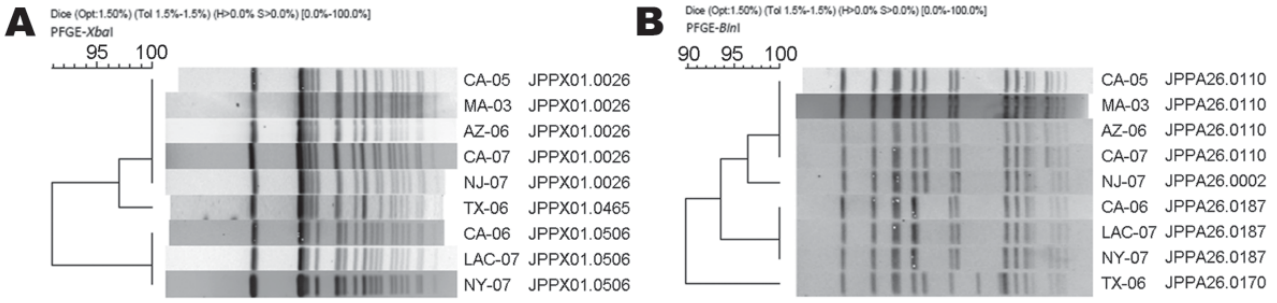

Figure. Pulsed-field gel electrophoresis (PFGE) *Xba*I (A) and *Bln*I (B) patterns of 9 ciprofloxacin-resistant *Salmonella enterica* serotype Typhi isolates detected in the National Antimicrobial Resistance Monitoring System, 1999–2008. PFGE pattern similarity was assessed by cluster analysis (Dice, UPGMA [unweighted pair group method using arithmetic average]) and band-matching applications of BioNumerics software (Applied Maths, Sint-Martens-Latem, Belgium) and confirmed by visual comparison. PulseNet only considers band markings found within the scale of the global standard, which are all bands between 20.5 kb and 1,135 kb. The cluster parameters are Dice coefficient and UPGMA with the tolerance of band position of 1.5% and optimization of 1.5%.

Six additional patients, who were detected in 2006 and 2007, also reported travel to India. Travel to the Indian subcontinent has been associated with nalidixic acid–resistant *S. enterica* serotype Typhi infection; however, ciprofloxacin-resistant infections are rarely reported by using current CLSI criteria (*4*,*5*,*11*). Other resistance patterns were first described in southern Asia, where the incidence of typhoid fever is high and antimicrobial agents are widely available without prescription, providing the opportunity for the development and selection of resistant strains (*8*).

Other than reports by 8 patients of travel to India, we have no information about possible shared exposures, such as specific locations visited, sources of food or water, or contact with carriers of *S. enterica* serotype Typhi. However, the indistinguishable PFGE *Xba*I and *Bln*I patterns and identical *gyrA* and *parC* mutations of isolates from the first 2 patients suggest that, although typhoid fever occurred nearly 2 years apart, the same ciprofloxacin-resistant strain is likely to have been involved. After 2005, different *Xba*I and *Bln*I patterns have been identified in ciprofloxacin-resistant isolates, indicating independent selection of ciprofloxacin resistance in different strains.

The *gyrA* and *parC* mutations of isolates from the first 2 patients were reported in ciprofloxacin-resistant *S. enterica* serotype Typhi in India (*11*). The 2 *gyrA* mutations are well characterized and known to be associated with quinolone resistance; 2 point mutations in *gyrA* and 1 in *parC* confer fluoroquinolone resistance (*8*,*10*–*12*). Further studies, including characterization of other resistance mechanisms,

Table 2. Patient and isolate description, resistance to other antimicrobial agents, PFGE pattern, and travel reported for 9 ciprofloxacin-resistant *Salmonella enterica* serotype Typhi infections detected in the National Antimicrobial Resistance Monitoring System, United States, 1999–2008*

Patient no. (isolate)	Age, y/ sex	Site	Specimen collection year	Specimen source	Resistance to other agents	PFGE *Xba*I pattern†	PFGE *Bln*I pattern‡	Travel§
1 (MA-03)	1/F	MA	2003	Blood	Cot, Fis, Nal, Tet	JPPX01.0026	JPPA26.0110	India
2 (CA-05)	2/F	CA	2005	Blood	Cot, Fis, Nal, Tet	JPPX01.0026	JPPA26.0110	India
3 (CA-06)	26/F	CA	2006	Blood	Nal	JPPX01.0506	JPPA26.0187	India
4 (TX-06)	8/M	TX	2006	Blood	Amp, Chl, Cot, Fis, Nal, Str	JPPX01.0465	JPPA26.0170	India, other
5 (AZ-06)	5/M	AZ	2006	Stool	Cot, Fis, Nal, Tet	JPPX01.0026	JPPA26.0110	India
6 (NY-07)	6/M	NYC	2007	Stool	Nal	JPPX01.0506	JPPA26.0187	India
7 (CA-07)	22/M	CA	2007	Stool	Cot, Fis, Nal, Tet	JPPX01.0026	JPPA26.0110	India
8 (NJ-07)	28/M	NJ	2007	Blood	Nal	JPPX01.0026	JPPA26.0002	India
9 (LAC-07)	48/F	LAC	2007	Blood	Nal	JPPX01.0506	JPPA26.0187	Unknown

*PFGE, pulsed-field gel electrophoresis. State/local public health laboratories that submitted isolates: MA, Massachusetts; CA, California; TX, Texas; AZ, Arizona; NYC, New York City; NJ, New Jersey; LAC, Los Angeles County, California. Resistance to antimicrobial agents other than ciprofloxacin: Cot, trimethoprim–sulfamethoxazole; Fis, sulfamethoxazole or sulfisoxazole; Nal, nalidixic acid; Tet, tetracycline; Amp, ampicillin; Chl, chloramphenicol; Str, streptomycin.
†National Molecular Subtyping Network for Foodborne Disease Surveillance (PulseNet)–designated PFGE patterns using restriction enzyme *Xba*I (data as of 2009 Oct 21): JPPX01.0026, the most common *Xba*I pattern among 3,233 isolates with reported *Xba*I pattern in PulseNet, was detected in 486 (15.0%); JPPX01.0465 and JPPX01.0506 each were detected in 12 (0.4%) isolates.
‡PulseNet-designated PFGE patterns using restriction enzyme *Bln*I (data as of 2009 Oct 21): JPPA26.0002, the most common *Bln*I pattern among 409 isolates with reported *Bln*I pattern in PulseNet, was detected in 61 (14.9%) isolates; JPPA26.0110 was detected in 5 (1.2%), JPPA26.0187 was detected in 3 (0.7%), and JPPA26.0170 was detected in 1 (0.2%).
§Travel outside the United States reported in the 30 d before illness onset; patient 4 also traveled to Bangladesh and the United Arab Emirates.

are needed to track the evolution of fluoroquinolone-resistant *S. enterica* serotype Typhi.

Although the ciprofloxacin resistance we detected using current CLSI criteria is rare in *S. enterica* serotype Typhi, nalidixic acid resistance, which correlates with decreased susceptibility to ciprofloxacin, has increased (*7*). Clinicians should be aware that infection with *Salmonella* spp. with decreased susceptibility to ciprofloxacin may not respond satisfactorily to this agent (*6*,*8*,*9*,*13*,*15*). In addition, identification of ciprofloxacin-resistant cases has been increasing. In the presence of quinolone resistance, third-generation cephalosporins, such as ceftriaxone, can be used (*2*,*6*,*8*,*15*). Recent clinical trials suggest that azithromycin might be useful for treating uncomplicated typhoid fever (*2,8,9,15*). Recommendations for empiric treatment of typhoid fever in the United States are best developed by using information about antimicrobial drug resistance trends in isolates from countries where the infection was acquired.

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High Vancomycin MIC and Complicated Methicillin-Susceptible *Staphylococcus aureus* Bacteremia

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We conducted a retrospective study of 99 patients with methicillin-suseptible *Staphylococcus aureus* catheter-related bacteremia in which vancomycin MIC was determined by Etest. High vancomycin MIC (≥1.5 μg/mL) was the only independent risk factor for development of complicated bacteremia caused by methicillin-susceptible *S. aureus* (odds ratio 22.9, 95% confidence interval 6.7–78.1).

Several studies have established a relationship between high vancomycin MIC and a worse prognosis for patients with bacteremia caused by methicillin-resistant *Staphylococcus aureus* (MRSA) (*1–5*). However, to our knowledge, the role that a high vancomycin MIC could play in the clinical course of a patient with methicillin-susceptible *S. aureus* (MSSA) bacteremia, has not been investigated, although a high vancomycin MIC has been also reported for strains of MSSA (*6*).

The Study

We retrospectively determined the MIC of vancomycin for the first MSSA blood culture isolate from a cohort of 99 adult patients with catheter-related bacteremia. These patients were consecutively evaluated from January 2002 through December 2004 (mean follow-up 3 years) in University Hospital 12 de Octubre in Madrid, Spain, a 1,000-bed university medical center.

We determined methicillin and vancomycin susceptibility by using broth microdilution according to Clinical Laboratory Standards Institute methods. Vancomycin MIC of was determined under blinded conditions by Etest in the first isolate by using a 0.5 McFarland inoculum streaked evenly with a swab onto Mueller-Hinton agar plates (*7*).

Complicated bacteremia was defined by one of the following events occurring after the first episode of bacteremia: 1) development of endocarditis, septic thrombophlebitis (defined by persistent MSSA bacteremia at least 72 hours after initiation of active antimicrobial drugs + documented thrombi), arthritis, spondylitis, as well as end-organ hematogenous spread of infection to other locations; or 2) infection involving vascular or osteoarticular prostheses (excluding intravascular catheter) not removed within 4 days. We also calculated the crude death rate in the first 30 days after the first positive blood culture (30-day mortality) and mortality rate attributable to *S. aureus* bacteremia (attributable death rate).

The Student unpaired *t* test was used to compare continuous variables, the Mann-Whitney U test to compare continuous variables with a nonnormal distribution, and the Fisher exact test to compare proportions. All statistical tests were 2-tailed and the threshold of statistical significance was $p<0.05$. To analyze the risk factors for development of complicated bacteremia, we performed a multivariate forward stepwise logistic regression model including all the clinically relevant variables with a p value of <0.05 and possible confounding factors with a p value of <0.1 detected in the univariate analysis (SPSS software version 15.0, SPSS, Chicago, IL, USA).

All 99 MSSA strains were susceptible to vancomycin (MIC ≤2 μg/mL) by the broth microdilution method. Our data showed that, in 23/99 (23.2%) strains, MICs of vancomycin were ≥1.5 μg/mL by Etest (range 1.5–1.7 μg/mL).

Comparative data of patients with or without a high vancomycin MIC MSSA strain are shown in Table 1. The incidence of severe sepsis/septic shock was similar in both groups (21.7% vs. 14.5%; $p = 0.69$), but patients with high vancomycin MIC strains had complicated bacteremia more frequently (78.3% vs. 13.2%; $p<0.0001$). Attributable death rate was higher in patients with high vancomycin MIC strains with a difference that nearly achieves statistical significance (17.4% vs. 3.9%; $p = 0.08$).

Comparative data between the 28 patients in whom complicated bacteremia developed and the remaining cohort are shown in Table 2. The percentage of isolates with vancomycin MIC ≥1.5 μg/mL was significantly higher in patients with complicated bacteremia (18/28 [64.3%] vs. 5/71 [7%]; $p<0.0001$). Initial treatment with glycopeptides was more frequent in patients in whom complicated bacteremia developed (82.1% vs 57.7%; $p = 0.042$). Among the 64 patients treated initially with glycopeptides, the rate of complicated bacteremia was significantly higher in patients with high vancomycin MIC isolates (15/18 [83.3%] vs. 8/46 [17.4%]; $p<0.0001$), as occurred in the 25 patients treated initially with β-lactams, (3/5 [60%] vs.

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Table 1. Differential characteristics of patients with bacteremia caused by MSSA strains with a MIC to vancomycin ≥1.5 μg/mL compared with <1.5 μg/mL by Etest, Hospital 12 de Octubre, Madrid, Spain, January 2002–December 2004*

Characteristic	MIC <1.5, n = 76	MIC ≥1.5, n = 23	p value
Mean age, y (SD)	63.55 (16.7)	62.9 (18.8)	0.87
M/F, %	69.7/30.3	56.5/43.5	0.36
Co-morbidity Charlson Index, mean (SD)	2.76 (2.7)	3.4 (3.7)	0.4
Previous valvular prosthesis	1 (1.3)	2 (8.7)	0.23
Other previous endovascular prosthesis	4 (5.3)	1 (4.3)	0.7
Previous osteoarticular prosthesis	3 (3.9)	0	0.79
Previous renal failure requiring hemodialysis	7 (9.2)	4 (17.4)	0.47
Type of IV catheter as the source of bacteremia			
Peripheral line	34 (44.7)	9 (39.1)	0.71
Transitory central catheter	34 (44.7)	10 (43.5)	0.82
Permanent central catheter	8 (10.6)	4 (17.4)	0.76
Vancomycin MIC of the first MSSA isolate, median (range)	1.2 (0.5–1.4)	1.5 (1.5–1.7)	<0.0001
Initial treatment with glycopeptides	46 (60.5)	18 (78.3)	0.19
Initial treatment with antistaphylococcal β-lactams†	20 (26.3)	5 (21.7)	0.87
Initial treatment with non–β-lactam anti-staphylococcal agents‡	7 (9.2)	0	0.29
Delay in initiation of active antibiotic treatment, d,§ mean (SD)	0.85 (1.06)	1.3 (1.6)	0.14
Duration of antibiotic treatment, d, mean (SD)	13.4 (8.24)	18.6 (12)	0.07
Prompt IV catheter removal¶	62 (81.6)	17 (73.9)	0.45
Conservative IV catheter management#	4 (5.3)	2 (8.7)	0.32
Development of severe sepsis/septic shock	11 (14.5)	5 (21.7)	0.69
Complicated bacteremia	10 (13.2)	18 (78.3)	<0.0001
Septic thrombophlebitis	5 (6.6)	8 (34.9)	0.002
Endocarditis	3 (3.9)	4 (17.3)	0.08
Osteoarticular	2 (2.6)	2 (8.7)	0.48
Pulmonary emboli	0	2 (8.7)	0.08
Other	0	2 (8.7)	0.08
Crude 30-day death rate	8 (10.5)	6 (26.1)	0.13
Attributable death rate	3 (3.9)	4 (17.4)	0.083

*Values are no. (%) except as indicated. MSSA, methicillin-susceptible *Staphylococcus aureus*; IV, intravenous.
†Antistaphylococcal β-lactams refers to parenteral cloxacillin, cefazolin, amoxicillin-clavulanate, piperacillin-tazobactam, or imipenem/meropenem.
‡Including non–β-lactam antibiotics with in vitro activity against MSSA (mostly levofloxacin, moxifloxacin or, clindamycin).
§Delay since isolation of MSSA in blood cultures.
¶Removal of catheter in the first 48 hours since isolation of MSSA in blood cultures.
#Catheter kept at least 7 days since isolation of MSSA in blood cultures.

2/20 [10%]; p = 0.064). Vancomycin MIC ≥1.5 μg/mL was the only variable independently related to the risk for complicated bacteremia (OR 22.9, 95% confidence interval 6.7–78.1) in the multivariate analysis.

Conclusions

The aim of our study was to evaluate whether vancomycin MIC has any influence on the death rates and outcomes of patients with catheter-related MSSA bacteremia. We chose a MIC ≥1.5 μg/mL as interpretive criteria for diminished susceptibility on the basis of the reported treatment failure for infections caused by organisms who have exhibited this level of vancomycin MIC (*5,8*). A first relevant finding of our study was the relatively high incidence of high vancomycin MIC among MSSA strains producing bacteremia (23.2%), a result similar to the percentage found for MRSA strains in our hospital (*9,10*).

Although a previous study found that vancomycin MICs for MSSA strains recovered from hemodialysis-dependent patients with bacteremia who had been treated with vancomycin did not seem to be related to their clinical outcomes (*11*), recently published in vitro data suggest that isolates of *S. aureus* with high vancomycin MICs could be less susceptible to cloxacillin or daptomycin (*6*). Our data showed that patients with MSSA bacteremia caused by strains with high vancomycin MIC were not related to a higher rate of severe sepsis/septic shock development but were associated with a higher rate of complicated bacteremia. In fact, complicated bacteremia was related to a vancomycin MIC ≥1.5 μg/mL but not with other factors such as age, acquisition of infection, severity of underlying disease, or catheter management, which was confirmed in the multivariate analysis.

The initial treatment most frequently associated with complicated bacteremia, in patients with and without high vancomycin MIC, was the use of glycopeptides alone or followed by antistaphylococcal β-lactams. A possible explanation for this finding is that the first hours of antibiotic treatment are crucial to avoid complications. Nevertheless,

Table 2. Comparative analysis of 99 patients with complicated vs. noncomplicated MSSA bacteremia, Hospital 12 de Octubre, Madrid, Spain, January 2002–December 2004*

Variable	Noncomplicated MSSA, n = 71	Complicated MSSA, n = 28	p value
Mean age, y (SD)	63.9 (17.4)	62 (16)	0.6
M/F, %	69/31	60.7/39.3	0.8
Co-morbidity Charlson Index, mean (SD)	2.92 (2.4)	2.93 (3.5)	0.9
Previous valvular prosthesis	1 (1.4)	2 (7.1)	0.39
Other previous endovascular prosthesis	4 (5.6)	1 (3.6)	0.9
Previous ostheoarticular prosthesis	2 (2.8)	1 (3.6)	0.8
Previous renal failure requiring hemodyalisis	8 (11.3)	3 (10.7)	0.8
Type of IV catheter as the source of bacteremia			
Peripheral line	32 (45.1)	11 (39.3)	0.7
Transitory central catheter	30 (42.2)	14 (50)	0.8
Permanent central catheter	9 (12.7)	3 (10.7)	0.9
Vancomycin MIC for the first MSSA isolate, median (range)	1.2 (0.5–1.7)	1.5 (1.0–1.7)	<0.0001
Vancomycin MIC ≥1.5 μg/mL for the first MSSA isolate	5 (7)	18 (64.3)	<0.0001
Initial treatment with glycopeptides	41 (57.7)	23 (82.1)	0.042
Initial treatment with antistaphylococcal β-lactams†	20 (28.2)	5 (17.9)	0.42
Initial treatment with non–β-lactam antistaphylococcal agents‡	7 (9.9)	0	0.19
Delay in initiation of active antibiotic treatment, d,§ mean (SD)	0.92 (1.3)	1.07 (1)	0.8
Delay >24 h at the start of effective antibiotics§	28 (39.4)	14 (53.6)	0.2
Duration of antibiotic treatment, d, mean (SD)	12.77 (8)	19.39 (11.4)	0.002
Prompt IV catheter removal¶	58 (81.7)	21 (75)	0.65
Conservative IV catheter management#	5 (7)	1 (3.6)	0.8
Development of severe sepsis/septic shock	9 (12.7)	7 (25)	0.23
Days of follow-up, mean (SD)	502 (441)	462 (463)	0.77
Crude 30-day death rate	9 (12.7)	5 (17.9)	0.9
Attributable death rate	3 (4.2)	4 (14.3)	0.18

*Values are no. (%) except as indicated. MSSA, methicillin-susceptible *Staphylococcus aureus*; IV, intravenous.
†Antistaphylococcal β-lactams refer to parenteral cloxacillin, cefazolin, amoxicillin-clavulanate, piperacillin-tazobactam, or imipenem/meropenem.
‡Including non–β-lactam antibiotics with in vitro activity against MSSA (mostly levofloxacin, moxifloxacin, or clindamycin).
§Delay since isolation of MSSA in blood cultures.
¶Removal of catheter in the first 48 h since isolation of MSSA in blood cultures.
#Catheter kept at least 7 days since isolation of MSSA in blood cultures.

in our opinion the greatest risk for complicated bacteremia related to strains of *S. aureus* with high vancomycin MIC should not only be attributed to the fact that these strains are more resistant to vancomycin because patients infected with strains with high vancomycin MIC that were initially treated with β-lactams also had a clear tendency to develop more complicated bacteremia. Nevertheless, the scarce number of patients who were treated initially with β-lactams limit our results. We hypothesize that certain structural modifications might also occur in the cell wall of strains with high vancomycin MIC, including a thicker cell wall as it has been described in MRSA (*12*). Thickness of the cell wall should not only hinder the action of vancomycin, but also the arrival to the target (penicillin binding proteins of β-lactams). If this hypothesis is correct, a vancomycin MIC of 1.5–2 μg/mL in MSSA could be not only a marker of poor response to vancomycin but also a subrogate marker of suboptimal response to β-lactams and even pathogenicity, as has been recently suggested in MRSA isolates (*13*).

Some limitations of this study deserve specific consideration. For most patients a treatment schedule including glycopeptides and β-lactams was used, so it is difficult to analyze the role played by each antimicrobial drug. All our strains had a vancomycin MIC <2 μg/mL, so we do not know which would be the outcome of MSSA bacteremia caused by more resistant strains. We did not specifically test clonality of strains with high vancomycin MIC because we had previously demonstrated that MSSA strains isolated from patients with bacteremia at our institution (which coincided with most of the strains included in the present study) were polyclonal (*14*). Finally, some important variables such as the previous use of vancomycin or vancomycin serum levels were not included in the analysis.

A high level of resistance to vancomycin is related with the development of complicated complicated bacteremia caused by MSSA, independent of the type of initial antibiotic treatment. Failure of glycopeptides does not appear to be the unique factor for the development of complicated bacteremia in patients with high vancomycin MIC isolates, and therefore intrinsic characteristics of these strains could also explain MSSA's pathogenic role in the development of complicated bacteremia.

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Characterization and Prevalence of a New Porcine Calicivirus in Swine, United States

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Real-time reverse transcription PCR revealed that new St-Valerien–like porcine caliciviruses are prevalent (2.6%–80%; 23.8% overall) in finisher pigs in North Carolina. One strain, NC-WGP93C, shares 89.3%–89.7% genomic nucleotide identity with Canadian strains. Whether these viruses cause disease in pigs or humans or are of food safety concern requires further investigation.

Viruses in the family *Caliciviridae* are nonenveloped, polyadenylated, single-stranded, positive-sense RNA viruses (*1*). They have been classified into 5 genera (*Norovirus*, *Sapovirus*, *Vesivirus*, *Lagovirus,* and *Nebovirus*) since 2009 (www.ictvonline.org). Later, the nonhuman primate Tulane virus (*2*) and the porcine St-Valerien–like viruses (*3*) were characterized as potential new genera in the *Caliciviridae* family.

The Study

Recently, we identified a St-Valerien–like virus, NC-WGP93C strain, from a healthy finisher pig in the United States by reverse transcription PCR (RT-PCR) with calicivirus universal primers p290/110 (*4,5*), followed by direct sequencing and nucleotide BLAST search (www.ncbi.nlm.nih.gov). We further sequenced the genome of NC-WGP93C strain by using primer walking, 3′ and 5′ rapid amplification of cDNA ends (RACE) methods (*3,6,7*). The NC-WGP93C strain was closely related genetically to the Canadian St-Valerien–like viruses, AB90, AB104, and F15–10 strains (*3*), sharing 89.3%–89.7% nt identity, without insertions or deletions, and similar genomic organization. Complete genomes of strains representing different *Caliciviridae* genera were selected for a phylogenetic tree (Figure 1). The NC-WGP93C strain grouped with the Canadian St-Valerien–like viruses to form a potentially new genus within the *Caliciviridae* family.

Next, we developed a real-time quantitative RT-PCR (RT-qPCR) for detection of St-Valerien–like viruses with primers (WGP93-polF1, 5′-TCTAAAG CGTGCACTCTGGGTCAT-3′; WGP93-polR1, 5′-ACC CTTTCTCCACCAGGAACTTCT-3′) and probe (WGP93-polP1, FAM-ACGAGTTTGTGGACTTCCTCTCGCA-BHQ) that targeted the RNA-dependent RNA polymerase (RdRp). The assay was performed by using the OneStep RT-PCR Kit (QIAGEN, Valencia, CA, USA) and a real-time thermocycler (RealPlex, Eppendorf, Germany). A plasmid DNA carrying the p290/110 amplicon of the NC-WGP93C strain was used to generate a standard curve. The detection limit was 10 genomic equivalents (GE) per 20-μL reaction (cycle threshold 37.71), corresponding to 4×10^4 GE/g of fecal sample (cut-off cycle threshold 38.00). No other porcine enteric caliciviruses, including sapoviruses (GIII/Cowden, GVI/JJ681, GVII/LL26 strains) and noroviruses (GII.11/QW48, GII.18/QW101, and GII.19/QW170 strains) (*8,9*), were detected. This RT-qPCR is sensitive and specific for the detection of St-Valerien–like caliciviruses.

Using the above RT-qPCR, we performed a prevalence study of St-Valerien–like viruses. Pig fecal samples (n = 1,567) were collected during May–November 2009 from a study of *Salmonella* infections in apparently healthy finisher pigs from 3 different swine production systems (3 farms per system, and 4 barns per farm, except for farm RW [3 barns]) located in North Carolina (Table 1) (*10*). Each barn was treated with 1 of 3 biocides—Biosentry (Biosentry, Inc., Stone Mountain, GA, USA), Synergize (Preserve International, Reno, NV, USA), or VirkonS (Dupont Animal Health Solutions, Sudbury, UK)—or with pressurized water as control (*11*). One pig per pen

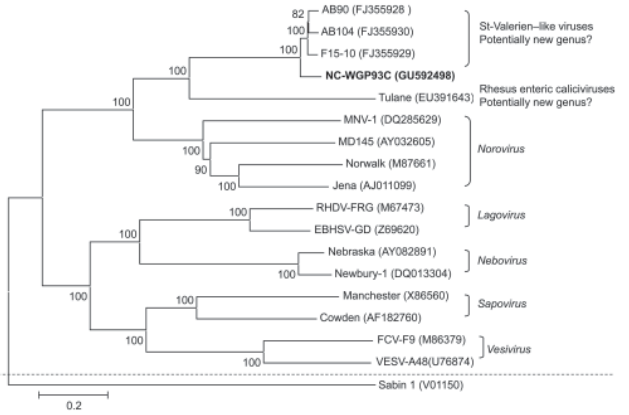

Figure 1. Neighbor-joining phylogenetic tree of caliciviruses based on the complete genomes (nucleotide). The newly identified St-Valerien–like virus NC-WGP93C strain is in **boldface**. The GenBank accession number of each strain is within parentheses. Bootstrap values are shown near branches. Human *Poliovirus* Sabin 1 was an outgroup control. Scale bar indicates nucleotide substitutions per site.

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Table 1. Prevalence of St-Valerien–like viruses in finisher swine farms in North Carolina, USA, 2009*

System code	Farm code	Barn treatment	Sampling month	No. individual samples	No. pooled samples	Barn level, no. positive/total (%)	Farm level, no. positive/total (%)†
BC1	BH	Water	Aug	48	10	4/10 (40.0)	8/40 (20.0)[bc]
BC1	BH	BIO	Aug	48	10	2/10 (20.0)	
BC1	BH	SYN	Aug	48	10	0/10	
BC1	BH	VIR	Aug	48	10	2/10 (20.0)	
BC1	EW	Water	Jul	42	10	0/10	1/39 (2.6)[c]
BC1	EW	BIO	Jul	39	9	0/10	
BC1	EW	SYN	Jul	47	10	1/10 (10.0)	
BC1	EW	VIR	Jul	48	10	0/10	
BC1	WL	Water	May	47	10	10/10 (100.0)	32/40 (80)[a]
BC1	WL	BIO	May	47	10	10/10 (100.0)	
BC1	WL	SYN	May	46	10	2/10 (20.0)	
BC1	WL	VIR	May	48	10	10/10 (100.0)	
BC2	DC	Water	Nov	39	9	4/9 (44.4)	13/37 (35.1)[b]
BC2	DC	BIO	Nov	45	10	7/10 (70.0)	
BC2	DC	SYN	Nov	35	8	2/8 (25.0)	
BC2	DC	VIR	Nov	48	10	0/10	
BC2	FF	Water	Oct	43	10	1/10 (10.0)	3/40 (7.5)[c]
BC2	FF	BIO	Oct	48	10	1/10 (10.0)	
BC2	FF	SYN	Oct	40	10	0/10	
BC2	FF	VIR	Oct	48	10	1/10 (10.0)	
BC2	RW	BIO	May	45	10	0/10	2/30 (6.7)[c]
BC2	RW	SYN	May	48	10	2/10 (20.0)	
BC2	RW	VIR	May	46	10	0/10	
BC3	GO	Water	Nov	46	10	1/10 (10.0)	8/40 (20)[bc]
BC3	GO	BIO	Aug	45	10	1/10 (10.0)	
BC3	GO	SYN	Nov	46	10	4/10 (40.0)	
BC3	GO	VIR	Aug	46	10	2/10 (20.0)	
BC3	TE	Water	Jul	47	10	4/10 (40.0)	12/39 (30.8)[b]
BC3	TE	BIO	Jul	38	9	2/9 (22.2)	
BC3	TE	SYN	Jul	40	10	4/10 (40.0)	
BC3	TE	VIR	Jul	47	10	2/10 (20.0)	
BC3	TT	Water	Jul	42	10	1/10 (10.0)	3/39 (7.7)[c]
BC3	TT	BIO	May	45	10	0 /10	
BC3	TT	SYN	Jul	38	9	2/9 (22.2)	
BC3	TT	VIR	May	46	10	0/10	
Total				1567	344	82/344 (23.8)	

*BIO, Biosentry (Bisentry, Inc., Stone Mountain, GA, USA); SYN, Synergize (Preserve International, Reno, NV, USA; VIR, VirkonS (Dupont Animal Health Solutions, Sudbury, UK).
†Superscript letters indicate significance, i.e., values between farms labeled with different letters differed significantly, but values between farms labeled with the same letter did not differ significantly. $p<0.05$ by binomial proportion test.

was sampled at 26–28 weeks of age. Fecal samples were collected directly from the rectums of selected individual pigs (based on convenience); sterile gloves and cups were used to prevent contamination from the environment and between samples. In the laboratory, 4–5 individual samples from the same barn were pooled and stored (–20°C), resulting in 344 pooled fecal samples for the prevalence study. RNA was extracted from 10% (wt/vol) fecal suspensions by using the 5× MagMAX-96 Viral 1 Kit and the RNA extraction robot MagMax Express Magnetic Particle Processor (Applied Biosystems, Foster City, CA, USA). The relationship and differences in prevalence among the various biocide treatments (barns), farms, and production systems were assessed by χ^2 and binomial proportion tests (SAS Institute, Inc., Cary, NC, USA). A p value of <0.05 was considered significant.

All 9 swine farms were positive for St-Valerien–like viruses. Overall prevalence was 23.8% (range 2.6%–80.0%) (Table 1). The prevalence in farm WL (32/40; 80.0%) was significantly higher than that in the other 8 farms, suggesting that an outbreak occurred at this farm during sampling. The prevalence in production system BC1 (41/119; 34.5%) was significantly higher than that in production system BC2 (18/107; 16.8%), but not BC3 (23/118; 19.5%). Differences among the 3 production systems were determined to be primarily due to the origin of the pigs. Each production system is fully independent with their own genetics/breeding units, farrowing sites where

Table 2. Prevalence of St-Valerien–like viruses from historical pig fecal samples collected during 2002–2005, United States*

Swine farm	% Pigs (no. positive/total no.)				
	% Nursing pigs, 1–3 wk	% Post-weaning pigs, 3–10 wk	% Finisher pigs, 10–24 wk	% Sows, >1 y	% Total
Ohio A	0 (0/14)	0 (0/12)	0 (0/22)	0 (0/13)	0 (0/61)
Ohio B	0 (0/31)	0 (0/45)	0 (0/45)	0 (0/30)	0 (0/151)
Ohio C	0 (0/15)	0 (0/12)	0 (0/6)	0 (0/28)	0 (0/61)
Ohio D	0 (0/8)	0 (0/10)	NA	NA	0 (0/18)
North Carolina A	NA	NA	0 (0/5)	NA	0 (0/5)
North Carolina B	NA	NA	53 (10/19)	NA	53 (10/19)
Michigan A	NA	NA	2 (1/60)	NA	2 (1/60)
Total	0 (0/68)	0 (0/79)	7 (11/157)	0 (0/71)	3 (11/375)

*NA, not available.

the sampled pigs originated, etc. Our findings suggest that breed differences or incidence of infection in pigs at earlier production stages might affect incidence at the sampled finisher stage. Overall, we found no statistically significant difference in prevalence among the treatments (biocides and water control) (data not shown). These results are consistent with the environmental stability of caliciviruses and their resistance to many disinfectants (*1*).

We also tested by RT-qPCR historical RNA samples extracted from pig fecal samples collected from December 2002 to March 2005 from 2 North Carolina farms, 4 Ohio farms, and 1 Michigan farm (n = 375) (Table 2). These RNA samples were from a previous study of the prevalence of porcine noroviruses and sapoviruses and have been stored at –70°C since 2005 (*12*). St-Valerien–like viruses were detected in 1 North Carolina swine farm in 2003 (10/19 samples; 53%). Only 1 of 60 samples collected in 2002 from the Michigan farm showed a weak positive result (3.3×10^5 GE/g). No St-Valerien–like viruses were detected in the pigs of different ages on the 4 Ohio farms. These results suggest regional differences in the distribution of this new virus.

Representative St-Valerien–like virus strains were selected on the basis of collection sites (Tables 1, 2): NC-WGP6A (BC2, FF, BIO), NC-WGP117 (BC1, WL, BIO), NC-WGP128 (BC1, WL, VIR), and 1 historic sample NC-QW266 (NC farm B). Because of low virus titers, the positive samples from production system BC3 and Michigan could not be further amplified. The 3′ end, 2,511-nt fragments, including the predicted partial RdRp, complete capsid viral protein (VP) 1, and minor structural protein VP2 genes were sequenced to examine the genetic variation among St-Valerien–like viruses. The 5 strains from the United States share 97.0%–99.9% nt identity in this region. Strains from the United States, the recently reported strain from Italy (25A), and the strains from Canada (*3,13*) share 96.4%–100%, 95.9%–100.0%, and 92.0%–100% aa identities for the partial RdRp (167 aa), VP1 and VP2, respectively. These results suggest that there is only 1 genotype within this potentially new genus (Figure 2), although the strain from Italy clusters with strains from the United States.

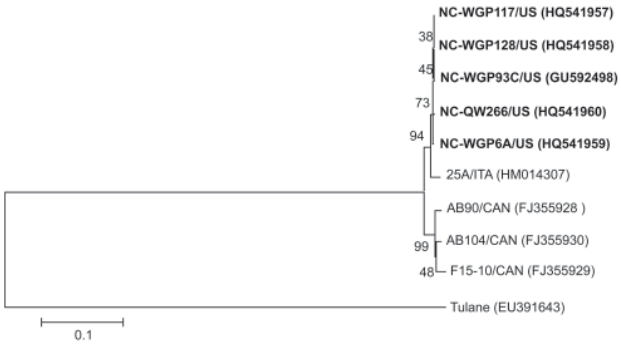

Figure 2. Neighbor-joining phylogenetic tree of St-Valerien–like viruses based on the predicted capsid viral protein 1 sequences (516 aa). The newly identified US St-Valerien–like virus strains are in **boldface**. The GenBank accession number of each strain is within parentheses. Bootstrap values are shown near branches. Rhesus monkey Tulane calicivirus was an out-group control. Scale bar indicates amino acids substitutions per site.

Conclusions

St-Valerien–like viruses have been detected in Canada, the United States, and Italy. The prevalence of St-Valerien–like viruses in finisher pigs in North Carolina was 23.8%. No such viruses were detected in swine samples from Ohio collected during March 2003–March 2005. For a proposed new genus, it is critical to determine if St-Valerien–like viruses are present in other regions or species and to examine the genetic diversity among strains. Because these viruses are genetically closest to Tulane virus and human noroviruses (*2,3*), this information is useful in examining their potential for interspecies transmission and in controlling the spread of new viruses. Whether St-Valerien–like viruses cause disease in pigs or humans or cause food safety concerns requires further investigation.

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Invasive *Streptococcus pneumoniae* in Children, Malawi, 2004–2006

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Of 176 invasive *Streptococcus pneumoniae* isolates from children in Malawi, common serotypes were 1 (23%), 6A/B (18%), 14 (6%), and 23F (6%). Coverage with the 7-valent pneumococcal conjugate vaccine (PCV) was 39%; PCV10 and PCV13 increased coverage to 66% and 88%, respectively. We found chloramphenicol resistance in 27% of isolates and penicillin nonsusceptibility in 10% (by using meningitis breakpoints); all were ceftriaxone susceptible.

Streptococcus pneumoniae causes a spectrum of disease, ranging from relatively mild otitis media to life-threatening pneumonia, meningitis, and septicemia. Recent estimates suggest that pneumococcal disease is responsible for 1 million deaths annually, >800,000 of which are in children <5 years of age in the developing world (*1*). Developing countries have the highest incidence of pneumococcal disease, and the spread of HIV, which increases the risk for pneumococcal disease up to 40-fold, has exacerbated the situation (*2*). In Malawi, in southern Africa, *S. pneumoniae* is 1 of the most common organisms isolated from blood and cerebrospinal fluid (CSF) cultures of children admitted to the hospital, and the case-fatality rate for invasive pneumococcal disease (IPD), pneumonia, septicemia, and meningitis is ≈25% (*3*,*4*).

The successful introduction of the 7-valent pneumococcal conjugate vaccine (PCV7) in several industrialized nations has led to plans to extend its use to sub-Saharan Africa (*2*). PCV7 contains the most commonly isolated 7 serotypes from IPD in children in the United States before vaccine implementation. However, these 7 serotypes account for <50% of IPD isolates from children in Africa (*5*). Surveillance of circulating serotypes is therefore essential information for developing policy about vaccine introduction.

In the United States, PCV7 has successfully reduced the incidence of IPD and antimicrobial drug resistance in vaccine serotypes; however, this decrease paralleled an increase in the incidence of IPD caused by nonvaccine serotypes, among which antimicrobial drug resistance is increasing (*6*). Resistance to penicillin and other antimicrobial agents in pneumococci complicates clinical management (*7*). Previous data from Malawi suggest that penicillin resistance in IPD is relatively low (*8*–*11*).

We report the serotypes of pneumococcal isolates from febrile children admitted to the largest hospital in Blantyre, Malawi, during April 2004–October 2006. We also report susceptibilities to antimicrobial drugs used to treat IPD.

The Study

We studied *S. pneumoniae* isolated from the blood or CSF of children 2 months–16 years of age, admitted to Queen Elizabeth Central Hospital (QECH), the main referral hospital for southern Malawi, during April 2004–October 2006. Blood cultures were performed for all children admitted with signs of pneumonia or meningitis, and CSF cultures were performed for all children with signs suggestive of meningitis during the collection period. QECH admits ≈25,000 children and 17,000 adults annually and serves a population of ≈1 million. It is a government-funded teaching and referral hospital with 1,250 beds, although the total number of patients can exceed 2,000. Participants were recruited to studies of the host and bacterial factors determining outcome in invasive pneumococcal infection (*3*). The College of Medicine Research Committee, Malawi, and The Liverpool School of Tropical Medicine Local Research Ethics Committee granted ethics approval for this study.

Blood and CSF were processed by standard microbiological methods (*3*). *S. pneumoniae* isolates were identified by colony morphology and α-hemolysis and then confirmed by Gram staining and determination of optochin susceptibility (Oxoid, Basingstoke, UK). Isolates were stored at –80°C after primary isolation in bead and broth cryopreservers (Pro-Lab Diagnostics, Richmond Hill, ON, Canada). Isolates were transported to Liverpool and later subcultured for serotyping and MIC determinations.

Serotyping was performed by multiplex PCR as described by Pai et al. (*12*). MICs were determined by the Etest (AB Biodisk, Solna, Sweden) according to the manufacturer's recommendation. Benzyl penicillin, ceftriaxone, and chloramphenicol were tested. *S. pneumoniae* ATCC 49619 was used as a quality control

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strain and gave values within an acceptable range. Antimicrobial drug susceptibility breakpoints were defined according to Clinical and Laboratory Standards Institute criteria (*13*).

We compared categorical values using Fisher exact test. A p value of <0.05 was considered significant. Statistical analysis was performed by using Stata 10 (StataCorp, College Station, TX, USA). When calculating serotype coverage, we assumed serotype 6A/B cross-protection for PCV7 and the 10-valent pneumococcal vaccine (PCV10).

A total of 180 isolates were collected from children admitted to QECH during the study period: 37 (21%) from CSF and 143 (79%) from blood. Four isolates did not remain viable during storage; 176 isolates were available for serotyping and MIC determination. Of these, 95 (54%) were from boys. Median age of patients was 2.5 years (range 2 months–14 years; interquartile range 8 months–7 years). Of the isolates studied, 100 (57%) were from HIV-positive children, and 71 (40%) were from HIV-negative children. Testing was declined for 5 (3%) children. The case-fatality rate was 25% for HIV-positive children and 21% for HIV-negative children (p = 0.68). Serotypes 1 and 6A/6B predominated, accounting for 23% and 18% of isolates, respectively (Table 1). Of the 176 isolates, 69 (39%), 116 (66%), and 154 (88%) had a serotype included in PCV7, PCV10, and PCV13, respectively.

Clinical and Laboratory Standards Institute breakpoints for penicillin and ceftriaxone varied for meningitis or nonmeningitis infections (Table 2). Using the meningitis breakpoints, we found 158 (90%) isolates were susceptible to penicillin, and 18 (10%) were resistant to penicillin. However, according to non-meningitis breakpoints, all isolates were penicillin susceptible. Of the 150 children with meningitis, isolates from 16 (10.7%) patients were resistant by meningitis breakpoints. All isolates were susceptible to ceftriaxone by both breakpoints. Chloramphenicol resistance was present in 47 (27%) of isolates. Chloramphenicol-resistant pneumococci were isolated from 28 (39%) of the 71 HIV-negative children and 18 (18%) of the 100 HIV-positive children (p = 0.0027). The 2 groups did not differ significantly in levels of resistance to the other antimicrobial agents.

Table 1. Serotypes of *Streptococcus.pnemoniae* isolated from children at Queen Elizabeth Central Hospital, Blantyre, Malawi, 2004–2006

Serotype	No. (%) isolates	HIV serostatus, no. (%)		
		Positive	Negative	Declined
1	41 (23)	16 (16)	24 (34)	1 (20)
6A/6B*	31 (18)	21 (21)	9 (13)	1 (20)
14*	11 (6)	8 (8)	3 (4)	0
23F*	11 (6)	7 (7)	4 (6)	0
12F	10 (6)	4 (4)	6 (8)	0
19F*	9 (5)	6 (6)	3 (4)	0
Sg18*	6 (3)	3 (3)	3 (4)	0
4*	5 (3)	5 (5)	0	0
7f	4 (2)	1 (1)	3 (4)	0
10A	3 (2)	2 (2)	1 (1)	0
16F	3 (2)	3 (3)	0	0
33F	3 (2)	1 (1)	2 (3)	0
35F	3 (2)	2 (2)	1 (1)	0
3	2 (1)	2 (2)	0	0
5	2 (1)	0	2 (3)	0
9V*	2 (1)	1 (1)	0	1 (20)
14/4	2 (1)	1 (1)	1 (1)	0
15a	2 (1)	1 (1)	1 (1)	0
19a	2 (1)	1 (1)	0	1 (20)
7c	1 (1)	0	1 (1)	0
8	1 (1)	0	1 (1)	0
34	1 (1)	1 (1)	0	0
11a	1 (1)	1 (1)	0	0
17f	1 (1)	1 (1)	0	0
35B	1 (1)	0	0	1 (20)
Not typeable	18 (10)	12 (12)	6 (8)	0
Total	176	100	71	5

*Serotypes in 7-valent pneumococcal conjugate vaccine: 4, 6B, 9V, 14, 18C, 19F, 23F. PCV10 adds serotypes 1, 5, and 7F. PCV13 adds serotypes 3, 6A, and 19A.

Conclusions

Our study describes recent pneumococcal serotyping and antimicrobial drug susceptibility data for children in Malawi. Serotype distributions suggest that PCV7 would provide poor potential coverage for these children; PCV7 includes only 39% of serotypes identified. This information is supported by a previous study that found that PCV7 would cover 41% of invasive pneumococcal isolates from children (*14*). Serotypes 1 and 5, long regarded as essential in vaccines for use in sub-Saharan Africa, accounted for 23% and 1% of all isolates in this study, respectively. Our data suggest that use of the 13-valent vaccine, which includes serotypes 1 and 5 and is due to be introduced into Malawi in late 2011, will substantially increase vaccine coverage. The nontypeable isolates included in the study may have been typeable by an alternative method. However, the serotyping method used includes all serotypes in PCV7, PCV10, and PCV13.

Our study is not a formal epidemiologic study because it did not comprise a true random selection of isolates; however, QECH is the only public hospital in this area and most children admitted to the hospital live within the local community. Furthermore, we studied all consecutive cases during the study period, both severe and nonsevere. The sample of pneumococcal disease in children studied is therefore likely to be representative of the incidence of disease in this area.

The high proportion of blood cultures studied implies that pneumonia was the primary clinical diagnosis, however, most had meningitis. Penicillin is the first-line treatment for pneumonia and presumed sepsis at QECH, and ceftriaxone is the first-line treatment for suspected meningitis. Use

Table 2. Antimicrobial drug susceptibilities of *Streptococcus pneumoniae* isolates from children at Queen Elizabeth Central Hospital, Blantyre, Malawi, 2004–2006*

Antimicrobial drug	MIC, mg/L					No. (%) isolates*			Breakpoint values		
	Minimum	Maximum	MIC_{50}	MIC_{90}	GM	S	I	R	S	I	R
Benzyl penicillin											
Meningitis breakpoints	<0.016	0.500	<0.016	0.094	0.025	156 (90)	–	18 (10)	≤0.06	–	≥0.12
Nonmeningitis breakpoints	<0.016	0.500	<0.016	0.094	0.025	176 (100)	0	0	≤2.0	4.0	≥8.0
Ceftriaxone											
Meningitis breakpoints	<0.016	0.250	<0.016	0.064	0.025	176 (100)	0	0	≤0.5	1.0	≥2.0
Nonmeningitis breakpoints	<0.016	0.500	<0.016	0.094	0.025	176 (100)	0	0	≤1.0	2.0	≥4.0
Chloramphenicol	0.380	30.000	3.000	25.000	4.660	129 (73)	–	47 (27)	≤4.0	–	≥8.0

*MIC_{50}, 50% MIC; MIC_{90}, 90% MIC; GM, geometric mean; S, susceptible; I, intermediate; R, resistant; –, no intermediate resistance values for these antimicrobial drugs, according to Clinical and Laboratory Standards Institute definitions.

of non-meningitis breakpoints in this study demonstrates no penicillin resistance. The susceptibility of all isolates to ceftriaxone confirms its suitability as a second-line treatment. Chloramphenicol resistance rates were high at 27%. The resistance levels reported here remain similar to those reported previously (*8*–*11*). Levels of resistance are comparable to those in other studies in sub-Saharan Africa but less than in many other areas in the world (*7*), possibly because antimicrobial drug use in Malawi is lower than that of other countries.

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Worldwide Distribution of Major Clones of *Listeria monocytogenes*

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Listeria monocytogenes is a worldwide pathogen, but the geographic distribution of clones remains largely unknown. Genotyping of 300 isolates from the 5 continents and diverse sources showed the existence of few prevalent and globally distributed clones, some of which include previously described epidemic clones. Cosmopolitan distribution indicates the need for genotyping standardization.

Listeria monocytogenes is a foodborne pathogen that can cause listeriosis, a severe invasive infection in humans with a particularly high case-fatality rate. Listeriosis is a major public health concern in all world regions, with an increasing incidence in Europe, especially among elderly persons (*1*,*2*).

L. monocytogenes is genetically heterogeneous (*3*–*5*). To help epidemiologic investigation and to define clones, i.e., groups of genetically similar isolates descending from a common ancestor, a variety of typing methods have been used, including pulsed-field gel electrophoresis (*5*,*6*), single nucleotide polymorphism typing (*7*), and multiple housekeeping and virulence gene sequencing (*8*,*9*). Some clones implicated in multiple outbreaks have been defined as epidemic clones (EC) (*3*,*5*,*9*–*11*). ECI and ECIV have been described in several countries (*3*,*5*), but because of the lack of standardization of genotyping, a definition of clones is not widely accepted, and current knowledge on the global distribution of *L. monocytogenes* clones is virtually absent. Multilocus sequence typing (MLST) is a reference method for global epidemiology and population biology of bacteria, and its application to *L. monocytogenes* (*12*) effectively allows isolate comparisons across laboratories (www.pasteur.fr/mlst). The aim of this study was to investigate the global distribution of *L. monocytogenes* MLST-defined clones.

The Study

Three hundred *L. monocytogenes* isolates were collected from different sources from 42 countries on 5 continents (online Appendix Table, www.cdc.gov/EID/content/17/6/1110-appT.htm). The isolates derived from 1) the collection of the World Health Organization Collaborating Center for *Listeria* and 2) the Seeliger *Listeria* Culture Collection. When available, up to 10 countries per continent were included. Only 1 isolate per documented outbreak was kept, and the isolates from a given country were selected from various sources, years, and serotypes. A total of 117 isolates were from humans, 107 from food, 28 from animals, 32 from the environment and vegetation, and 16 of undocumented origin. The relative proportion of isolates from distinct sources was similar among world regions (online Appendix Table), except that no animal isolate was available from the Western Hemisphere and that the ratio of human to food isolates was lower from this continent.

Each isolate was hemolytic when streaked for isolation on blood agar. Genomic DNA was extracted by using Promega Wizard Genomic DNA purification kit (Promega, Madison, WI, USA). Serotype information was confirmed by PCR serogrouping (*13*). MLST was performed as described (*12*). Alleles and sequence types (STs) are publicly available at www.pasteur.fr/mlst. Clonal complexes (CC) were defined as groups of STs differing by only 1 gene from another member of the group (*12*) and were considered as clones. The θ estimator of the Fst statistic, which measures population differentiation, was determined on the basis of ST frequency by using FSTAT (www2.unil.ch/popgen/softwares/fstat.htm).

The 300 isolates represented 111 STs (diversity index 95.4%) grouped into 17 CCs (online Appendix Figure, www.cdc.gov/EID/content/17/6/1110-appF.htm). Phylogenetic analysis of the concatenated genes (not shown) indicated that 199, 98, and 3 isolates belonged to lineages I, II, and III, respectively (*12*). In lineage I, 3 CCs were highly prevalent: CC1 (47 isolates, serotype 4b), CC2 (64 isolates, 4b,) and CC3 (32 isolates, 1/2b). The remaining isolates of lineage I were of serotype 4b or 1/2b (Table). In lineage II, CC9 (28, all with serotype 1/2c, except one 1/2a isolate) was the most frequent, followed by CC7 (15 1/2a isolates). All other lineage II isolates had serotype 1/2a.

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Table. Distribution of the major *Listeria monocytogenes* clonal complexes in lineages I and II among sources

Lineage or clonal complexes	No. (%), by source						Human/ food ratio
	Total	Human	Food	Animal	Environment	Unknown	
Lineage I, total	199 (100)	88 (44)	60 (30)	16 (8)	24 (12)	11 (6)	1.60:1
CC1 (4b)	47 (100)	26 (55)	10 (21)	4 (9)	3 (6)	4 (9)	2.60:1
CC2 (4b)	64 (100)	36 (56)	13 (20)	6 (9)	5 (8)	4 (6)	2.77:1
CC3 (1/2b)	32 (100)	11 (34)	17 (53)	1 (3)	2 (6)	1 (3)	0.65:1
Other 4b	20 (100)	8 (40)	6 (30)	1 (5)	4 (20)	1 (5)	1.33:1
Other 1/2b	36 (100)	7 (19)	14 (39)	4 (11)	10 (28)	1 (3)	0.50:1
Lineage II, total	98 (100)	29 (30)	45 (46)	11 (11)	8 (8)	5 (5)	0.64:1
CC9 (1/2c)	28 (100)	7 (25)	14 (50)	2 (7)	3 (11)	2 (7)	0.50:1
Other, lineage II (1/2a)	70 (100)	22 (31)	31 (44)	9 (13)	5 (71)	3 (4)	0.71:1

Comparisons of populations from different sources (Table) showed a clear partitioning of genotypic diversity between clinical isolates on the one hand and food or environmental isolates on the other (θ = 0.033 and 0.050, respectively; $p<0.0002$). Consistent with common knowledge (*4*,*5*), and even though recent outbreaks in Canada and Austria/Germany were caused by 1/2a strains, isolates of serotype 4b were, compared with other serotypes, relatively more frequent in human cases than in food. This difference in source distribution was further demonstrated for individual clones because the human/food ratio of both CC1 (2.6) and CC2 (2.8) differed significantly from those of CC3 (0.65) and CC9 (0.5) (χ^2 $p<0.01$ for the 4 comparisons).

A global distribution of *L. monocytogenes* clones was evident (Figure). Frequent clones were found in many countries (up to 30 countries for CC2; online Appendix Table) and were globally distributed. Remarkably, CC1 and CC2 were predominant in all world regions except northern Africa for CC1 (Figure). CC3 ranked among the 4 most common clones in all regions, whereas CC9 ranked third in Europe and the Western Hemisphere. Altogether, these 4 clones represented 54 (50%) food isolates and 80 (68%) clinical isolates. Our results show that the same few clones account for a large fraction of nonepidemic *L. monocytogenes* isolates in distant world regions. However, continents and sources were not equally represented in our sample, and larger studies are needed to confirm our hypothesis that the clonal composition is similar across world regions and countries. Consistent with their cosmopolitan distribution, 15 of the 17 clones found herein (except CC199 and CC315, with only 6 and 3 isolates, respectively) included isolates from our previous analysis of 360 isolates, mostly from France (*12*).

Conclusions

This study provides the first global view of *L. monocytogenes* clonal diversity. Our results clearly demonstrate the worldwide distribution and high prevalence of a few frequent clones in distinct world regions. In the current debate on the phylogeography of bacterial species (*14*), major *L. monocytogenes* clones clearly fit in the "everything is everywhere" group, as do other pathogens in the environment, e.g., *Pseudomonas aeruginosa* (*15*). Dispersal by human travel, animal or food trade, wild animal migration, or wind and dust all might contribute to the global diffusion of *L. monocytogenes* clones. However, finer phylogenetic resolution will possibly subdivide

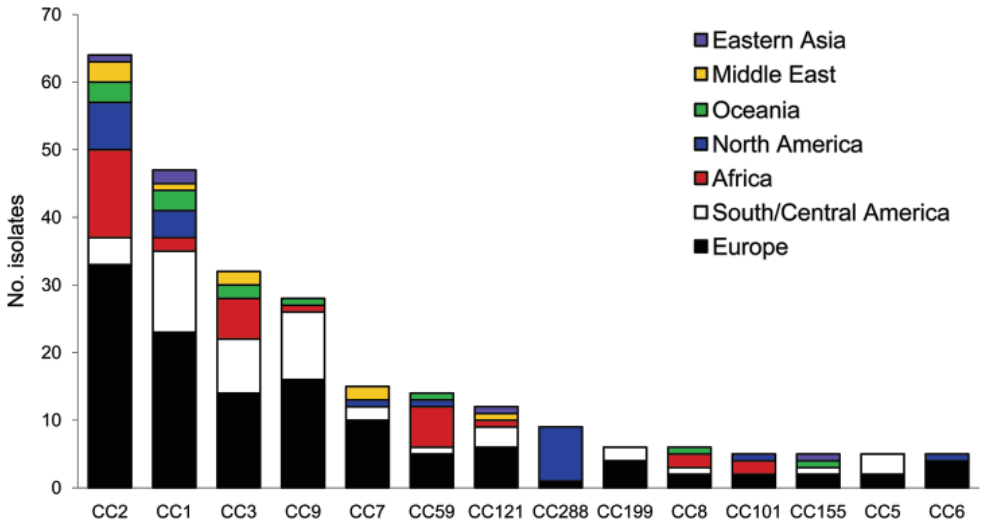

Figure. Number of isolates from 7 world regions where the most prevalent clones of *Listeria monocytogenes* are found.

widespread MLST-defined clones into subclades that might exhibit phylogeographic partitioning and will better clarify the rate and patterns of strain dispersal.

Remarkably, some ECs correspond with highly prevalent clones. ECII, described relatively recently (*6*), and ECIII, involved in outbreaks from a single plant, correspond to 2 clones (CC6 and ST11, respectively [*12*]), that were rare herein (5 and 0 isolates, respectively), suggesting that both clones experienced particular conditions that favored their diffusion on specific occasions. In contrast, the outbreaks caused by ECI and ECIV, reference strains of which belong to CC1 and CC2, respectively (*12*), could have been favored by their high prevalence in sources. One important question for future research is whether ECs correspond entirely to MLST-defined clones (i.e., CCs) or whether, on the contrary, they represent a genotypic subset thereof. The cosmopolitan distribution of clones, which protects them against extinction resulting from local disturbances, further highlights the crucial need to standardize *L. monocytogenes* genotyping to improve global epidemiologic knowledge and monitoring of current emergence trends.

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Klebsiella pneumoniae Bacteremia and Capsular Serotypes, Taiwan

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Capsular serotypes of 225 *Klebsiella pneumoniae* isolates in Taiwan were identified by using PCR. Patients infected with K1 serotypes (41 isolates) had increased community-onset bacteremia, more nonfatal diseases and liver abscesses, lower Pittsburgh bacteremia scores and mortality rates, and fewer urinary tract infections than patients infected with non–K1/K2 serotypes (147 isolates).

Klebsiella pneumoniae bacteria cause a variety of infections (*1,2*). Geographic differences in this organism have been recognized, and a high prevalence of liver abscesses has been observed for >20 years in persons in Taiwan infected with *K. pneumoniae* (*3,4*). K1 and K2 are the major capsular serotypes that cause liver abscesses and have increased virulence (*4–7*). In contrast, only limited information is available about serotypes causing *K. pneumoniae* bacteremia (*3,5*).

Yu et al. grouped K1 and K2 serotypes and compared clinical characteristics for patients with *K. pneumoniae* bacteremia with those for patients infected with non–K1/K2 serotypes (*3*). Recent evidence suggests that K1 is a major cause of primary liver abscesses and has greater potential for causing metastasis, and that K2 is a major cause of secondary liver abscesses (*6,8*). We examined the distribution and clinical characteristics of serotypes that cause *K. pneumoniae* bacteremia from 225 patients (*9*) and performed PCR-based genotyping to identify capsular serotypes (*10*).

The Study

The study was conducted at Far-Eastern Memorial Hospital in Taipei, Taiwan. Patients with *K. pneumoniae* bacteremia were identified during January 1–December 31, 2007. Identification of *K. pneumoniae* was based on colony morphologic features and biochemical reactions (*11*). Data on time until positive blood culture results were obtained from the automated blood culture system at the hospital. Data for each patient were included only once (at the time of the first detection of bacteremia). Patients <18 years of age and those not admitted to our hospital were excluded. Inactive malignancy was not included as an underlying illness. In-hospital and 14-day mortality rates were assessed. For 225 available bacterial isolates, *cps* genotyping was performed (*10*).

A total of 231 patients with *K. pneumoniae* bacteremia were observed at the hospital during the study; 225 isolates from 225 patients were used. A total of 133 (59%) of these patients had community-onset bacteremia (bacteremia identified in an emergency department). The in-hospital mortality rate was 32.4%. Among 225 isolates, 41 (18.2%) were identified as K1 serotype, 37 (16.4%) as K2, 15 (6.7%) as K57, and 8 (3.6%) as K54. The K1 serotype was found predominantly in community-onset infections (36 [87.8%] of 41 patients compared with 75 [51.0%] of 147 patients infected with non–K1/K2 serotypes; odds ratio [OR] 6.91, 95% confidence interval [CI] 2.57–18.60) (online Appendix Table 1, www.cdc.gov/EID/content/17/6/1113-appT1.htm).

Underlying illness was classified as nonfatal in 75.6% of patients with K1 bacteremia (53.7% of patients with non–K1/K2 bacteremia; OR 2.67, 95% CI 1.22–5.84). A lower percentage of patients with K1 bacteremia had surgery in the previous 3 months (9.8% vs. 30.6%; OR 0.25, 95% CI 0.09–0.73). Patients with K1 bacteremia had lower mean ± SD Pittsburgh bacteremia scores than those with non–K1/K2 bacteremia (2.7 ± 3.1 vs. 4.4 ± 4.7; OR 0.90, 95% CI 0.81–0.99), but the time until a positive blood culture was obtained was not different. K1 serotype was more common in patients with liver abscesses (46.3% vs. 4.1%; OR 20.3, 95% CI 7.31–56.40) and less common in patients with urinary tract infections (UTIs) (4.9% vs. 20.4%; OR 0.20, 95% CI 0.05–0.88). The in-hospital mortality rate for patients with K1 bacteremia was lower that that for patients with non–K1/K2 bacteremia (14.6% vs. 34.7%; OR 0.32, 95% CI 0.13–0.82).

No differences were found in clinical characteristics for patients with K2 bacteremia and those with non–K1/K2 bacteremia except for a higher frequency of liver abscesses in patients with K2 bacteremia (13.5% vs. 4.1%; OR 3.67, 95% CI 1.06–12.8). For patients infected with K54 and K57 serotypes, 1 K57 serotype caused liver abscesses; no abscesses were found in patients infected with a K54 serotype. The in-hospital mortality rate was 50% (4/8) for patients with K54 bacteremia and 53.3% (8/15) for patients with K57 bacteremia.

Patients infected with a K1 serotype had lower mean ± SD Pittsburgh bacteremia scores (2.7 ± 3.1 vs. 5.0 ± 5.3;

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OR 0.88, 95% CI 0.78–0.98, p = 0.002) and lower 14-day and in-hospital mortality rates (9.8% vs. 27.0%; OR 0.29, 95% CI 0.08–1.03, p = 0.06; and 14.6% vs. 43.2%; OR 0.23, 95% CI 0.08–0.67, p = 0.007) than patients infected with K2 serotypes. A higher percentage of patients with K1 bacteremia had liver abscesses at the site of infection (46.3% vs. 13.5%; OR 5.53, 95% CI 1.80–17.02, p = 0.003).

Characteristics of patients with community-onset *K. pneumoniae* bacteremia were also analyzed (online Appendix Table 2, www.cdc.gov/EID/content/17/6/1113-appT2.htm). Patients infected with a K1 serotype were more likely to have liver abscesses and less likely to have UTIs or biliary tract infections (OR 11.5, 95% CI 3.99–33.20; OR 0.20, 95% CI 0.04–0.92; and OR 0.25, 95% CI 0.07–0.91, respectively).

In our patients, K1 and K2 serotypes were found at similar frequencies (18.2% and 16.4%, respectively), which differs from results of Fung et al., in which the K1 serotype was more common (K1 30.8% and K2 5.1%) (*12*). Despite reported virulence of the K1 serotype, it was primarily responsible for community-onset bacteremia in patients with less severe underlying illness and associated with lower mortality rates. Moreover, the K1 serotype is associated with liver abscesses and lower mortality rates (*2–7*). Liver abscesses were found in 46% of patients with K1 bacteremia, and a K1 serotype was found in 63.3% of patients with liver abscesses.

Conclusions

Management of liver abscesses has improved in Taiwan because of increased physician awareness (*13*). Mortality rates for patients with *K. pneumoniae* bacteremia were lower in patients with UTIs or biliary tract infections (*5,14*), which were less common in patients infected with a K1 serotype. Thus, patient outcomes depend more on underlying conditions and severity of sepsis than on bacterial serotypes (*5,9,14*).

In our previous study of the interval until a positive blood culture for *K. pneumoniae* bacteremia was obtained (*9*), we found that higher Pittsburgh bacteremia scores, a time until a positive blood culture <7 hours, and active malignancy were associated with death. In this study, we found no difference in time until a positive blood culture was obtained for patients infected with different serotypes. This interval for patients infected with K1 serotypes was slightly longer than that for patients infected with K2 and non–K1/K2 serotypes. This finding may have resulted from a higher percentage of community-onset infections and liver abscesses and less severe underlying illness in patients infected with a K1 serotype.

Studies investigating *K. pneumoniae* bacteremia have grouped K1 and K2 serotypes (*3,7*). However, such grouping may be problematic because evidence suggests that the K1 serotype is the major cause of primary liver abscesses (*6*). Another report showed that the genetic background of serotype K2 is diversified, and only 1 of the 2 major K2 clones was highly virulent in mice (*15*). These findings are consistent with our clinical observations. Differences in symptoms of patients infected with K2 and non–K1/K2 serotypes were minimal, despite slightly more liver abscesses among patients infected with K2 serotypes, which was lower than for patients infected with K1 serotypes. Because of different serotyping methods used (*3,5,15*), caution is required when interpreting data from various studies.

Despite greater virulence of the K1 serotype, it is predominant in patients with community-onset infections and in those with less severe underlying illness. Although the K1 serotype is the major cause of liver abscesses, it results in a lower mortality rate, which can be attributed to host factors.

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Association of Patients' Geographic Origins with Viral Hepatitis Co-infection Patterns, Spain

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To determine if hepatitis C virus seropositivity and active hepatitis B virus infection in HIV-positive patients vary with patients' geographic origins, we studied co-infections in HIV-seropositive adults. Active hepatitis B infection was more prevalent in persons from Africa, and hepatitis C seropositivity was more common in persons from eastern Europe.

Since the introduction of highly active antiretroviral treatment (HAART), non-AIDS defining conditions have become major causes of illness and death in HIV-infected patients. In particular, liver disease has emerged as a major cause of death in the HAART era (*1*,*2*). In HIV-infected patients, chronic liver conditions are mostly caused by hepatitis C virus (HCV) and hepatitis B virus (HBV) (*3*,*4*).

Worldwide distribution of both viruses is heterogeneous because of different patterns of transmission (*5*,*6*). In addition, HBV immunization programs at birth were implemented in some countries during the 1990s, which has led to a decrease in the proportion of chronic HBV carriers (*6*). As a consequence of these different patterns of risk and immunization, HCV and HBV prevalence vary across countries and even across regions in the same country (*7*,*8*). Several studies have addressed HBV and HCV prevalence in migrants and ethnic minorities (*9,10*), but few studied viral hepatitis co-infections in HIV-infected persons according to the patients' geographic origins (*11*,*12*).

In Spain, as in other high-income countries, migrants from developing countries represent a growing proportion of persons with HIV-infections (*13*). The question we addressed in our study was whether HCV seropositivity and active HBV infection in HIV-positive patients vary with the patients' geographic origins.

The Study

To assess this question, we defined active HBV infection as the presence in serum of hepatitis B surface antigen (HBsAg) and defined HCV seropositivity as the presence of HCV antibodies. Then we described the prevalence of HCV seropositivity and active HBV infection in HIV-positive patients from the Cohort of the Spanish AIDS Research Network (CoRIS) who had never received HAART, according to their geographic origin. In addition, we explored the association between HCV seropositivity and active HBV infection with geographic origin, taking into account potential confounders.

CoRIS is an open, prospective cohort, which integrates data from 31 centers from 13 of the 17 autonomous communities in Spain. CoRIS inclusion criteria for patients are the following: >13 years of age, new to the center, and previously untreated with HAART. A detailed description of this cohort has been previously reported (*14*). Patients signed informed consent and the study was approved by the ethics committees at each participant hospital. For the purpose of this study, we collected data from all 4,419 HIV-positive HAART-naive patients included in CoRIS from January 1, 2004, through November 30, 2008.

Serologic tests for HBV and HCV were done by the clinical laboratories associated with each of the participating sites by using commercially available ELISAs to detect HBsAg. HCV antibody testing was performed with a commercial ELISA, and positive results were confirmed by immunoblot. For the HBV analyses, we considered only those patients who had positive HBsAg results at study entry (n = 3,824). Similarly, for the HCV analyses, we only considered patients with positive HCV antibody test results at entry (n = 3,867).

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[1]A complete listing of the members of the Spanish AIDS Research Network is provided in the online Technical Appendix (www.cdc.gov/EID/content/17/6/1116-Techapp.pdf)

CoRIS collected the following variables at cohort entry: gender (male or female), age (<31, 31–40, or >40 years), transmission category (injection drug users [IDU], men who have sex with men [MSM], heterosexual contact, and other/unknown), educational level (no studies, primary school, secondary school, university, and unknown), geographic origin (Spain, non-Spanish western Europe, eastern Europe and Russia, sub-Saharan Africa, North Africa, Latin America, and other/unknown origin), serologic markers (positive, negative, and unknown).

Description of baseline characteristics was done by frequency distributions. A χ^2 test was used to compare proportions between geographic origins. We calculated univariate odds ratios of association of co-infection with sex, transmission category, age at entry into cohort, educational level, and geographic origin. Trend score tests were used with age and educational level. Multivariate logistic regression analysis was used to estimate the association of geographic origin with HCV and active HBV co-infections. Taking into account previous studies (*7–11*), we decided to include the following variables in the multivariate analyses: gender, transmission category, age at entry to cohort, and educational level. We used likelihood ratio tests to address the adequacy of the model.

Differences at baseline according to geographic origin are shown in Table 1. In all studied populations, prevalence of HCV seropositivity was 21.8% (95% confidence interval [CI] 20.5%–23.1%). Compared with Spaniards, for whom prevalence was 26.5%, HCV seropositivity was higher in migrants from eastern Europe, 45.9% (p<0.01), and lower in persons from sub-Saharan Africa, North Africa, and Latin America, for whom numbers were 10.1% (p<0.01), 16.4% (p = 0.09), and 4.9% (p<0.01), respectively. No significant differences were observed when seropositivity was compared with that of persons from Western Europe, 22.3% (p = 0.30).

In all studied populations, active HBV infection was 5.8% (95% CI 5.1%–6.6%); when compared to prevalence of infections in persons born in Spain (4.9%), active HBV infection was more common in persons from western Europe, sub-Saharan Africa, and North Africa with 11.2% (p<0.01), 11.1% (p<0.01), and 10.9% (p<0.05), respectively. No significant differences were observed between prevalence in Spain and prevalence in persons from eastern Europe and Russia, 8.4% (p = 0.15) and Latin America, 5.6% (p = 0.46).

Marked differences in HCV seropositivity and active HBV infection prevalence according to transmission category were also observed, showing higher prevalence of co-infection in IDU (HCV 89.5%; HBV 7.8%) than in heterosexual persons (HCV 13.0%, p<0.01; HBV 5.1%, p<0.05) or MSM (HCV 3.5%, p<0.01; HBV 5.8%, p = 0.09). Active HBV infection was more common in MSM (8.2%) than in heterosexual persons (2.8%, p<0.01) only in HIV-positive patients from Latin America.

In analyses adjusted for age group (<31, 31–40, or >40 years), gender, transmission category (heterosexual, IDU, MSM, other) and level of education (no studies, primary school, secondary school, university, unknown), geographic origin remains a strong risk factor for HCV seropositivity (Table 2). Geographic origin in eastern Europe and Russia was significantly associated with higher prevalence of HCV

Table 1. Sociodemographic characteristics of patients in Cohort of the Spanish Aids Research Network by geographic origin, Spain, 2004–2008*

Patient characteristic	No. (%) patients							
	All	Spain	Western Europe	Eastern Europe	Sub-Saharan Africa	North Africa	Latin America	Other/UNK
Total	4,419	3,023	136	90	315	67	740	48
Female sex	1,003 (22.70)	574 (18.99)	15 (11.03)†	41 (45.56)‡	182 (57.78)‡	24 (35.82)‡	166 (22.43)†	1 (2.08)
Age, y								
<31	1,344 (30.42)	751 (24.84)	23 (16.91)	56 (62.22)‡	147 (46.67)‡	18 (26.87)	330 (44.59)‡	19 (39.58)
31–40	1,750 (39.61)	1,197 (39.60)	76 (55.88)‡	23 (25.56)	105 (33.33)	32 (47.76)	301 (40.68)	16 (33.33)
>40	1,325 (29.98)	1,075 (35.56)	37 (27.21)	11 (12.22)	63 (20.00)†	17 (25.37)	109 (14.73)‡	13 (27.08)
Transmission								
Heterosexual	1,666 (37.70)	960 (31.76)	30 (22.06)	48 (53.33)‡	284 (90.16)‡	50 (74.63)‡	282 (38.11)†	12 (25.00)
IDU	721 (16.32)	641 (21.20)	25 (18.38)	24 (26.67)	5 (1.59)	6 (8.96)	14 (1.89)	6 (12.50)
MSM	1,852 (41.91)	1,301 (43.04)	78 (57.35)†	12 (13.33)†	2 (0.63)	6 (8.96)	427 (57.70)‡	26 (54.17)
Other/UNK	180 (4.07)	121(4.00)	3 (2.21)	6 (6.67)	24 (7.62)	5(7.46)	17 (2.30)	4 (8.33)
Level of studies								
No studies	303 (6.86)	152 (5.03)	5 (3.68)	9 (10.00)	78 (24.76)‡	15 (22.39)‡	41 (5.54)	3 (6.25)
Primary	1,416 (32.04)	998 (33.01)	30 (22.06)	33 (36.67)	92 (29.21)	19 (28.36)	236 (31.89)	8 (16.67)
Secondary	1,203 (27.22)	839 (27.75)	42 (30.88)	28 (31.11)	39 (12.38)†	14 (20.90)	231 (31.22)	10 (20.83)
University	642 (14.53)	459 (15.18)	39 (28.68)†	7 (7.78)	10 (3.17)	7 (10.45)	113 (15.27)	7 (14.58)
Unknown	855 (19.35)	575 (19.02)	20 (14.71)	13 (14.44)	96 (30.48)†	12 (17.91)	119 (16.08)	20 (41.67)†

*IDU, injection drug user; MSM, men who have sex with men; UNK, unknown.
†p<0.05, χ^2 test for the difference between proportions of persons from each place of origin and persons born in Spain.
‡p<0.01.

Table 2. Frequencies of hepatitis C virus seropositivity and/or active hepatitis B virus-HIV coinfection in HIV-infected patients and multivariate odds ratio of association to sociodemographic variables, Spain, 2004–2008*

	HCV seropositivity		HBsAg seropositivity	
Variable	No. (%) patients	Adjusted OR (95% CI)	No. (%) patients	Adjusted OR (95% CI)
Sex				
M	3,012 (21.18)	NA	2,952 (6.47)	1.00
F	855 (24.09)		872 (3.67)	0.44 (0.28–0.68)
Age, y				
<31	1,168 (9.76)	1.00	1,119 (5.36)	NA
31–40	1,532 (24.22)	2.60 (1.84–3.67)	1,515 (5.54)	
>40	1,166 (30.79)	3.85 (2.72–5.46)	1,189 (6.64)	
Transmission category				
Heterosexual	1,400 (13)	1.00	1,467 (5.11)	1.00
Injection drug use	639 (89.51)	50.67 (36.85–69.68)	618 (7.77)	1.71 (1.11–2.63)
Men who have sex with men	1,670 (3.53)	0.33 (0.24–0.45)	1,584 (5.81)	1.33 (0.89–1.98)
Other/unknown	158 (19.62)	1.50 (0.97–2.33)	155 (5.16)	0.97 (0.45–2.09)
Level of studies				
No studies	272 (32.72)	1.00	262 (8.78)	1.00
Primary school	1,286 (31.18)	0.80 (0.52–1.24)	1,244 (6.75)	0.82 (0.5–1.36)
Secondary school	1,108 (13.09)	0.51 (0.32–0.82)	1,054 (4.27)	0.48 (0.27–0.84)
University	591 (5.75)	0.42 (0.24–0.76)	560 (5.54)	0.61 (0.33–1.12)
Unknown	610 (28.69)	1.05 (0.66–1.67)	704 (5.68)	0.64 (0.37–1.11)
Geographic origin				
Spain	2,650 (26.53)	1.00	2,628 (4.91)	1.00
Western Europe	121 (22.31)	1.01 (0.51–2.03)	116 (11.21)	2.38 (1.29–4.39)
Eastern Europe	85 (45.88)	3.76 (2.06–6.83)	83 (8.43)	2.15 (0.96–4.84)
Sub-Saharan Africa	268 (10.07)	0.60 (0.38–0.96)	280 (11.07)	3.63 (2.22–5.92)
North Africa	55 (16.36)	0.51 (0.2–1.34)	55 (10.91)	2.96 (1.21–7.23)
Latin America	648 (4.94)	0.45 (0.29–0.69)	622 (5.63)	1.30 (0.87–1.93)
Other/unknown	40 (17.5)	0.81 (0.26–2.5)	40 (5.00)	0.98 (0.23–4.15)

*Variables included in the multivariate analyses: gender, transmission category, age at entry into cohort, and educational level. HCV, hepatitis C virus; HBsAg, hepatitis B virus surface antigen; OR, odds ratio; CI, confidence interval; NA, not associated in the model.

seropositivity than was origin in Spain, and sub-Saharan African, North African, and Latin American origins were associated with lower prevalence of HCV seropositivity. Nonetheless, IDU transmission category was the factor that showed the greatest association with HCV seropositivity. Analyses of association of geographic origin to active HBV infection, with data adjusted for age, gender, transmission category, and level of education (Table 2), showed that origin in Western Europe, sub-Saharan Africa, and North Africa was associated with a significantly higher prevalence of active HBV infection than origin in Spain.

CoRIS annually undertakes both internal and external quality audits. The cohort represents the HIV-positive population that initiates care at hospitals in Spain, i.e., those whose conditions have been newly diagnosed and, therefore, they have not begun HAART at the time of entry at cohort. Geographic origin and transmission categories are collected as reported by the patient, which could produce some misclassification. However, our results are consistent, and the association of both co-infections with geographic origin is unlikely to be biased. We could not hypothesize about risks at origin since data on exposure in country of origin (e.g., vaccination, occupation, health care received) are not collected in our database.

Conclusions

Geographic origin of HIV-positive patients influences the epidemiology of both HCV seropositivity and active HBV infection in HIV-positive patients who begin HIV clinical care in Spain. Although injection drug use remains the main risk factor for HCV seropositivity as reported by other studies (*15*), differences by geographic origin are maintained in multivariate analyses. For active HBV infection, geographic origin is the major risk factor shown by HIV-positive patients who seek clinical care for HIV in Spain. Our findings suggest that the background prevalence of HCV and HBV co-infections in different migrant communities does play a role in shaping the epidemiology of both co-infections in HIV-positive patients.

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Possible Novel Nebovirus Genotype in Cattle, France

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To determine if bovine caliciviruses circulate in France, we studied 456 fecal samples from diarrheic calves. We found a 20% prevalence of genogroup III noroviruses and a predominance of genotype III.2. Neboviruses, with a prevalence of 7%, were all related to the reference strain Bo/Nebraska/80/US, except for the strain Bo/DijonA216/06/FR, which could represent a novel genotype.

In the *Caliciviridae* family, genogroup III noroviruses (NoVsGIII) and neboviruses are associated with enteric disease in cattle (*1*), while genogroups I and II noroviruses (NoVsGI, NoVsGII) are a major cause of viral gastroenteritis in humans. Because of these common taxonomic and clinical features, molecular epidemiologic studies have been conducted on cattle worldwide to investigate possible zoonotic transmission. In France, little is known about the prevalence and genetic diversity of NoVsGIII and neboviruses circulating in cattle and possibly in humans.

The Study

We collected 456 fecal samples from diarrheic calves (mean age 9 days, median 8 days) from 415 farms in Burgundy, France, during December 2005 through September 2008 to screen for these viruses: 1 sample each was collected for 377 outbreaks; 2 and 3 samples were collected for 35 and 3 outbreaks, respectively. Reverse transcription PCR, targeting the 3′ end of the polymerase gene of NoVsGIII and neboviruses, was done with the QIAGEN One Step RT-PCR kit (QIAGEN, Hilden, Germany), according to the manufacturer's instructions, by using the primer sets CBECU-F/CBECU-R and NBU-F/NBU-R (*2*). The complete capsid gene of a selection of neboviruses was amplified by using NBcap-F3/NBcap-R primers (*3*). The amplified products of 532 bp, 549 bp, and 1,692 bp were sequenced by using the same primers.

In addition, we collected human stool samples in Burgundy and other regions of France during June 2006 through September 2008 and analyzed them for the presence of NoVsGIII and neboviruses. Samples included 60 samples from 21 gastroenteritis outbreaks of unknown etiology and 50 samples from 13 gastroenteritis outbreaks related to the consumption of oysters or water contaminated by human noroviruses and other enteric viruses.

Among the 456 samples from cattle, 114 (25%) were positive: 89 (20%) and 34 (7%) for NoVsGIII and neboviruses, respectively, with 9 (2%) samples infected by both viruses. These findings corresponded to 83 and 32 outbreaks positive for NoVsGIII and neboviruses, respectively, among which 9 presented co-infections in the same samples. The prevalence of NoVsGIII in similar studies range from 4% in the Netherlands (*4*) to 80% in Michigan, USA (*5*). These variations can be partly explained by differences in sampling strategies. The prevalence of neboviruses in our study was similar to that reported in other countries, e.g., 8% in the United Kingdom (*6*) and 9% in South Korea (*7*), but lower than in Ohio, USA (29%) (*2*). Furthermore, similar to our results, Smiley et al. (*2*) found a predominance of NoVsGIII compared to nebovirus, whereas in the United Kingdom and South Korea the prevalence of the 2 viruses was similar (*6*–*9*).

Sequencing and phylogenetic analyses of 89 NoVsGIII strains enabled them to be classified into 2 groups: 25 strains, representing 5% of the 456 specimens analyzed, were homologous to each other. They clustered with the genotype 1 reference strain Bo/Jena/80/DE on the gene fragment analyzed (Table 1, phylogenetic analyses not shown). The other 64 strains, 14% of the 456 samples, clustered with the genotype 2 reference strain Bo/Newbury2/76/UK (Newbury Agent [NA] 2). Partial polymerase sequences of a selection of these strains were submitted to the GenBank database under the accession nos. GU259570–GU259580 and FJ974131–FJ974136. The high number of sequences obtained in our study allowed us to highlight the existence of 2 distinct genotypes within NoVsGIII, as proposed by Ando et al. (*10*) and confirmed by others (*2*,*5*,*9*). Furthermore, the predominance of genotype 2 observed in our study is in keeping with numerous data (*2*,*4*,*8*,*9*). One study reported similar prevalence for the 2 genotypes, with a slight predominance of genotype 2 (*5*). All these results suggest that genotype 1 could be a minor circulating genotype and genotype 2 the main genotype worldwide.

Molecular and phylogenetic analyses of the partial polymerase region of 34 detected neboviruses revealed that 33 strains were homologous to each other and to the reference strain Bo/Nebraska/80/US (Nebraska strain [NB]) (*11*) (Figure 1; Table 2). No strain was related to the reference strain Bo/Newbury1/76/UK (NA1) (*1*,*6*). The same observation was made in the United States and South Korea, where only NB-like neboviruses were identified (*2*,*7*). A few studies have presented data about the

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Table 1. Genogroup III norovirus nt and aa identities calculated on the 3′ end polymerase*

Variable	Group 1, France		Group 2, France		Bo/Jena/80/DE (genotype 1)		Bo/Newbury2/76/UK (genotype 2)	
	nt identity	aa identity	nt identity	aa identity	nt identity	aa identity	nt identity	aa identity
Group 1 (25 strains)	80.4–100	94.6–100	71.3–78.0	84.6–90.4	83.4–90.8	96.4–100	71.7–76.8	85.6–88.5
Group 2 (64 strains)	71.3–78.0	84.6–90.4	83.6–100	97.6–100	72.0–76.1	85.6–88.5	82.8–91.9	97.6–100

*Identity expressed as a percentage calculated by using MEGA 4.0 (www.megasoftware.net). nt, nucleotide; aa, amino acid.

epidemiology of neboviruses, but it seems that NA1-like neboviruses have only been identified in UK cattle (*1*,*6*). The 34th strain of our study, Bo/DijonA216/06/FR, was not related to NB or NA1 and appeared on a quite separate branch of the neboviruses.

To gain more insight into the classification of neboviruses, we analyzed the complete capsid sequences of a selection of French neboviruses, including Bo/DijonA216/06/FR. While NB, NA1, and all but 1 of the French strains were closely related (Table 2; Figure 2), Bo/DijonA216/06/FR again presented low levels of identity with reference strains and appeared on a different branch on the phylogenetic tree. To our knowledge, there is no clear definition of a nebovirus genotype based on nucleotide or amino acid sequence identities. Oliver et al. (*6*) compared sequences of different NB-like and NA1-like strains and showed that strains of the same polymerase genotype presented >88% nt (>95% aa) identity in the polymerase region, while nucleotide sequence identities among strains from distinct polymerase type were <78% (88% aa) in this region. In a study from Korea, the minimum polymerase nucleotide identity between strains related to the NB-like viruses was 80.9% (84.5% aa), while these strains presented a maximum of 78.4% nt (82.8% aa) identity with NA1-like strains (*7*). Using capsid sequences, Oliver et al. (*6*) showed that nucleotide sequence identities within 1 type ranged from 92% to 94% (96% to 99% aa), whereas in Korea nucleotide sequence identities ranged from 85.7% to 87.7% (92.3 to 93.8% aa) (*7*). According to these data, Bo/DijonA216/06/FR appears to fall into a new genotype. This hypothesis is reinforced by the phylogenetic analysis of the amino acid sequences: Bo/DijonA216/06/FR clusters

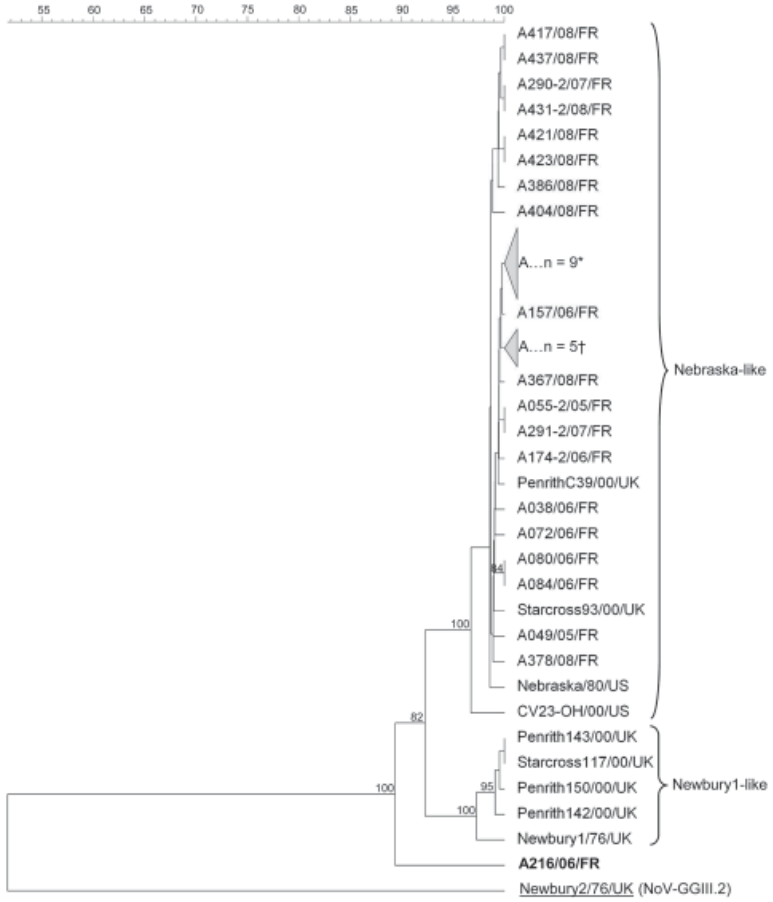

Figure 1. Nebovirus phylogenetic tree based on the deduced 167-aa–length sequences covering the 3′ end polymerase region. Possible novel strain is shown in **boldface**. Sequence alignments and clustering were performed by the unweighted-pair group method by using arithmetic average with Bionumerics software (Applied Maths, Sint-Martens-Latem, Belgium). Bootstrap values calculated with 1,000 replicate trees are given at each node when >70%. Collapsed branches are made up of the following nebovirus strains from France: *A042/06/FR, A051/05/FR, A058/05/FR, A130–2/06/FR, A143–2/06/FR, A311–2/08/FR, A381/08/FR, A445–2/08/FR, and A448/08/FR; †A281/07/FR, A355/07/FR, A356/07/FR, A364/08/FR, and A365/08/FR. Partial polymerase sequences of the French nebovirus strains were submitted to the GenBank database under accession nos. GU259537–GU259569 (A complete list is available from the authors.) GenBank accession nos. of calicivirus reference strains used in this tree are the following: AY082890 (CV23-OH/00/US), AY082891 (Nebraska/80/US), NC_007916 (Newbury1/76/UK), AF097917 (Newbury2/76/UK), DQ228162 (PenrithC39/00/UK), DQ228160 (Penrith142/00/UK), DQ228161 (Penrith143/00/UK), DQ228157 (Penrith150/00/UK), DQ228165 (Starcross93/00/UK), and DQ228164 (Starcross 117/00/UK). Underlined strain is an outgroup. Scale bar indicates the percentage of similarities between strains.

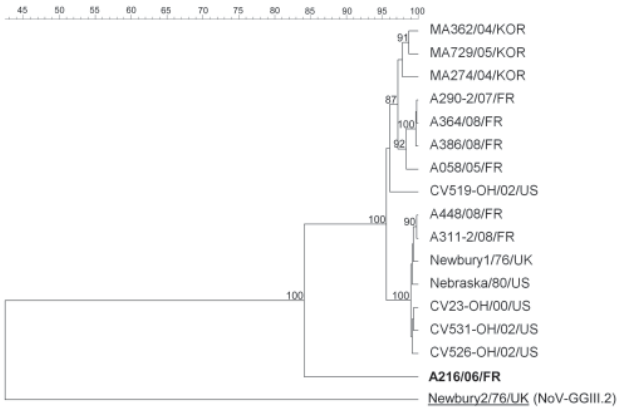

Figure 2. Nebovirus phylogenetic tree based on the deduced 549-aa–length sequences of the complete capsid. Possible novel strain is shown in **boldface**. Sequence alignments and clustering were performed by the unweighted-pair group method by using arithmetic average with Bionumerics software (Applied Maths, Sint-Martens-Latem, Belgium). Bootstrap values calculated with 1000 replicate trees are given at each node when >70%. Capsid sequences of the French nebovirus strains were submitted to the GenBank database under the following accession nos.: GU259542 (DijonA058/05/FR), FJ687386 (DijonA216/06/FR), GU259551 (DijonA290–2/07/FR), GU259553 (DijonA311–2/08/FR), GU259556 (DijonA364/08/FR), GU259561 (DijonA386/08/FR), and GU259569 (DijonA448/08/FR). GenBank accession nos. of Calicivirus reference strains used in this tree are: AY082890 (CV23-OH/00/US), AY549169 (CV519-OH/02/US), AY549170 (CV526-OH/02/US), AY549171 (CV531-OH/02/US), EF528561 (MA274/04/KOR), EF528564 (MA362/04/KOR), EF528569 (MA729/05/KOR), AY082891 (Nebraska/80/US), NC_007916 (Newbury1/76/UK), and AF097917 (Newbury2/76/UK). Underlined strain is an outgroup. Scale bar indicates the percentage of similarities between strains.

on a separate branch for both the polymerase and capsid regions, whereas the NB-like and NA1-like viruses cluster on separate branches in the polymerase region, but together in the capsid region. Given the capsid analyses, the new *Nebovirus* genus in the *Caliciviridae* family should thus comprise 2 genotypes: one would include the reference strains Bo/Nebraska/80/US and Bo/Newbury1/76/UK and the second would include Bo/DijonA216/06/FR. This hypothesis awaits confirmation, and the complete genome of this strain is now being characterized in our laboratory.

Conclusions

The existence of animal reservoirs for human NoVs has been suggested (*4*,*8*,*12*) but, to our knowledge, only Mattison et al. (*13*) detected NoVsGII in fecal samples from pigs and cattle. In our study, we found no evidence of zoonotic transmission, since no NoVsGIII or neboviruses were detected in any samples from humans, which, for NoVsGIII, is consistent with the results of Wolf et al. (*14*). However, in contrast to these observations, Widdowson et al. (*15*) detected serum antibodies raised against NoVsGIII in humans, which suggests that NoVsGIII do infect humans.

This study found that NoVsGIII are more frequent than neboviruses in diarrheic calves in France. Our results confirmed the predominance of genotype 2 NoVsGIII, as previously reported (*2,4,8,9*), and showed that NB-like viruses are the major circulating strains of neboviruses. In addition, substantial genetic diversity of neboviruses was demonstrated with the existence of a possible novel strain, which could represent a new genotype.

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Table 2. Nebovirus nucleotide and amino acid identities calculated on the 3′ end polymerase and capsid*

	Nebovirus, France		Bo/Nebraska/80/US		Bo/Newbury1/76/UK	
Variable	nt identity	aa identity	nt identity	aa identity	nt identity	aa identity
Polymerase						
Nebovirus, France (33 strains)	88.4–100	96.0–100	87.9–90.2	96.6–98.0	76.8–79.5	87.9–89.3
Bo/DijonA216/06/FR strain	75.9–78.1	83.9–84.6	76.1	84.6	80.4	87.2
Capsid						
Nebovirus, France (6 strains)	85.3–97.7	92.9–99.6	85.2–91.1	92.9–99.0	84.6–92.3	92.9–99.2
Bo/DijonA216/06/FR strain	68.5–69.3	75.1–75.7	69.6	74.9	68.0	75.1

*Identity expressed as a percentage calculated by using MEGA 4.0 (www.megasoftware.net). nt, nucleotide; aa, amino acid.

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Vibrio cholerae in Traveler from Haiti to Canada

To the Editor: A nationwide outbreak of cholera caused by *Vibrio cholerae* O1 serotype Ogawa began in Haiti in October 2010 and has since resulted in >200,000 illnesses and 4,000 deaths (*1*). Additional cases of cholera attributed to the outbreak strain have subsequently been reported in the neighboring Dominican Republic and in Florida and New Jersey in the United States. In these instances, illness was related to travel to Haiti or consumption of contaminated water on the island of Hispaniola (which is shared by Haiti and Dominican Republic).

In Canada, the province of Québec has a large Haitian immigrant population. In early November 2010, the Québec public health authorities provided clinicians and laboratories with recommendations regarding the diagnosis of *V. cholerae* infections. We report a case of *V. cholerae* O1 serotype Ogawa in Canada related to the outbreak in Haiti. It was diagnosed in Montréal, Québec, on January 5, 2011.

A 49-year-old Canadian woman traveled to Haiti with her 5 brothers and sisters during December 22–29, 2010, to attend her mother's funeral. While in Haiti, they stayed with family members. She came to the emergency department of the Centre Hospitalier de l'Université de Montréal on January 1, 2011, with abdominal cramps and diarrhea of moderate intensity that had started on December 29, the day she returned from Haiti. The patient was asthenic, but vital signs and results of a physical examination were normal. A complete blood count, levels of serum electrolytes and serum creatinine, and results of liver function tests were within reference ranges. A fecal sample was submitted and the patient received intravenous fluids and 1 dose (300 mg) of doxycycline. She improved rapidly and was discharged on January 3. The patient returned to the outpatient clinic on January 7, and she had recovered from her illness. Control fecal specimens obtained on January 9 and 10 were negative for *V. cholerae*. Family members that traveled with her did not get ill, and there were no secondary cases among her family members in Montréal.

The fecal culture of the sample provided on December 29 contained *V. cholerae.* The isolate was confirmed as toxigenic *V. cholerae* serogroup O1, serotype Ogawa, biotype El Tor, and matched the Haiti outbreak strain when tested by pulsed-field gel electrophoresis (PFGE). Antimicrobial drug susceptibility testing was performed by continuous gradient dilution (Etest; AB Biodisk, Solna, Sweden), and results were interpreted according to standard criteria (*2*). The strain was susceptible to azithromycin (0.25 mg/L), ciprofloxacin (0.5 mg/L), and tetracycline (1 mg/L) and resistant to trimethoprim/sulfamethoxazole (>32/608 mg/L).

Cases of diagnosed cholera are rare in Canada (0–3 laboratory-confirmed isolations of serogroup O1 and O139 *V. cholerae* per year (*3*). All cases in Canada have been associated with travel to cholera-endemic areas, including Africa and Southeast Asia. Monitoring of cholera in Canada is completed through the National Notifiable Diseases Program and through the public health laboratory network. Biochemical identification, serotyping, and PFGE testing are performed on all suspected *V. cholerae* isolates. Confirmed isolations of a serogroup O1 or O139 *V. cholerae* strain that produces cholera toxin are also reported through the International Health Regulations focal point.

Whole genome sequencing has been completed for several isolates to investigate the origin of the Haiti cholera outbreak (*4*,*5*). However, PFGE remains one of the primary tools for defining the outbreak strain (*4*). The highly standardized methods of PulseNet International for generating, analyzing, and comparing PFGE patterns are used worldwide to track the temporal and geographic distribution of *V. cholerae* (*6*,*7*).

PFGE for *V. cholerae* was performed by using restriction enzymes *Sfi*I and *Not*I (*8*). The PFGE patterns of this travel-associated case matched patterns of the representative Haiti cholera outbreak strain that was deposited into the American Type Culture Collection (Manassas, VA, USA) by the Centers for Disease Control and Prevention (Atlanta, GA, USA; strain BAA-2163; CDC isolate 2010EL-1786) (Figure). PulseNet Canada *Sfi*I and *Not*I PFGE pattern designations were VCSFI.0006 and VCNTI.0006, respectively, and were equivalent to PulseNet USA patterns KZGS12.0088 and KZGN11.0092.

Prevention, treatment, and control efforts are currently under way in Haiti. Loss of infrastructure during the earthquake of January 12, 2010, has affected implementation of sanitation and public health measures. Travel

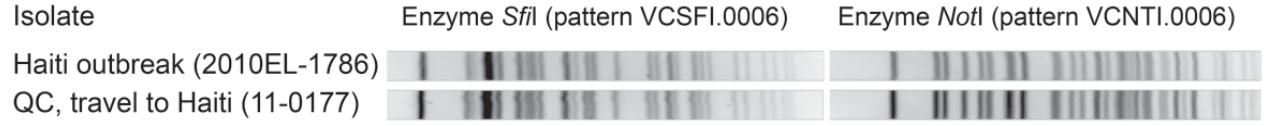

Figure. Pulsed-field gel electrophoresis (PFGE) of cholera outbreak strain from Haiti and travel-associated isolate in the patient, by using *Sfi*I and *Not*I and PulseNet Canada PFGE pattern designations.

advisories and travel health precautions were subsequently released, including those from Canada and United States (*9*,*10*). These precautions recommended that preventative measures such as vaccination and safe food and water consumption practices be adhered to by residents and visitors to affected regions. Although the public health community anticipated that travel-associated cases would be diagnosed in Québec, this report of a documented case (supported by laboratory and epidemiologic data) emphasizes the domestic and international public health risk caused by the nationwide outbreak in Haiti. It also illustrates the need for an accurate travel history in clinical and laboratory diagnosis of cholera infections.

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Easy Diagnosis of Invasive Pneumococcal Disease

To the Editor: Invasive pneumococcal disease (IPD) causes many cases of severe disease and death among children <5 years of age, mostly in developing countries (*1*,*2*). Before conjugate vaccines can be introduced in developing countries, information about disease epidemiology is urgently needed. The lack of laboratories equipped to perform pneumococcal serotyping leads to the need to send isolates to reference laboratories. Good sample preservation is necessary to prevent samples from arriving at the laboratory in poor condition. We evaluated the usefulness of multiplex real-time PCR from strains and blood samples kept at room temperature on dried blood spot (DBS) filter paper for detecting and serotyping *Streptococcus pneumoniae*. DBS screening is a reliable method that requires only a small amount of blood; it is used for the diagnosis of several human diseases (*3*,*4*).

To validate the technique, we selected 15 pneumococcus clinical isolates representing 15 serotypes (1, 5, 19A, 19F, 14, 3, 7F, 4, 6A, 6B, 8, 9N, 18C, 23A, 23F) obtained during 2009 from patients at Hospital Sant Joan de Déu, in Barcelona. These isolates, used as controls, had been serotyped by quellung reaction at the Instituto de Salud Carlos III, Majadahonda-Madrid, Spain. These strains were cultured overnight at 35°C in 5% carbon dioxide on Columbia agar plates with 5% sheep blood (bioMérieux SA, Marcy l'Etoile, France). A suspension of each strain was adjusted to match a 0.5 McFarland standard (equivalent to 10^8 colony-forming units (CFU)/mL). Stock solutions of pneumococcus culture for each previously identified serotype were injected into blood

previously extracted from 2 healthy volunteers. Serial dilutions of 100,000 CFU/mL to 1,000 CFU/mL (1,000 to 10 CFU equivalents/PCR) were performed. A total of 100 µL of blood was applied to DBS filter paper, and another 100 µL was used for DNA extraction from fresh blood. All DBS samples were air dried for 1 week. The procedure was also performed on negative control blood samples.

DNA was extracted from DBS and fresh blood samples by using the NucliSense easyMAG automated extraction platform (bioMérieux, Boxtel, the Netherlands) according to the manufacturer's instructions. DNA detection of the *pneumolysin* (*ply*) gene by real-time PCR was performed according to a published assay (*5*). In addition, we performed a multiplex real-time PCR for molecular serotype detection of serotypes 1, 3, 5, 4, 6A, 6B, 7FA, 8, 9VANL, 14, 15BC, 18CB, 19A, 19FBC, 23F, 23A and the conserved capsular gene *wzg* as described by Tarrago et al. (*6*). DNA extracts were amplified with the Applied Biosystems 7300 Real-time PCR System (Applied Biosystems, Foster City, CA, USA). Negative results were defined as those with cycle threshold >40.

To evaluate the reliability obtained with this in vivo approach, we performed identification and serotyping of *S. pneumoniae* in 25 DBS samples from 25 children at Saint John of God Hospital in Mabesseneh-Lunsar, Sierra Leone. This hospital does not perform blood cultures. IPD was confirmed when DNA of a *pneumolysin (ply)* gene and an additional capsular gene of *S. pneumoniae* were detected by multiplex real-time PCR of DBS samples.

Detection of *ply*, *wzg*, and the specific gene for molecular serotype showed that both fresh blood and DBS samples yielded correctly positive results from the 10-fold serial dilutions analyzed (Table). With respect to the 25 (11 female and 14 male) patients from Sierra Leone who had suspected IPD, the median age was 25.71 months (range 15 days to 96 months); all had a diagnosis of fever without apparent source, and 16 also had malaria. Of these 25 children, DBS samples from 15 (60%) yielded a positive result for the *ply* and *wzg* genes, so they were considered confirmed episodes of IPD. A serotype included in 13-valent conjugate vaccine was detected in 6 (40%) of 15 positive samples: serotypes 3, 7FA, 19A, 6A, 6B, and 9VNL (1 sample each). In the remaining 9 samples, the results for *ply* gene and *wzg* gene were positive, but none of the 24 tested serotypes was detected.

This preliminary study enabled us to demonstrate that DBS screening is a reliable and easy method for diagnosing IPD and also for epidemiologic surveillance of the more frequent serotypes. The main limitation of our study is the small number of DBS samples sent from Saint John of God Hospital in Sierra Leone.

In conclusion, the DBS technique enables reproducible transport of samples for identification and serotyping of *S. pneumoniae* by multiplex PCR. The use of DBS on filter paper is an attractive alternative method for storing samples at room temperature and easily transporting them. Additional studies, including evaluation of the relative sensitivity of this method compared to direct culture, are necessary.

Table. Sensitivity of real-time PCR for detecting *Streptococcus pneumoniae ply* or *wzg* genes or a specific gene for molecular serotype from fresh or dried blood spot samples*

	Serial dilutions correctly detected, CFU equivalent/PCR†	
Gene or serotype	Fresh blood	Dried blood spot
ply gene	1.10^1–1.10^3	1.10^1–1.10^3
wzg gene	1.10^1–1.10^3	1.10^1–1.10^3
Serotype 1	1.10^1–1.10^3	1.10^1–1.10^3
Serotype 5	1.10^1–1.10^3	1.10^1–1.10^3
Serotype 19A	1.10^1–1.10^3	1.10^1–1.10^3
Serotype 19F	1.10^1–1.10^3	1.10^1–1.10^3
Serotype 14	1.10^1–1.10^3	1.10^1–1.10^3
Serotype 3	**1.10^2–1.10^3**	1.10^1–1.10^3
Serotype 7F	1.10^1–1.10^3	1.10^1–1.10^3
Serotype 4	1.10^1–1.10^3	1.10^1–1.10^3
Serotype 6A	1.10^1–1.10^3	**1.10^2–1.10^3**
Serotype 6B	1.10^1–1.10^3	1.10^1–1.10^3
Serotype 8	1.10^1–1.10^3	1.10^2–1.10^3
Serotype 9N	1.10^1–1.10^3	1.10^1–1.10^3
Serotype 18C	1.10^1–1.10^3	1.10^1–1.10^3
Serotype 23A	1.10^1–1.10^3	1.10^1–1.10^3
Serotype 23F	1.10^1–1.10^3	1.10^1–1.10^3

*Serial dilutions (10–1,000 CFU equivalent/PCR) of 15 pneumococcus cultures mixed with 15 negative-control blood samples were analyzed. **Boldface** indicates results that differ from others.
†10 CFU equivalent/PCR = 1,000 CFU equivalents/mL blood.

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Mimivirus-like Particles in Acanthamoebae from Sewage Sludge

To the Editor: Mimivirus is a giant, double-stranded DNA virus. Its 650-nm diameter and 1.2-Mb genome make it the largest known virus (*1*). In 2003, mimivirus was isolated from a water cooling tower in Bradford, UK, after a pneumonia outbreak and was reported to infect *Acanthamoeba polyphaga* amebae (*2*). Subsequently, a small number of additional isolates have been reported (*3*).

Mimivirus has been associated with pneumonia, and this association was strengthened after antibodies to mimivirus were found in serum samples from patients with community- and hospital-acquired pneumonia and after mimivirus DNA was found in bronchoalveolar lavage specimens (*4*). More direct evidence of pathogenicity was illustrated when a pneumonia-like disease developed in a laboratory technician who worked with mimivirus and showed seroconversion to 23 mimivirus-specific proteins (*5*).

We report finding mimivirus-like particles during our molecular study of *Acanthamoeba* spp. abundance and diversity in final-stage conventionally treated sewage sludge from a wastewater treatment plant in the West Midlands, UK. Using metagenomic DNA extracted from the sludge (*6*), we estimated the abundance of *Acanthamoeba* spp. by using real-time PCR (*7*) and found it to be $\approx 1 \times 10^2$/g sludge. To assess species diversity, we amplified an *Acanthamoeba* spp.–specific 18S rRNA target, which resulted in products of ≈450 bp (*8*). PCR products were cloned and sequenced, revealing low *Acanthamoeba* spp. diversity with a predominance of clones most similar to *A. palestinensis* (22/25 clones), which fall within the T6 clade according to the classification of Stothard et al. (*9*). A small number (3/25) of clones showed closest similarity to acanthamoebae belonging to the T4 clade, which includes strains considered to be human pathogens, including some *A. polyphaga* strains.

Acanthamoebae were isolated from fully digested sewage sludge by inoculating diluted sludge onto cerophyl-Prescott infusion agar and subculturing onto nonnutrient agar plates streaked with heat-killed *Escherichia coli*. Cultures were incubated at 20°C and 30°C and examined under an Axioskop 2 microscope (Zeiss, Oberkochen, Germany) at 100× magnification; cells of interest were examined at 1,000× magnification. One clonal population of an *Acanthamoeba* sp. isolated at 20°C, which demonstrated typical trophozoite and cyst morphology, contained large numbers of particles either within vacuoles or within the cytoplasm (Figure). Vacuoles were densely packed with particles that appeared to be constantly moving; vacuole size varied from that typical of food vacuoles to large vacuoles that occupied most of the cell volume (expanded online Figure, panels B, D, and G, www.cdc.gov/EID/content/17/6/1127-F.htm). Because the particles were assumed to be bacterial pathogens, efforts were made to produce an axenic culture of the ameba isolate, and 16S rRNA PCR was performed to identify any intracellular bacteria. DNA was extracted by using a phenol chloroform method according to Griffiths et al. (*6*). However, no 16S rRNA PCR products were amplified.

Months later, an image review led to recognition of unusual arrangements of intracellular particles in a lattice-like structure in which each particle was surrounded by 6 others. Measurement of rows of particles, assuming tight packing, gave an average particle size of 620 nm. At this point, we realized that the particles were virus-like

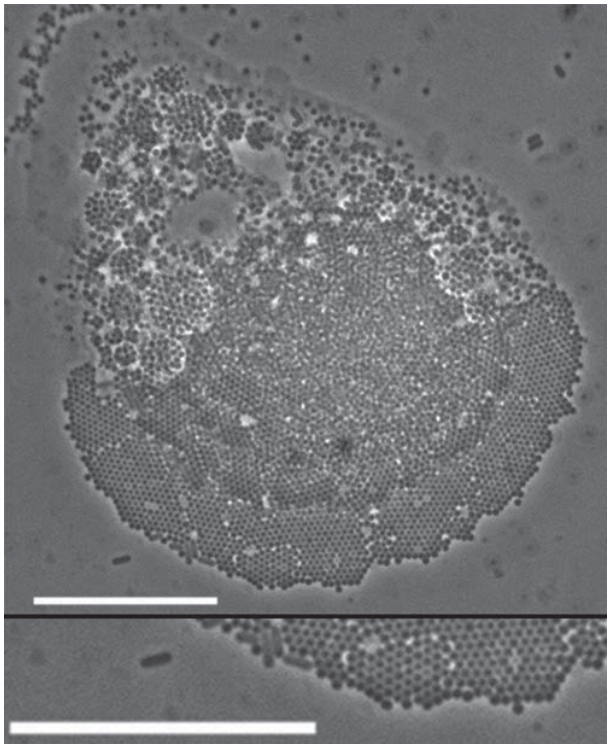

Figure. Light micrograph of acanthamoebae infected with mimivirus-like particles, showing cell packed with mimivirus-like particles. Enlargement of region of image shown at bottom of original image. Scale bar = 10 μm. An expanded version of this figure with additional images is available online (www.cdc.gov/EID/content/17/6/1127-F.htm).

and closely resembled mimivirus. Subsequent efforts to resuscitate the infected *Acanthamoeba* spp. culture were unsuccessful, and performing specific PCR for mimivirus sequences was not possible. Sewage sludge samples collected later were tested for mimivirus by using PCR (*4*); however, no amplification was observed, indicating either that mimivirus was present only transitorily, that mimivirus was below detection limits, or that the target primer sites were not conserved.

The density of virus-like particles within acanthamoebae cells was extremely high (Figure). The advantage of in situ observation of amebae on the surface on which they were cultivated is that the cell is not disturbed. The virus-like particles are arranged in tightly packed, flat sheets, indicative of an icosahedral structure. Toward the bottom of the Figure A, a single sheet of particles can be seen, corresponding to the hyaline zone at the anterior of the cell, and in the center of the cell are multiple layers of tightly packed virus-like particles. Toward the anterior of the cell, vacuoles containing particles were apparently being egested at the uroid. Dense 3-dimensional aggregations of particles (expanded online Figure, panel E) resembled previously described virus factories (*10*). Free mimivirus-like particles (expanded online Figure, panels B, D) indicate egestion by amebae or the result of amebal lysis, a phenomenon observed in cocultures. Prevalence of infection was high and infection was immediately obvious, even when cultures were observed at low magnification (100×).

Although we did not confirm the identity of the mimivirus-like particles by molecular methods or electron microscopy, the nature of the light micrographs enabled close examination of the particles. These particles demonstrated close similarity to mimivirus in size and shape as indicated by the lattice arrangement in which 1 particle was surrounded by 6 others, as seen previously (*10*). Our study illustrates that acanthamoebae that survive sewage treatment can harbor mimivirus-like particles, which could be disseminated to agricultural land and surface waters.

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Letters

Letters commenting on recent articles as well as letters reporting cases, outbreaks, or original research are welcome. Letters commenting on articles should contain no more than 300 words and 5 references; they are more likely to be published if submitted within 4 weeks of the original article's publication. Letters reporting cases, outbreaks, or original research should contain no more than 800 words and 10 references. They may have 1 Figure or Table and should not be divided into sections. All letters should contain material not previously published and include a word count.

Rabies Immunization Status of Dogs, Beijing, China

To the Editor: In the People's Republic of China, >3,000 persons die of rabies each year; most were infected by dog bites (*1*). Since 2000, the dog population in Beijing has increased dramatically, and the exact vaccination coverage and immunization status of dogs are not known.

During 2006–2009, to assist with governmental rabies control, Fengtai District was selected as a geographically representative area in Beijing in which to conduct a survey of rabies antibody titers in domestic dogs. Blood samples were randomly collected from 4,775 dogs in Fengtai District, which account for 3% of all registered dogs in the district. Rabies virus neutralization antibody (VNA) titers were detected by fluorescent antibody virus neutralization (*2*). In brief, VNA titers ≥0.5 IU indicated positive immunization, implying that the dog had an adequate level of antibody, and VNA <0.5 IU indicated negative immunization (*3*). The data were analyzed by 2-tailed χ^2 test; $p<0.05$ was considered significant. Vaccination coverage and antibody levels were categorized either by dog's function (guard or pet) or residence (urban or suburban) (Figure).

Most dogs with a history of vaccination were positively immunized (68.1%) (Figure, bar A), compared with 16.4% in the unvaccinated group (Figure, bar B), demonstrating that compulsory immunization is crucial to rabies control (*4*). Of 944 dogs with unclear vaccination history, 221 (23.4%) (Figure, bar C) had adequate antibody levels, possibly from undocumented vaccination or contact with rabies hosts. However, for 2006, 2007, 2008, and 2009, immunization coverage in the district was 55.0%, 53.8%, 67.4%, and 54.4%, respectively, all below the >70% criterion recommended by the World Health Organization (*5*). The results imply that much work still needs to be done by the Beijing government, not only to meet the World Health Organization immunization baseline but also to keep risk for a rabies epidemic in Beijing low.

Immunization coverage ratios differed significantly ($p<0.05$) between guard (39.3%) and pet dogs (69.5%) (Figure, bars D, E) and between urban (81.7%) and suburban areas (27.6%) (Figure, bars F, G). Consequently, the number of negatively immunized guard dogs was 1.68× lower than that for pet dogs (Figure, bars D, E) ($p<0.05$), and the number of positively immunized dogs in urban areas was 2.5× higher than that in suburban areas (Figure, bars F, G) ($p<0.05$).

In Beijing, guard dogs are usually raised by villagers to protect the house, whereas pet dogs are usually raised by city dwellers who treat dogs as friends. As a result, in urban areas dogs are registered and vaccinated in a timely manner by authorized pet hospitals (*6*). In suburban areas, however, dog management is deficient. For example, guard dogs in suburban areas are sometimes not vaccinated because the owner or veterinarian cannot safely restrain the dog for vaccination.

According to our study, >10% of unregistered dogs with no clear history of vaccination are not vaccinated during yearly vaccination programs. In Beijing during 2007–2009, of 9 cases of rabies in humans, 6 were associated with stray dogs (*7*), and most stray dogs were found in suburban areas. Hence, strategies to either reduce stray dogs in the city or to get such dogs under official management (e.g., include stray dogs in compulsory annual vaccination programs) are urgently needed.

In our opinion, policies related to dog registration, vaccination recording, and vaccination strategies

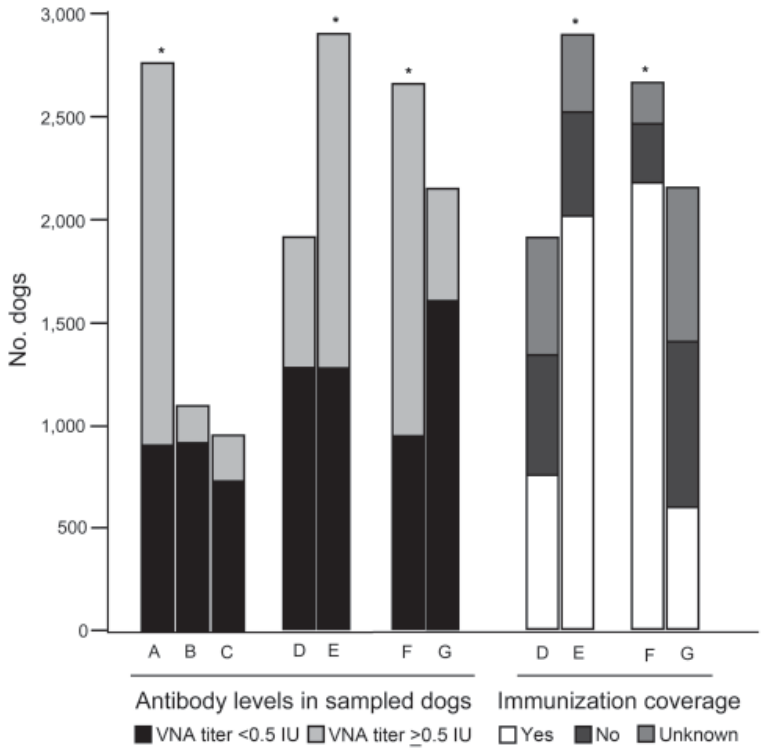

Figure. Rabies immunization status of dogs, Fengtai, Beijing, China. Immunization status and vaccination coverage were categorized according to dog vaccination background and rabies antibody level in each dog. A) Vaccinated, B) never vaccinated, C) unclear vaccination history; D) guard dog, E) pet dog; F) in urban areas, G) in suburban areas. *Significant difference ($p<0.05$) for dogs with positive antibody levels between A, B, and C; between D and E; or between F and G; or a significant difference ($p<0.05$) in dog immunization coverage between D and E or between F and G. A color version of this figure is available online (www.cdc.gov/EID/content/17/6/1129-F.htm).

need improvement in Beijing, especially in suburban areas. Although our report only focused on the Fengtai District, the findings could be helpful for the Beijing government for establishing strategies to control the rabies epidemic in the entire city.

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Effect of Media Warnings on Rabies Postexposure Prophylaxis, France

To the Editor: Rabies was officially declared eliminated in nonflying mammals in metropolitan France during 2001–2008 by the World Organisation for Animal Health; the last case of rabies was believed to occur in a fox in 1998. However, rabies remained a public health concern because of the risk of translocation of infected dogs from enzootic areas and the natural circulation of bat rabies–associated lyssaviruses (BRALVs). In 2008, France temporarily lost its rabies-free status following evidence of an indigenous case of rabies in a dog, linked to an index case in a dog infected in Morocco. In 2007, a domestic cat was found infected with a BRALV, an indication that, although bats are the primary hosts of this pathogen, other mammals may be infected. (*1*). Rabies is a tragic and frightening disease, and bats have a sinister image. Therefore, possible transmission of rabies from bats to humans represents a particularly terrifying threat in which emotional distortion may play a key role in public responses. Patient demand for rabies postexposure prophylaxis (RPEP) has been associated with media-communicated health alerts in France (*2*,*3*) and French Guiana (*4*).

We compared the number of RPEP treatments in humans after bat-related exposures in the south of France with newspaper reports about rabies-related events over an 8-year period. In France, primary health care management of patients seeking RPEP is delivered through an official network of antirabies medical centers. All centers in the southern half of France were asked to provide the number of RPEP treatments that followed bat-related exposures in mainland France during 2002 through 2009. Of 22 centers, 18 participated in the study, reporting 326 RPEP treatments (Figure). Two marked peaks were observed: in September 2004 and in September 2008. The number of patients reporting bat-related exposures that occurred during the summer period (June–September) showed marked annual variations with a 2.1-fold increase in 2004 (44 cases) and a 4.7-fold increase in 2008 (96 cases) compared with the 2002–2009 average of 20.5 cases/summer (range 7–31). Most cases in 2008 were reported by the Marseille and Bordeaux centers. In 2004, 3 cases of illegally imported dogs with rabies were observed in France, in February, May, and August (*2*,*5*). Newspapers reported extensively on the third case, with 54 articles published in the 3 major national newspapers (Le Monde, Le Figaro, Libération) (*2*), after an alert was issued in late August (Figure). On July 30, 2008, in response to a familial cluster of RPEP following bat bites near Marseille, a media-communicated health alert was organized by the Marseille center in the major regional newspaper (La Provence) to warn people about the potential risk of rabies after bat-related exposures and the necessity of seeking advice when such events occur. The alert was developed by the Agence France Presse, released by the majority of national and regional newspapers, and included in a large number of websites (Figure). On August 28, 2008, an additional alert was released in Bordeaux because of 2 BRALV-infected bats in the region (Figure). The alert was published in the regional newspaper (Sud-Ouest) (*3*). No other rabies-related events were intensively reported in French media during the study period.

The pattern of spikes in RPEP in the south of France seen after bat-rabies media reports supports the results of other studies that found effective newspaper reporting increases patient demand for RPEP (*2*–*4*). However, the increase is of short duration. Our results may indicate that media reports bring out the worried well who were not truly exposed but still request RPEP. The results may also indicate that bat-related injuries or contacts are underreported in the absence of media events.

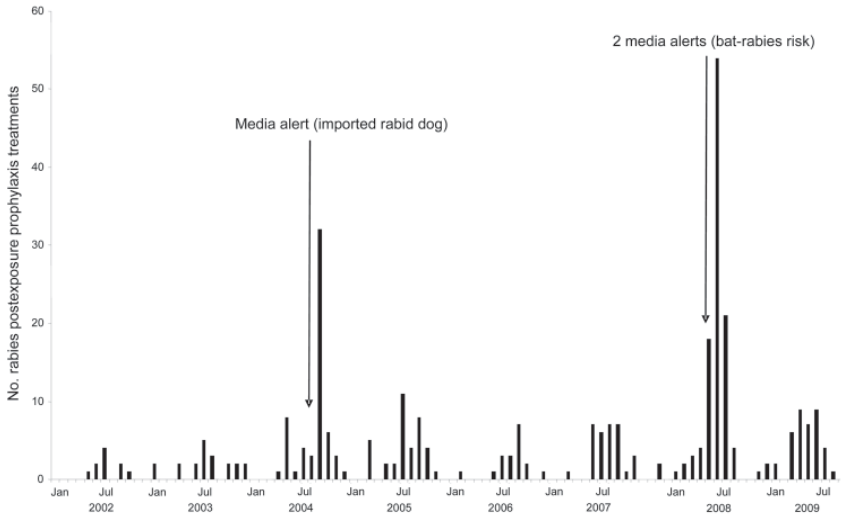

Figure. Number of rabies postexposure prophylaxis treatments caused by bat-related exposures as reported by 18 antirabies medical centers in southern France, by time of first visit, 2002–2009. Centers responding were Annecy, Annonay, Aurillac, Bastia, Bordeaux, Chambéry, Grenoble, Le Puy en Velay, Limoges, Lyon, Marseille, Nice, Pau, Perpignan, Poitiers, Roanne, Saint-Etienne, and Toulouse.

Classical rabies virus has been transmitted to humans by bats in South America (*6*). In Europe, European bat lyssavirus type 1 (EBLV-1) has been isolated from bats in Germany, France, the Netherlands, Denmark, Yugoslavia, Spain, and Poland and EBLV-2 from bats in the Netherlands, United Kingdom, Switzerland, Germany, and Finland (*6*,*7*). Only 3 cases of rabies in humans caused by bat bites have been reported in Europe (*6*). In France, health authorities recommend that all cases of confirmed bat-related exposures (bites, scratches, exposures to a mucous membrane) receive RPEP with both vaccine and immunoglobulin (*8*), based on World Health Organization guidelines (*9*). Whether RPEF is needed for other types of exposures is subject to considerable debate (*10*). The centers in southern France are directed to provide RPEP only for known exposures.

Based on results of study we conducted in 2008 when RPEP increased 4.7 fold after 2 media releases within several weeks, it is possible that only 20% of persons with bat exposure typically seek RPEP in periods without media reports. Although the risk of human rabies acquired through exposure to European bats is rare, information should be provided to the French public to avoid direct contact with bats, including handling when found inside homes during the summertime. When available, bats should be submitted for rabies testing to determine whether RPEP is needed. Following these procedures should minimize both the potential risk for transmission and the number of expensive RPEP treatments.

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Bedbugs as Vectors for Drug-Resistant Bacteria

To the Editor: Over the past 10 years in North America and western Europe, a resurgence of bedbugs (*Cimex lectularius*) has occurred (*1*). Although the basis for this resurgence is unclear, large bedbug infestations have been attributed to increased worldwide travel, altered insecticide management, and increased resistance to pesticides (*2*). Marginalized populations in large urban centers appear to be disproportionately affected; ≈30% of shelters in Toronto, Ontario, Canada, report bedbugs (*1*). We report recovery of methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus faecium* (VRE) from bedbugs in Vancouver, British Columbia.

Three patients, all residents of Vancouver's Downtown Eastside—an impoverished community in Vancouver with high rates of homelessness, poverty, HIV/AIDS, and injection drug use (*3*)—were hospitalized and found to be infested with bedbugs. Hypothesizing that

these parasites may be vectors for the transmission of antimicrobial drug–resistant pathogens, we collected 5 bedbugs and tested them for drug-resistant organisms. The bedbugs were homogenized and streaked onto standard microbiological media, including 5% sheep blood agar. Bacterial colonies were identified by using conventional and automated microbiological methods, and susceptibility testing was performed in accordance with Clinical Laboratory Standards Institute guidelines (*4*). For 2 patients, VRE was isolated from 1 bedbug each. These bacterial isolates were also resistant to ampicillin, teicoplanin, and aminoglycosides but susceptible to linezolid, quinupristin/dalfopristin, and tetracycline. For 1 other patient, MRSA was isolated from 3 bedbugs. All MRSA isolates had susceptibility patterns consistent with pulsed-field gel electrophoresis type USA300 (susceptible to vancomycin, clindamycin, trimethoprim/sulfamethoxasole, tetracycline, and rifampin; resistant to erythromycin).

Despite investigations of transmissibility of numerous infectious agents, including transmissible blood-borne pathogens such as HIV and hepatitis B and C viruses, to our knowledge, no conclusive evidence has demonstrated disease transmission by bedbugs (*5*,*6*). Clinically, bedbug bites have been associated with cutaneous manifestations, most commonly pruritic wheals with a central hemorrhagic punctum (*6*). Excoriation at the site of a bite can cause further skin abrasion, thereby providing an entry point for colonizing bacteria, which can potentially result in folliculitic or cellulitic superinfections (*5*). *S. aureus*, which is commonly found on the skin and can cause cellulitis, has been reported to colonize the salivary glands of bedbugs for as long as 15 days (*7*). However, although transient and persistent forms of colonization may play a role in disease transmission, we did not differentiate these forms because the clinical bedbug specimens were processed at the time of receipt in the laboratory.

Similar to other cities worldwide, Vancouver has seen an alarming increase in bedbugs, particularly in the Downtown Eastside, where 31% of residents have reported bedbug infestation (*8*). Enterococci commensally occupy the gastrointestinal tract. The recovery of VRE from bedbugs could result from the relatively unhygienic living conditions encountered in the Downtown Eastside and the high level of interconnectedness between St. Paul's Hospital (where a large pool of patients with VRE colonization/infection reside) and the surrounding community.

MRSA is also a substantial problem in the Downtown Eastside; it has been cultured from 54.8% of skin and soft tissue infections of patients seen at the emergency department of St. Paul's Hospital (*9*). More recently, among wound infections in injection drug users in this community, 43% were colonized or infected with MRSA consistent with USA300, the predominant community-associated MRSA strain in Vancouver (*10*). The phenotype of the MRSA recovered from the bedbugs was consistent with community-associated MRSA and identical to that found on antibiograms from patients with MRSA infection who reside in this community. Given the high prevalence of MRSA (particularly USA300) in hotels and rooming houses in Vancouver's Downtown Eastside, bedbugs may become colonized with community-associated MRSA. Consequently, these insects may act as a hidden environmental reservoir for MRSA and may promote the spread of MRSA in impoverished and overcrowded communities.

Bedbugs carrying MRSA and/or VRE may have the potential to act as vectors for transmission. Further studies are needed to characterize the association between *S. aureus* and bedbugs. Bedbug carriage of MRSA, and the portal of entry provided through feeding, suggests a plausible potential mechanism for passive transmission of bacteria during a blood meal. Because of the insect's ability to compromise the skin integrity of its host, and the propensity for *S. aureus* to invade damaged skin, bedbugs may serve to amplify MRSA infections in impoverished urban communities.

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Community Vaccinators in the Workplace

To the Editor: Adult vaccination rates are low (*1*), and workplaces are a useful location for increasing vaccination (*2*). In 2008, only 41% of US workers 50–64 years of age reported vaccination against influenza virus (*3*). Workplace vaccination is common and increases with employer size (*4*). Among adults, the workplace is the most common site for influenza vaccination for persons 18–49 years of age and second most common for persons 50–64 years (*2*). Offering vaccination in the workplace increases vaccination coverage (*5*).

Consistent with guidelines and economic incentives, employers have focused workplace vaccination on seasonal influenza (*4*), but the workplace has also been a key site for vaccination against influenza A pandemic (H1N1) 2009 and could be a site for other adult vaccinations. The most recent guidelines from the Advisory Committee on Immunization Practices recommend annual influenza vaccination of all adults (*6*). In most years, the seasonal influenza vaccine and predominant circulating viruses are well matched, and employers have an economic incentive to decrease worker absenteeism by increasing influenza vaccination (*7*). The workplace is also potentially a site for delivery of herpes zoster, pneumococcal, and tetanus-diphtheria-pertussis vaccines (*6*).

Our experience with employers suggests that most contract with external organizations (i.e., community vaccinators) to provide workplace vaccination, but we found little or no information about these organizations in the literature. Therefore, we interviewed community vaccinators about their 2009 experience with workplace vaccination against seasonal influenza virus and pandemic (H1N1) 2009 virus, their business practices, barriers encountered, and delivery of other adult vaccines.

We selected a diverse study population of community vaccinators. We combined the 10 US Department of Health and Human Services regions to create 5 study regions. Beginning with a list of vaccinators provided by the Centers for Disease Control and Prevention (Atlanta, GA, USA) and searching with Google for "on-site vaccinators," we identified 17 national and 28 local vaccinators (full list available from the authors). We selected at least 1 national and 1 local vaccinator from each region and then purposively sampled them to increase geographic and organizational diversity. Our sample comprised 5 national vaccinators, 7 local vaccinators serving urban and rural workplaces, a mobile-clinic vaccinator, a visiting nurses association, and an occupational health specialist.

The qualitative study (*8*) used a structured telephone interview with community vaccinators' lead personnel responsible for worker vaccination. Our theoretical approach was content analysis. After 2 pilot interviews, 2 interviewers completed 10 additional interviews. Because the questions in the pilot and final interviews were similar, we analyzed both groups together and report here on all 12 interviews. We designed the interviews to last <20 minutes and conducted them during March and April 2010. One interviewer used Atlas.ti software (Atlas.ti Software Development, Berlin, Germany) to code the interviews, with review and concurrence from the second interviewer. The Human Subjects Division of the University of Washington approved this study as exempt from review.

Challenges reported for the 2009 influenza vaccination season included the need for workers to receive 2 vaccines (seasonal and pandemic [H1N1] 2009) and a mismatch between vaccine demand and supply, resulting in delayed or lost business (9/12 respondents). Some vaccinators found the season more challenging than in prior seasons (4/5 national; 2/7 local), yet most reported having added clients (4/5 national; 4/7 local).

Vaccinators' reported business practices include vaccinating at sites in addition to workplaces, for example, churches and faith-based settings (9 vaccinators), schools (9), and community centers (8). Most (9) reported vaccinating on multiple work shifts and at multiple worksites. Ten vaccinators also reported they can help employers publicize workplace vaccination events. Most did not report patient-level vaccination information to health plans (10 vaccinators), primary-care providers (9), or registries (8). Many directly bill Medicare (8) and private insurers (7) if asked.

Additional findings related to barriers and delivery of other vaccines. Commonly reported barriers

to increasing workplace vaccination rates were worker reluctance (voiced as "I'm too busy," "I don't need it," or "It gives me the flu") (10 vaccinators); worker out-of-pocket costs (9); and low worker awareness of workplace vaccination events (5). Other vaccines offered by these workplace vaccinators included the following: tetanus-diphtheria-pertussis (10 vaccinators), pneumococcal (10), hepatitis A and B (7), and herpes zoster (4).

This qualitative study, although small and not necessarily representative, found remarkable consistency across community vaccinators. Vaccinators were challenged by the pandemic (H1N1) 2009 vaccination season, but the season also provided new clients. Most reported vaccinating at diverse sites in addition to workplaces, and most already vaccinated against diseases other than influenza. Vaccinators consistently identified workers' reluctance and out-of-pocket costs, and poor publicizing of workplace vaccination events as remediable barriers to vaccination. Tackling of these barriers is supported by the literature (*9*,*10*) and the Guide to Community Preventive Services (*5*).

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Methicillin-Resistant *Staphylococcus aureus* in Retail Meat, Detroit, Michigan, USA

To the Editor: Because methicillin-resistant *Staphylococcus aureus* (MRSA) has been identified in retail meat worldwide (*1*–*4*), the potential exists for its transmission to humans. Of the various meat products surveyed, pork had the highest contamination rate in the United States and Canada (*1*,*2*), as did beef in South Korea (*3*) and poultry in the Netherlands (*4*). The study in South Korea also observed MRSA from chicken, which demonstrated sequence type (ST) 692 by multilocus sequence typing (MLST), a type distinct from that isolated in beef and pork. Despite sample size variations, these studies suggested that MRSA contamination in different meat categories can vary by location and that molecular distinction may exist among MRSA isolates in meat of different origin.

We collected 289 raw meat samples (156 beef, 76 chicken, and 57 turkey) from 30 grocery stores in Detroit, Michigan, USA, during August 2009–January 2010. Up to 3 presumptive *S. aureus* colonies per sample were identified by coagulase test and species-specific PCR (*1*). Antimicrobial drug MICs were determined and interpreted according to Clinical and Laboratory Standards Institute guidelines (*5*). *S. aureus* were characterized by pulsed-field gel electrophoresis (PFGE), *mecA* identification, staphylococcal cassette chromosome (SCC) *mec* typing, Panton-Valentine leukocidin identification, *agr* typing, MLST, and *spa* typing as described (*1*,*6*).

Sixty-five (22.5%) samples yielded *S. aureus*: 32 beef (20.5%), 19

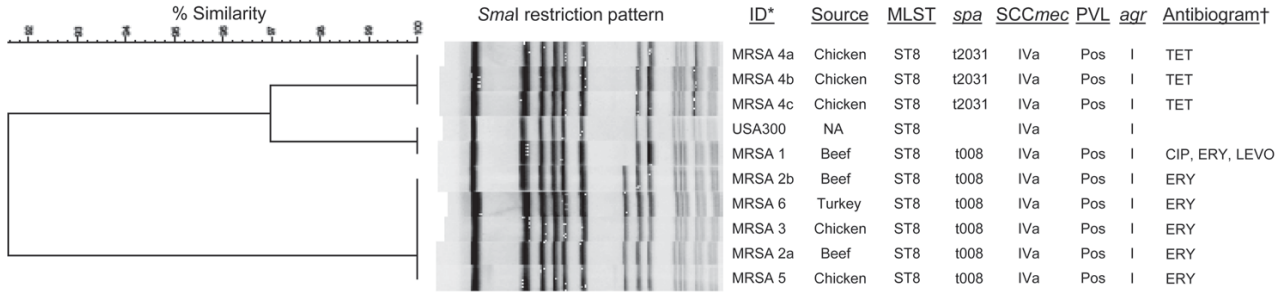
chicken (25.0%), and 14 turkey (24.6%) samples. Six samples, consisting of 2 beef (1.3%), 3 chickens (3.9%), and 1 turkey (1.7%), were positive for MRSA as evidenced by the presence of *mecA*. The overall lower prevalence of *S. aureus* and MRSA than that found in a previous study in the United States (40% and 5%, respectively) (*1*) might be explained by our exclusion of pork because pork and swine production have been major reservoirs of MRSA (*4*,*7*). However, different geographic location and cold sampling seasons in this study also might have caused the variations. The only multidrug-resistant MRSA isolate in this study (MRSA1) was from beef and was resistant to β-lactams, macrolides, and fluoroquinolones (Figure).

Although an extra band was generated in MRSA 2a, 2b, 3, 5, and 6 by PFGE, all 9 MRSA isolates belonged to USA300 (Figure). Multiple isolates from the same samples (MRSA 2a and 2b; MRSA 4a, 4b, and 4c) demonstrated indistinguishable PFGE patterns and other characteristics, which suggested identical MRSA clones. Moreover, MLST, SCC*mec* typing, *agr* typing, and *pvl* detection showed all strains to be positive for ST8, SCC*mec* IVa, *agr* I, and Panton-Valentine leukocidin, which are typical characteristics of USA300 clones. However, *spa* typing identified 2 distinct *spa* types, t008 (11–19–12–**21–17**–34–24–34–22–25) and t2031 (11–19–12–**12–34**–34–24–34–22–25) (repeat variants in **boldface**), which differed by 5 nucleotides. t008, the most common *spa* type of USA300, was identified in 6 isolates of beef, chicken, and turkey origin, whereas t2031 was recovered from MRSA4a, 4b, and 4c from a chicken sample. The nucleotide variation in t2031 caused amino acid changes from glycine-asparagine in t008 to asparagine-lysine. The single nucleotide difference between repeats 12 (GGT) and 21 (GGC) and repeats 34 (AAA) and 17 (AAG) resulted in no amino acid change, with glycine and lysine encoded, respectively.

Unlike studies in Europe, where researchers have reported the animal MRSA clone ST398 from various meat products (*4*), all MRSA isolates in our study were USA300, which suggests a possible human source of contamination during meat processing (*1*). The failure to identify ST398 in the US retail meat also indicates that the human MRSA clones might be better adapted in meat processing than ST398 in this country. Since ST398 is widespread in animals and meat in Europe and has been isolated from other parts of the world (*8*), it is not too bold to predict that ST398 might appear in US meat in the future, especially after the recent report of ST398 from US swine (*7*).

The 5-nt difference between t2031 and t008 implicates multiple MRSA clones in poultry. Previous studies have shown *spa* variants of USA300 from clinical cases associated with distinctive symptoms (*9*,*10*). A single repeat variant, t024, showed substantial genetic, epidemiologic, and clinical differences from t008 in Denmark (*10*). Researchers in Japan also recovered 2 *spa* variants of USA300: t024, which causes blood infections, and t711, which is associated with subcutaneous abscesses (*9*). In both studies, t024 behaved as hospital-associated MRSA, suggesting that *spa* variants of USA300 could lead to different clinical outcomes. Therefore, we can reasonably assume that variants with a meat origin also might have different public health implications; further research on their virulence potential would be helpful to elucidate this possibility.

Despite the recovery of MRSA from retail chicken and t2031 that has an antibiogram distinct from t008, except for β-lactam resistance, several questions remain about whether more *spa* variants are present in poultry (or meat). These include whether t2031 is more adaptable to chicken production because of the 2 amino acid difference from t008, or whether t2031 is linked with specific antimicrobial drug resistance phenotypes other than β-lactam resistance.



ID*	Source	MLST	*spa*	SCC*mec*	PVL	*agr*	Antibiogram†
MRSA 4a	Chicken	ST8	t2031	IVa	Pos	I	TET
MRSA 4b	Chicken	ST8	t2031	IVa	Pos	I	TET
MRSA 4c	Chicken	ST8	t2031	IVa	Pos	I	TET
USA300	NA	ST8		IVa		I	
MRSA 1	Beef	ST8	t008	IVa	Pos	I	CIP, ERY, LEVO
MRSA 2b	Beef	ST8	t008	IVa	Pos	I	ERY
MRSA 6	Turkey	ST8	t008	IVa	Pos	I	ERY
MRSA 3	Chicken	ST8	t008	IVa	Pos	I	ERY
MRSA 2a	Beef	ST8	t008	IVa	Pos	I	ERY
MRSA 5	Chicken	ST8	t008	IVa	Pos	I	ERY

Figure. Dendrogram showing comparison of *Sma*I pulsed-field gel electrophoresis patterns, staphylococcal cassette chromosome (SCC) *mec* type, Panton-Valentine leukocidin (PVL) content, and *agr* type of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from meat samples. All MRSA isolates were resistant to β-lactam antimicrobial drugs (ampicillin, penicillin, and oxacillin) and grew on the 6 μg/mL of cefoxitin for screening methicillin resistance. *Isolates with the same arabic numbers were from the same sample; †only resistance to non–β-lactam antimicrobial drugs was listed. ID, identification; MLST, multilocus sequence typing; ST, sequence type; pos, positive; TET, tetracycline; NA, not available; CIP, ciprofloxacin; ERY, erythromycin; LEVO, levofloxacin.

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Suspected Horse-to-Human Transmission of MRSA ST398

To the Editor: Methicillin-resistant *Staphylococcus aureus* (MRSA) is spreading worldwide among humans and animals, including horses. Many reports of MRSA colonization and infection in horses come from Canada and involve MRSA of sequence type (ST) 8, classified by pulsed-field gel electrophoresis (PFGE) as Canadian MRSA-5 or USA500. ST8 is thought to be a human epidemic clone that has adapted to horses (*1*). Another MRSA type, ST398, has recently begun spreading in Europe and North America and is associated with livestock (*2*). In the Netherlands, MRSA of ST8 (*spa*-type t064) and ST398 (*spa*-type t011), which belong to the livestock–associated CC398, predominate in clinical samples from horses (*3*). To date, human clinical infections with livestock–associated MRSA are uncommon in persons who have not had contact with pigs or calves (*2*). In this case study, we describe the suspected transmission of MRSA ST398 between a horse and a girl, which resulted in infection of the girl's right foot.

In the Netherlands, a 16-year-old girl with spinal muscular atrophy type II (wheelchair-bound and needing artificial ventilation) sought treatment at a hospital for an infected wound on her right foot thought to be caused by an insect bite (online Appendix Figure, www.cdc.gov/EID/content/17/6/1137-appF.htm). The girl was treated as an outpatient. The infection did not respond to empirical treatment with clindamycin and ciprofloxacin. From the infected wound, a MRSA strain that was resistant to clindamycin, ciprofloxacin, erythromycin, gentamicin, kanamycin, tetracycline, and trimethoprim/sulfonamide, and susceptible to rifampin and fusidic acid, was isolated 39 days after initial treatment. Identification of the bacteria and susceptibility testing were performed by using Vitek 2 (bioMérieux, Marcy l'Etoile, France). The girl did not have a history of hospital admission in other countries, nor contact with pigs or calves, but had had intensive contact with a foal. No information was available about hand hygiene practices the girl used after stroking the foal.

Because the girl was a frequent visitor to the hospital, according to the national hospital MRSA guidelines, decolonization therapy was indicated. Before therapy began, her 3 household members and their animals (7 adult Friesian horses, 2 dogs, and 2 cats) were screened for MRSA by enrichment culturing. Nasal swabs were taken from the animals; nasal, throat, and perineal samples were taken from the humans. MRSA with an identical susceptibility pattern

was isolated from a sample taken from the nares of the girl's healthy Friesian foal. The foal had been hospitalized at a horse clinic 2 months earlier because of a wound infection and had been treated with antimicrobial drugs, but no samples had been taken from the horse's wound at that time. All other screening samples were negative for MRSA. The girl's wound healed after application of mupirocin ointment to the nares and perineum (3×/d for 5 days), washing of the body with chlorhexidine shampoo (1×/d for 5 days), and oral administration of fusidic acid and rifampin for 7 days; samples taken were negative for MRSA. The girl was advised not to touch the foal until it too was negative for MRSA. Without therapy, and within 3 months, the foal was negative for MRSA (confirmed by 3 repeated negative cultures of nasal samples by enrichment culturing).

Isolates from the girl and the horse were further investigated by Martineau PCR targeting the *tuf* gene (*4*), *mecA* PCR (*5*), ST398-specific PCR (*6*), *spa* typing (*7*), and PFGE using *Sma*I and *Cfr*9I as restriction enzymes (*8*). Both isolates were identified as *S. aureus*, were *mecA* positive, belonged to ST398, were *spa* type t011, were nontypeable by PFGE using *Sma*I, and had indistinguishable PFGE patterns using *Cfr*9I.

Colonization of persons in contact with infected or colonized horses has been widely reported (*1*–*3*). Clinical MRSA infections of humans associated with horse contact, however, are rare and, to our knowledge, only 2 reports have been published. The first report of a human infection came from Canada and concerned a veterinarian who had a tattoo site infection with Canadian MRSA-5, (ST8, SCC*mec* type IV, *spa* type t007) (*9*). Human skin infections with Canadian MRSA-5 associated with horse contact were also reported from 3 persons who worked in a foal nursery (*10*). MRSA ST398 *spa*-type t011 are cultured regularly from equine samples at the horse clinic (*3*); therefore, the foal probably became colonized during its hospitalization. Livestock-associated MRSA infections are rare in humans in the region where the girl lives, and human-to-human transmission of MRSA ST398 is uncommon. In addition, the girl was severely handicapped and could not travel freely. Therefore, we theorize that the foal, which was stabled in a barn at her home, was the most likely source of the infection. It is also possible that the girl and the foal contracted MRSA from an unidentified common source or that the foal was exposed by the girl, although this is less likely. Close collaboration between the pediatrician, infection control practitioner, veterinarians, and the human microbiologist was necessary to identify the suspected source of infection.

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Screening for Pandemic (H1N1) 2009 Virus among Hospital Staff, Spain

To the Editor: After the emergence of pandemic (H1N1) 2009 virus, measures for its control were taken quickly (e.g., isolation of affected patients and use of gowns, gloves, and N95 respirators) when a clinical suspicion of pandemic influenza was established (*1*). One population group frequently exposed to this virus is health care staff. These circumstances prompted us to implement a screening program for the pandemic (H1N1) 2009 virus among personnel working at our hospital in Marbella, Spain.

Costa del Sol Hospital is a 250-bed, second-level center located on the Mediterranean coast. A proposal was made to staff working in the emergency and internal medicine areas that nasal and pharyngeal samples to identify the virus by real-time PCR should be taken weekly over 12 consecutive weeks, from the third week of September 2009 to the third week of December. In addition to providing samples, each worker would be asked to complete a health-status questionnaire regarding his or her vaccination record and the presence of signs or symptoms. Signs and symptoms to be reported in the questionnaires included fever, runny nose, painful swallowing, coughing, sore throat, diarrhea, vomiting, headaches, muscle pains, and general indisposition; 1 question also asked whether, during the previous week, a confirmed diagnosis of influenza with a positive PCR for pandemic (H1N1) 2009 virus had been made in the respondent's household.

At the outset, 60 members of the hospital staff volunteered to participate. Those who missed >4 sample tests, or >2 consecutive ones, were considered to have abandoned the study. Of the 36 staff members who completed the study, 27 were women (75%). The participants' average age was 37 years (CI 95%: 34.8–39.4). Sixteen were doctors, 16 were nurses, 2 were nursing auxiliary staff, and 2 were hospital orderlies. During the monitoring period, 5 (13%) subjects exhibited coughing, 7 (20%) had runny noses, 3 (8%) experienced painful swallowing, 6 (16%) had headaches, and 1 (2%) felt generally unwell. Nearly 75% stated they washed their hands with antiseptic lotion ≥20×/d. Three workers were vaccinated against seasonal and pandemic influenza, while only 1 was vaccinated against pandemic (H1N1) 2009 alone. None took oseltamivir. Five positive samples were identified (13.8% of the study population) being obtained from four doctors and one nurse, all women. The 4 doctors had signs and symptoms for 24–48 hours consisting of fever, general indisposition, and coughing; none of the 4 required hospitalization. The nurse was a woman 26 years of age with no influenza symptoms and with a positive PCR result on week 5. None of these 5 workers had received any influenza vaccination.

Three workers reported that a diagnosis of pandemic (H1N1) 2009 influenza had been made with respect to a member of their household, but none of the workers had a positive PCR result. The distribution of positive PCR results in our hospital during the study is shown in the Figure.

It had previously been hypothesized that the incidence of asymptomatic cases would be higher than the incidence of symptomatic cases (*2*) overall in persons with high exposure (*3*). However, among the study population, only 1 person with positive PCR results was asymptomatic.

Health care workers may have been exposed in a gradual manner from the beginning of the outbreak to a few symptomatic forms, which would explain why so few of them were actually affected. Of the workers in the emergency department who

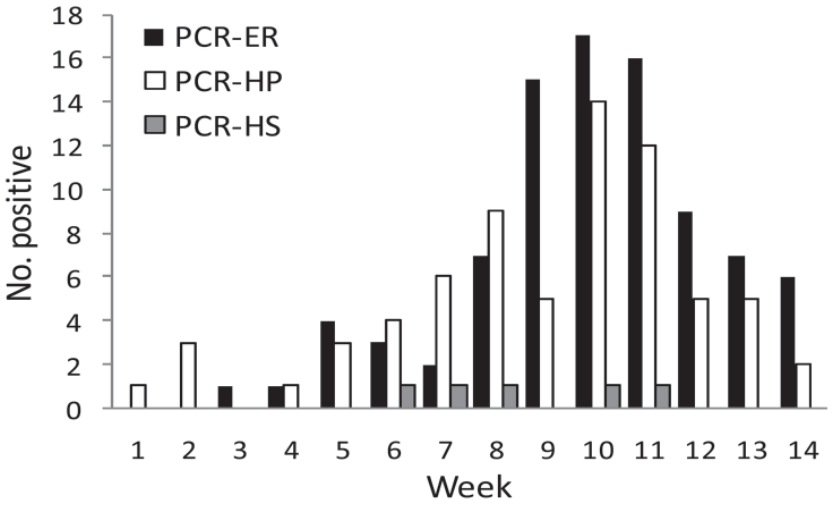

Figure. Number of PCR-confirmed cases of pandemic (H1N1) 2009 virus infection in the emergency department (PCR-ER), hospitalized patients (PCR-HP), and participants (PCR-HS) in a study of screening for pandemic (H1N1) 2009 virus among health care workers, Spain, September–December 2009.

were not part of the study, none were diagnosed with pandemic (H1N1) 2009 during the study period.

Our study began during the week in September 2009 in which the overall rate of incidence of pandemic (H1N1) 2009 in Spain reached 77.8 cases per 100,000 inhabitants (*4*), a level that was above the threshold established for the previous influenza season, and ended during the week in which influenza activity fell below this threshold level (*5*). Therefore, the study spanned the full cycle of the epidemic. The national peak, with an overall rate of incidence of 372.7 cases per 100,000 inhabitants, occurred in week 10 of our study.

This series included 1 asymptomatic carrier. We do not know if that finding could reflect a false-positive test or a low-virulence viral presence.

Notably, among the population of health care workers taking part in the study, only 4 (11%) had been vaccinated against the novel form of the influenza A virus, and none of them had positive PCR results for pandemic (H1N1) 2009 virus. On the other hand, 5 (15%) of workers not vaccinated had a positive PCR result. This finding suggests that, despite the climate of uncertainty concerning the evolution of the influenza outbreak, hospital workers had a greater fear of possible side effects of the vaccine than of the disease itself.

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Pandemic (H1N1) 2009 and HIV Infection

To the Editor: In the United States during spring and fall of 2009, pandemic (H1N1) 2009 influenza A virus resulted in 2 major outbreaks of disease. Initial reports identified immunosuppression, including HIV infection, as a risk factor for the development of severe influenza (*1*–*5*). Subsequent reports did not confirm this association, but the number of HIV-infected patients in these studies was small (*6*,*7*). We describe the clinical course of pandemic (H1N1) 2009 in HIV-infected persons in a US hospital.

During 2009, 23 cases of laboratory-confirmed pandemic (H1N1) 2009 in HIV-infected persons were identified at Harborview Medical Center (Seattle, WA, USA) by querying the University of Washington HIV Information System (a database that enables complete capture of all HIV testing results at Harborview Medical Center) and by querying the Harborview Infection Control Registry for influenza subtype H1N1 infections. Most cases occurred during October and November. Baseline patient characteristics are noted in the Table. Most patients who sought care had fever and cough; median duration of symptoms before seeking care was 4 days. Overall mortality rate for the entire cohort was 8.7%.

Of the 23 patients, only 2 were not treated for influenza; each had mild signs and symptoms and neither required hospital admission. Each of the remaining 13 outpatients received a 5-day course of treatment with oseltamivir. The 8 patients who required hospitalization received therapy for a median of 6 (range 1–22) days.

Overall mortality rate among HIV-infected patients hospitalized for pandemic (H1N1) 2009 infection was 25% (2 of 8 patients). The 2

Table. Baseline characteristics for HIV-infected patients with pandemic (H1N1) 2009, Seattle, Washington, USA, 2009*

Patient characteristics	All patients, n = 23	Inpatients, n = 8	Outpatients, n = 15	p value†
Demographics				
Male sex	19 (83)	5 (63)	14 (93)	0.1
Median age, y (range)	43 (22–72)	46 (34–72)	42 (22–64)	0.5
Race/ethnicity				1.0
White	14 (61)	5 (63)	9 (60)	
Black	5 (22)	2 (25)	3 (20)	
Hispanic	4 (17)	0	4 (27)	1.0
Other/refused to answer	4 (17)	1 (13)	3 (20)	
History				
Received 2009–10 seasonal influenza vaccine before illness	14 (61)	6 (75)	8 (53)	0.1
Ever smoked	15 (65)	7 (88)	8 (53)	0.2
HIV-associated factors				
CD4 count, cells/μL, median (range)	308 (32–1,024)	147 (32–1,024)	438 (119–833)	0.06
Undetectable HIV-1 RNA	19 (83)	6 (75)	13 (87)	0.6
Receiving antiretroviral therapy	21 (91)	8 (100)	13 (87)	0.5
Predisposing risk factors	15 (65)	6 (75)	9 (60)	0.5
Prior lung disease	8 (35)	5 (63)	3 (20)	0.07
Cardiovascular disease	4 (17)	3 (38)	1 (6.7)	0.1
Obesity, body mass index >30	4 (17)	1 (13)	3 (20)	1.0
Neutropenia	3 (13)	2 (25)	1 (6.7)	0.3
Receiving immunosuppressive agent	3 (13)	3 (38)	0	0.03
Malignancy	4 (17)	2 (25)	2 (13)	0.6
Diabetes	2 (8.7)	0	2 (13)	0.5
Signs and symptoms				
Fever	18 (78)	7 (88)	11 (73)	0.6
Fatigue	5 (22)	2 (25)	3 (20)	1.0
Malaise	11 (48)	7 (88)	4 (27)	0.009
Myalgia	10 (43)	3 (38)	7 (47)	1.0
Sore throat	5 (22)	2 (25)	3 (20)	1.0
Cough	21 (91)	8 (100)	13 (87)	0.5
Dyspnea	8 (35)	4 (50)	4 (27)	0.4
Nausea/vomiting	10 (43)	2 (25)	8 (53)	0.4
Median duration of symptoms before seeking care, d (range)	4 (0–30)	4.5 (0–10)	3 (1–30)	0.6
Physical examination findings				
Median temperature, °C (range)	38.0 (35.7–40.2)	39.1 (37.3–40.2)	37.7 (35.7–38.5)	0.001
Median heart rate, beats/min (range)	96 (69–129)	109 (80–127)	94 (69–129)	0.1
Mean arterial blood pressure, mm Hg (range)	94 (66–116)	89 (66–98)	97 (75–116)	0.05
Median respiratory rate, breaths/min (range)	20 (14–40)	22 (18–40)	19 (14–36)	0.04
Abnormal lung sounds	11 (48)	8 (100)	3 (23)	0.001
Laboratory findings				
Leukocyte count, cells × 10^9/L (range)	4.53 (0.53–10.8)	3.2 (0.53–10.8)	5.4 (2.8–9.4)	0.4
Leukopenia, <5,000 cells/μL	9 (39)	5 (63)	4 (40)	0.6
Chest radiograph findings, new infiltrate	6 (26)	5 (63)	1 (13)	0.1
Care received				
Antiviral treatment for influenza	21 (91)	8 (100)	13 (87)	0.5
Intensive care unit admission	3 (13)	3 (38)	NA	
Mechanical ventilation	2 (8.7)	2 (25)	NA	
Vasopressors	2 (8.7)	2 (25)	NA	
Outcomes				
Secondary pneumonia	3 (13)	3 (38)	0	0.03
Thrombotic complications	1 (4.6)	1 (13)	0	0.4
Died	2 (8.7)	2 (25)	0	0.1

*Values are no. (%) patients except as indicated. Among the 15 outpatients, 13 had a lung examination documented, 10 had a leukocyte count performed, and 8 had a chest radiograph taken. NA, not applicable.
†For comparison of characteristics between inpatients and outpatients.

inpatients who died had each received ≥14 days of therapy with oseltamivir. Three inpatients were admitted to the intensive care unit (ICU); of these, 2 had hypoxemic respiratory failure and bilateral infiltrates at the time of admission and a later diagnosis of acute respiratory distress syndrome, and 1 was hospitalized with fever and hemodynamic instability. Each patient with acute respiratory distress syndrome subsequently died; 1 had methicillin-resistant *Staphylococcus aureus* pneumonia at the time of admission, and 1 had severe hypoxemic respiratory failure requiring the use of rescue therapies (e.g., prone positioning and inhaled nitric oxide) and later treatment for ventilator-associated pneumonia. Of the 2 patients who died, 1 had concurrent conditions, including preexisting interstitial lung disease (believed to be associated with crack cocaine use) and a low CD4 cell count of 127 cells/μL, and 1 had a preserved CD4 cell count >1,000 cells/μL, but 8 days passed before anti-influenza therapy was started, and thrombotic complications developed before death. The lengths of ICU stay for the patients who died were 13 and 29 days.

Our findings are similar to those reported by others, suggesting that HIV infection alone does not appear to be a risk factor for severe pandemic (H1N1) 2009, provided that patients are not severely immunocompromised, do not have other risk factors associated with poor outcomes, and are treated for influenza soon after signs and symptoms develop (*6*–9). Most of the 23 patients described here had mild disease and were treated as outpatients. Only 3 required ICU admission, and 2 of these died. Although the mortality rate reported here is higher than that reported in other studies, our sample size was relatively small, and the patients who died had additional risk factors for poor outcomes.

Our study has several limitations. It is a retrospective study, and HIV-infected patients at Harborview Medical Center were not all prospectively tested for pandemic (H1N1) 2009. Most pandemic (H1N1) 2009 virus was detected by reverse transcription PCR of nasal swab specimens; this testing was only available after October 2009, during the second wave of influenza. Infections occurring during the spring were diagnosed by insensitive testing with fluorescent antibody and culture, diagnosed by clinical criteria alone and not included in this analysis, or missed altogether.

Because of differences in pandemic (H1N1) 2009 virus testing, we were unable to compare the incidence of pandemic (H1N1) 2009 virus infection and outcomes between HIV-infected and HIV-uninfected patients. A total of 189 persons received a diagnosis of pandemic (H1N1) 2009 at Harborview Medical Center in 2009, and 79 were hospitalized. A total of 8 (10%) of 79 patients with pandemic (H1N1) 2009 died, including the 2 HIV-infected patients reported here. However, during the peak of the epidemic, many HIV-infected outpatients, who were receiving antiretroviral therapy and had preserved CD4 cell counts, were advised to remain at home if they had mild influenza-like symptoms and were therefore not tested for influenza. This circumstance could have produced a bias toward diagnosing and reporting only more severe disease. Outpatients who had influenza-like symptoms were tested and treated empirically pending test results. Our case series of HIV-infected patients with pandemic (H1N1) 2009 at a single institution in the United States suggests that HIV itself does not appear to be as major a risk factor for severe disease as are other previously reported concurrent conditions, delays in treatment, and development of secondary bacterial pneumonia.

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Swine Influenza Virus A (H3N2) Infection in Human, Kansas, USA, 2009

To the Editor: Triple-reassortant swine influenza viruses (SIVs), which contain genes from human, swine, and avian influenza A viruses, have been enzootic among swine herds in the United States since the late 1990s (*1*). Although uncommon, occasional transmission of triple-reassortant SIVs from swine to humans has occurred (*2*–*4*). Before April 2009, only limited, nonsustained human-to-human transmission of SIVs had been reported (*5*–*7*). Although an animal source for pandemic (H1N1) 2009 virus has yet to be identified, the pandemic strain resulted from the reassortment of 2 different lineages of SIV (*8*).

On July 28, 2009, a 12-year-old Kansas boy sought treatment for fever, cough, and sore throat. Results of an influenza rapid antigen test were positive, and a specimen was sent to the Kansas Department of Health and Environment for further testing. Real-time reverse transcription PCR (rRT-PCR) testing determined the virus contained the surface hemagglutinin (HA) gene of influenza A (H3) and the internal nucleoprotein gene common to all triple-reassortant SIVs (*9*). The specimen was sent to the Centers for Disease Control and Prevention (Atlanta, GA, USA) on August 3 and identified as swine-origin influenza virus A (H3N2) by rRT-PCR and sequence analysis.

The patient reported that during July 23–25, 2009, he touched healthy-appearing swine multiple times while attending a county fair. The boy received a standard treatment course of oseltamivir and recovered completely. None of his 3 household contacts attended the fair, and none reported signs or symptoms of illness in the weeks afterward.

The Kansas Department of Health and Environment and the local health department collaborated with the county extension office to identify and interview swine exhibitors at the county fair, focusing on influenza symptoms among exhibitors and household contacts during the week before and after the fair. Twenty-seven (79%) of 34 exhibitors participated in the survey; none reported signs or symptoms of influenza-like illness, defined as fever (temperature ≥100°F) accompanied by either cough or sore throat. Two household contacts of separate exhibitors each reported a low-grade fever (<100°F) and sore throat in the week after the fair. Both touched swine while attending the fair. Both visited a physician's office; neither was tested for influenza; and symptoms of both resolved without treatment. The veterinarian overseeing the swine barn reported no signs of respiratory illness among the swine during the fair. Most swine exhibited were slaughtered at the fair's conclusion.

On August 7, the Kansas Animal Health Department collected nasal swab specimens and blood samples from 13 swine belonging to 7 exhibitors. All samples were delivered to the Kansas State Veterinary Diagnostic Laboratory for analysis by influenza matrix rRT-PCR and virus isolation on nasal swab samples and hemagglutination inhibition (HI) assays against classical swine influenza virus (H1N1) (A/swine/Iowa/73) and the prototype swine influenza virus (H3N2) (A/swine/Texas/98) on serum samples. In addition, the influenza virus (H3N2) "county fair" isolate, A/Kansas/13/2009 (H3N2), was sent from the Centers for Disease Control and Prevention, amplified, and used to develop a second HI assay that included the original swine serum samples as well as paired convalescent-phase samples from 3 of the swine (Table).

Influenza matrix RT-PCR and virus isolation on nasal swab samples were negative. HI assays demonstrated little or no antibody against the influenza (H1N1) indicator virus and low-level antibody reaction against the prototype swine influenza virus (H3N2). However, HI titers against the "county fair" influenza virus (H3N2) showed consistently elevated titers, which suggested that the animals might have been exposed to the virus 2 weeks earlier, during the time of the fair. The swine may have cleared the virus by the time the nasal swabs were collected, but without positive RT-PCR or virus isolation results, the situation remain inconclusive.

We compared the HA gene segment of A/Kansas/13/2009 (H3N2) with recent animal and human influenza (H3N2) viruses by using the neighbor-joining method, and it clustered with the HA from recent triple-reassortant SIV (H3N2) isolates (online Appendix Figure, www.cdc.gov/EID/content/17/6/1143-appF.htm) (*10*). A/Kansas/13/2009 (H3N2) shares >97% nucleotide identity with 2 swine viruses reported to have caused human infections, A/Ontario/RV1273/2005 and A/Ontario/1252/2007, and >90% nucleotide identity with currently circulating seasonal (H3N2) viruses, such as A/Perth/16/2009. Sequence analysis for the remaining 7 gene segments confirmed A/

Table. Hemagglutination inhibition assay titers for 3 influenza strains from swine exhibited at county fair, by date of blood draw, Kansas, 2009*

Swine ID no.	August 7 titers			August 31 titers	
	Subtype H1N1†	Subtype H3N2‡	"County fair," subtype H3N2§	Subtype H3N2‡	"County fair," subtype H3N2§
1	<10	20	320	¶	¶
2	<10	10	160	¶	¶
3	<10	40	640	¶	¶
4	<10	40	640	¶	¶
5	<10	80	640	¶	¶
6	<10	40	640	¶	¶
7	<10	320	640	¶	¶
8	10	10	160	¶	¶
9	<10	80	640	¶	¶
10	<10	40	320	<10	80
11	<10	80	320	10	320
12	<10	20	160	20	80
13	10	<10	40	¶	¶

*HI, hemagglutination inhibition; ID, identification.
†Strain A/Swine/Iowa/79 (H1N1).
‡Strain A/Swine/Texas/98 (H3N2).
§Strain A/Kansas/13/2009 (H3N2).
¶Ten of the original 13 swine were slaughtered before convalescent-phase serum could be collected.

Kansas/13/2009 as triple-reassortant SIV (H3N2), belonging to the same lineages as the genes for the reference virus A/swine/Texas/98 (data not shown). A full genome sequence for A/Kansas/13/2009 (H3N2) has been submitted to GenBank (accession nos. GU937743–GU937750).

This case emphasizes the importance of epidemiologic and laboratory surveillance in areas where humans and swine are in close contact. When novel influenza A infection occurs in humans, joint investigations by local, state, and federal public health and animal health agencies are key to determining the source of infection and extent of transmission. The worldwide spread of pandemic (H1N1) 2009 virus emphasizes the ongoing public health threat of interspecies influenza transmission. Improved surveillance among swine may lead to early identification of novel viruses with pandemic potential and provide early opportunities to implement control measures.

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Severe Leptospirosis Similar to Pandemic (H1N1) 2009, Florida and Missouri, USA

To the Editor: Leptospirosis is caused by pathogenic spirochetes of the genus *Leptospira* and transmitted through direct contact of skin or mucous membranes with urine or tissues of *Leptospira*-infected animals or through indirect contact with contaminated freshwater or soil. Leptospirosis shares common clinical signs with influenza, including fever, headache, myalgia, and sometimes cough and gastrointestinal symptoms. During 2009, acute complicated influenza-like illness (ILI) and rapid progressive pneumonia were often attributed to pandemic (H1N1) 2009; however, alternative final diagnoses were reported to be common (*1*). We report 3 cases of severe leptospirosis in Florida and Missouri with clinical signs similar to those of pandemic (H1N1) 2009.

Patient 1 was a 40-year-old Florida man who sought treatment at an emergency department after a 4-day history of fever, myalgia, calf pain, malaise, and headache in July 2009. ILI was diagnosed. Laboratory testing was not performed, and the patient was instructed to take ibuprofen. Three days later, jaundice developed. He was admitted to an intensive-care unit with a diagnosis of hepatitis and acute renal failure. The man raised horses, goats, and chickens on his farm and was frequently employed to control rat infestations at an auto parts store and warehouse. Leptospirosis was suspected. Doxycycline was administered, and the man recovered and was discharged on the eighth day of hospitalization. *Leptospira*-specific immunoglobulin M antibodies were detected by dot blot (ARUP Laboratories, Salt Lake City, Utah, USA) on the second of paired consecutive blood specimens.

Patient 2 was a 17-year-old Missouri woman with a history of obesity. She was hospitalized in August 2009 with a 5-day history of fever, myalgia, calf pain, malaise, headache, nausea, vomiting, dyspnea, and cough, complicated by acute renal failure. The diagnosis on admission was viral infection. On the third day of hospitalization, severe pneumonia and respiratory failure developed, and she was administered vancomycin, piperacillin/tazobactam, levofloxacin, and doxycycline. She died the same day. Ten days before illness onset, she had swum in a creek near her residence.

Patient 3 was a 59-year-old Florida man with a history of obesity and diabetes mellitus. He sought treatment at a clinic in September 2009 and reported a 5-day history of fever, myalgia, malaise, nausea, abdominal pain, and dyspnea. He was treated for gastritis. Two days later, he came to an emergency department and was admitted to the hospital with severe pneumonia and multiorgan failure; he died the next day. The man had frequently engaged in activities to control rat infestations on the farm where he raised chickens, pigs, and goats.

Although patients 2 and 3 were neither tested nor treated for influenza before they died, their clinical signs and rapidity of death prompted postmortem suspicion of pandemic (H1N1) 2009. Autopsies were performed and formalin-fixed tissues were submitted to the Centers for Disease Control and Prevention (Atlanta, GA, USA). Histopathologic evaluation of both patients demonstrated extensive pulmonary hemorrhage and interstitial nephritis (Figure, panels A and B), features consistent with leptospirosis. Immunohistochemical tests for leptospirosis, spotted fever group rickettsiae, and influenza A were performed on multiple tissues obtained from patients 2 and 3. Immunohistochemical evidence of leptospiral infection was identified in lung, liver, kidney, heart, and spleen tissue in both patients (Figure, panels C and D).

These cases of severe leptospirosis were reported during the 2009 influenza pandemic. Although pulmonary hemorrhage (experienced by patients 2 and 3) is increasingly recognized as a severe manifestation of leptospirosis (*2*), it is also a known complication of influenza (*3*). ILI was initially diagnosed in patient 1, but symptom progression and

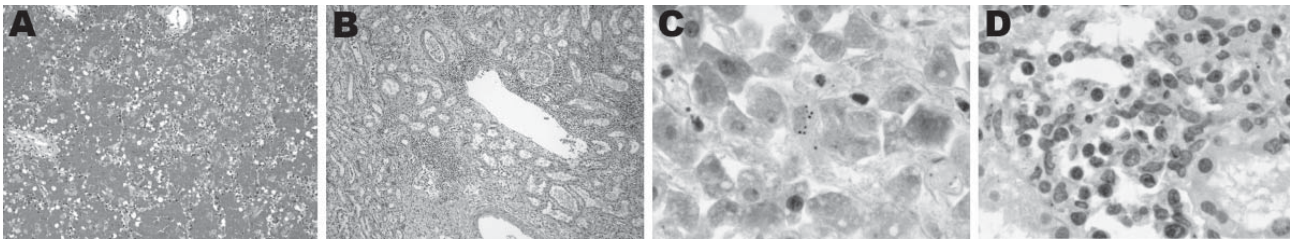

Figure. Photomicrographs of lung, liver, and kidney sections from patient 2 during study, Missouri and Florida, USA, 2009. Hematoxylin and eosin stain showed pulmonary hemorrhage (A) (original magnification ×10) and interstitial nephritis (B) (original magnification ×5), 2 characteristic pathologic findings of leptospirosis. Immunohistochemical testing showed scattered granular leptospiral antigens in liver (C) and kidney (D) (original magnification ×63). A color version of this figure is available online (www.cdc.gov/EID/content/17/6/1145-F.htm).

clinical complications, combined with a history of animal exposure, prompted the physician to consider leptospirosis and to initiate appropriate antimicrobial drug therapy.

Autopsies are critical in determining the reasons for death after undiagnosed illness. Pulmonary involvement in cases of leptospirosis is characterized by congestion and hemorrhage, usually without prominent inflammatory infiltrates (*4*); pulmonary involvement in cases of severe pandemic (H1N1) 2009 typically manifests as diffuse alveolar damage (*5*). Postmortem diagnosis of leptospirosis was supported by characteristic histopathologic findings, including pulmonary hemorrhage and interstitial nephritis, and was confirmed by immunohistochemical tests. Our report illustrates the need for autopsies in unexpected deaths, even if the cause appears obvious in a specific clinical and epidemic setting.

Leptospirosis ceased being nationally notifiable in the United States in 1994 and is likely underdiagnosed because it is not routinely considered in differential diagnoses. However, outbreaks with exposures similar to the case-patients we studied have been periodically reported in the United States (*6*–*8*). Because leptospirosis commonly manifests as acute febrile illness, cases can be underrecognized during infectious-disease epidemics (e.g., dengue) (*9*). Leptospirosis should be included in the differential diagnosis of acute febrile illness in the United States and other industrialized countries. Epidemiologic clues include recreational or occupational water exposure; animal exposure (including rodents) in the home or the workplace, travel to tropical areas, and water exposure during travel. These risk factors for leptospirosis are increasing in industrialized countries (*10*). Thorough patient-history reviews and consideration of alternative diagnoses are needed for cases of respiratory illness during an influenza pandemic.

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Coronavirus HKU1 in Children, Brazil, 1995

To the Editor: Coronavirus HKU1 is a newly identified human coronavirus (HCoV) that was reported first in 2005 in Hong Kong Special Administrative Region, People's Republic of China; later in Australia, Europe, and the United States, and more recently in Brazil, demonstrating a global distribution (*1*–*3*). We examined the circulation of HCoV in Brazil and the possible presence of the new HCoV types, with special attention to coronavirus HKU1, in samples collected back to 1995, tested by using universal coronavirus PCR.

The epidemiologic profile of HCoV was retrospectively investigated with samples collected during March–December 1995 in a pediatric ward of University Hospital, São Paulo University, São Paulo, Brazil. The Ethics Committee on Research Involving Human Subjects of the Institute of Biomedical Sciences, University of São Paulo, approved the study. Samples of nasopharyngeal aspirates were collected from 169 hospitalized children, ages 7 days–15 years, of whom 104 had respiratory symptoms, 23 had enteric disease, and 3 had both (*4*). The mean age of the study population was 19.6 months (median 7 months). Viral nucleic acid was extracted from specimens by using Trizol (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. Total RNA was then submitted to reverse transcription PCR with a High-Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA, USA) by using random primers according to the manufacturer's instructions. The cDNA obtained was screened with primers able to amplify a 220-bp product in the conserved polymerase region of all known HCoVs (*5*) and other coronaviruses (e.g., bovine coronavirus). We used cDNA obtained from cultured human rectal tumor cell line HRT-18G cells inoculated with bovine coronavirus strain Kakegawa as positive controls for PCR.

In an attempt to improve the sensitivity of HKU1 detection, we analyzed samples with negative results by PCR with a nested PCR specific for coronavirus HKU1. This nested assay was designed on an alignment of our HKU1-positive sample sequence (BRA169) and different HKU1 genotype sequences deposited in GenBank. Primers Fn-HKU1 (forward 5′-CGTGCYA TGCCAAATATTTTGCG-3′, HKU1-NC_006577, nt 15433–15454) and Rn-HKU1 (reverse 5′-TAGCAACC GCCACACATAAC-3′, HKU1-NC_ 006577, nt 15562–15581) produced an amplicon of 149 bp. The nested PCR was run in a 50-μL reaction comprising 10 μL of PCR product, 1.5 units of DNA Polymerase (Biotools, Madrid, Spain), 1 μmol/L of each primer, 200 μmol/L of each dNTP (Applied Biosystems), 2 mmol/L $MgCl_2$, and 1× buffer. PCR mixtures were heated to 95°C for 5 min, followed by 35 cycles of 1 min at 95°C, 30 s at 62°C, and 40 s at 72°C, followed by a final 10 min at 72°C. Clinical samples positive for HKU1 were used as positive controls in the HKU1 nested PCR. All fragments obtained from PCR and nested PCR were analyzed in a 2% (wt/vol) agarose gel by electrophoresis, stained with 0.5 μg/mL of ethidium bromide, and subsequently sequenced to confirm the type of coronavirus. Nucleotide sequencing reactions were performed on both amplicon strands by using an ABI PRISM Big Dye Cycle Sequencing Kit with the ABI PRISM 3100 automatic sequencer (Applied Biosystems).

Six (3.6%) samples tested positive for HCoV-HKU1: 2 samples by PCR and 4 by nested PCR. HCoV types 229E, OC43, and NL63 were not detected in any sample by PCR. Samples positive for HCoV were associated with pertussis, pneumonia, bronchiolitis, and diarrhea (Table).

In a recent review, an analysis of 18 studies indicated that the median (range) incidence of HCoV-HKU1 was 0.9% (0%–4.4%) (*2*), which is similar to the detection rate in our study. To our knowledge, the only study that has screened for HKU1 in Brazil found that 0.48% of children were positive for HKU1 (*3*), which is lower than our results.

Although we did not detect other HCoV types, all HCoV types were detected previously in Brazil in samples collected during 2006–2008 (*3*,*6*). The absence of detection of 229E, OC43, and NL63 HCoV might have resulted from the seasonality and natural viral year cycle or from the characteristics of the children studied

Table. Epidemiologic and laboratory data of children with coronavirus infection, Brazil, 1995*

Specimen no.: HCoV strain by pol analyses	Age/sex	Sample collection date	Clinical diagnosis	Co-infections	Detection method, fragment sequenced
09: HKU1A	3 mo/F	Mar	Pertussis	ND	Nested PCR, 143 bp
37: HKU1A	2 mo/M	Apr	Bronchiolitis plus bronchopneumonia	RSV	Nested PCR, 143 bp
90: HKU1B	4 mo/M	Jul	Upper respiratory infection	ND	Nested PCR, 143 bp
99: HKU1 B	9 y/M	Jul	Pleural effusion pneumonia	ND	Nested PCR, 143 bp
104: HKU1 A	2 mo/F	Jul	Pertussis	ND	Pancoronavirus PCR, 143 bp
169: HKU1B	3 y/F	Nov	Fever, diarrhea	Worms	Pancoronavirus PCR, 173 bp

*HCoV, human coronavirus; ND, not detected; RSV, respiratory syncytial virus.

because we included samples from children hospitalized with or without respiratory disease.

BLAST search (www.ncbi.nlm.nih.gov/blast/Blast.cgi) and phylogenetic analysis of amplicons from PCR and nested PCR indicated that samples were positive for HKU1 genotype B (samples BRA169, BRA90, and BRA99) or HKU1 genotype A (samples BRA09, BRA37, and BRA104). The sequences obtained in this study have been deposited in GenBank under accession nos. FJ931534.1 (BRA169), GU904424 (BRA37), GU904427 (BRA104), GU904423 (BRA09), GU904425 (BRA90), and GU904426 (BRA99).

This may be the oldest collection of human samples in which HKU1 has been detected. To our knowledge, the oldest previous sample positive for HCoV-HKU1 was detected in children in Finland during 1996–1998, without an exact date specified (*7*). Retrospective studies also have been conducted in the United States and Greece that showed the HKU1 virus in different countries in Europe and North America before its discovery (*8*,*9*). We have confirmed the circulation of HKU1 coronaviruses in children in Brazil in 1995.

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Macrolide Resistance–associated 23S rRNA Mutation in *Mycoplasma genitalium*, Japan

To the Editor: *Mycoplasma genitalium* is now recognized as a serious pathogen in sexually transmitted infections (*1*,*2*). Azithromycin regimens have been commonly used for treatment of *M. genitalium* infections (*3*). However, failure of azithromycin treatment has been reported in cases of *M. genitalium*–positive nongonococcal urethritis (NGU) (*4*,*5*), and macrolide-resistant strains of *M. genitalium* have been isolated from case-patients in Australia, Sweden, and Norway for whom azithromycin treatment has failed (*4*,*5*). In these strains, mutations in the 23S rRNA gene were associated with macrolide resistance, and mutations in ribosomal protein genes L4 and L22 were also found (*5*). Surveillance for antimicrobial resistance of *M. genitalium* is essential to identify antimicrobial resistant strains and to then determine appropriate treatment. Coculture of patient specimens with Vero cells has improved the primary isolation rate of *M. genitalium* from clinical specimens and offered some current clinical strains for antimicrobial drug susceptibility testing (*6*). To determine their antimicrobial susceptibilities, a molecular real-time PCR method has been developed (*7*,*8*). However, isolating *M. genitalium* from clinical specimens and antimicrobial drug susceptibility testing of clinical isolates remain labor-intensive, time-consuming tasks. In addition, no methods are available to directly determine antimicrobial drug susceptibilities of *M. genitalium* in clinical specimens. To monitor macrolide susceptibilities in clinical

strains of *M. genitalium* in Japan, therefore, we examined *M. genitalium* DNA found in the urine of men with NGU for the presence of macrolide resistance–associated mutations in the 23S rRNA gene and the ribosomal protein genes L4 and L22.

This retrospective study was approved by the Institutional Review Board of the Graduate School of Medicine, Gifu University, Gifu, Japan. We collected pretreatment urine specimens from 308 men with NGU who had visited a urologic clinic (iClinic) in Sendai, Japan, during 2006 through 2008 and stored the specimens at –70°C. Each man gave informed consent. Twenty-five of 58 urine specimens confirmed to be positive for *M. genitalium* by PCR-based assay were randomly chosen for this study and subjected to DNA purification. The 23S rRNA gene and the ribosomal proteins genes L4 and L22 of *M. genitalium* were amplified from the purified DNA by PCR as reported previously and then sequenced (*5*).

In 1 specimen, we found an A-to-G transition at nucleotide position 2072 in the 23S rRNA gene of *M. genitalium*, corresponding to position 2059 in *Escherichia coli* (Table). An A2059 (*E. coli* numbering) residue in region V of the 23S rRNA gene is critical for the binding of macrolides (*9*). Mutations of A2058, A2059, and other 23S rRNA residues within the macrolide-binding site can confer a high-level resistance to macrolides in several bacterial species, including *M. genitalium* (*5*,*9*). Therefore, *M. genitalium* strains that harbor the A2059G (*E. coli* numbering) mutation in the 23S rRNA gene could be highly macrolide resistant. We also found a T-to-G transition at nucleotide position 2199 in the 23S rRNA gene of *M. genitalium*, corresponding to position 2185 in *E. coli*, in 3 specimens, but this mutation has not been associated with macrolide resistance in other bacterial species (*9*).

We found amino acid changes in L4 and L22 ribosomal proteins in *M. genitalium* in 9 specimens. L4 and L22 ribosomal proteins each have extended loops, which converge to form a narrowing in the exit tunnel adjacent to the macrolide-binding site (*10*). Therefore, macrolide resistance–associated missense mutations in L4 and L22 tend to be localized to Gln62–Gly66 in L4 and Arg88-Ala93 in L22 of *E. coli*, which are closest to the macrolide-binding site (*10*). All of the amino acid changes in L4 of *M. genitalium* found in this study corresponded to those at the downstream regions from Gln62-Gly66 in L4 of *E. coli*. Of the amino acid changes in L22 of *M. genitalium*, the only Gly93Glu change found in *M. genitalium* harboring the A2059G (*E. coli* numbering) mutation in the 23S rRNA gene was located within the region corresponding to Arg88-Ala93 in L22 of *E. coli*. In this strain, therefore, the Gly93Glu change in L22 might contribute to the increase of macrolide resistance. The patient with NGU, whose specimen exhibited this strain of *M. genitalium* that harbored both the A2059G (*E. coli* numbering) mutation in the 23S rRNA gene and in which the Gly93Glu change in L22 was detected, was given a single dose of 1 g azithromycin and was clinically cured of NGU. However, the present study suggests that *M. genitalium* strains with high-level macrolide resistance might have already emerged in clinical settings in Japan. The emergence and spread of such a clinical mutant could threaten the ability of macrolides to treat *M. genitalium* infections. We should continue monitoring macrolide resistance of *M. genitalium* clinical strains. The nonculture approach used in our study will be useful until culturing of mycoplasmas from clinical specimens and antimicrobial drug susceptibility testing can be performed easily in laboratories.

Table. Mutations in the 23S rRNA gene and amino acid changes in L4 and L22 ribosomal proteins of 25 *Mycoplasma genitalium* strains in the pretreatment urine specimens of men with nongonococcal urethritis, Japan

No. urine specimens	Mutation in the 23S rRNA gene*	Amino acid change	
		L4	L22
1	A2059G	–	Gly93Glu/Asp109Glu
1	T2185G	Val84Gly	–
1	T2185G	GLu128Gly	–
2	T2185G	–	–
1	–	Pro81Ser	–
1	–	Tyr135Pro	–
1	–	–	Ser81Thr
1	–	–	Met82Lys
1	–	–	Asn112Asp
1	–	–	Arg114Lys
14	–	–	–

*Nucleotide position in the 23S rRNA gene is according to *Escherichia coli* numbering. –, identical to the type strain.

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Saffold Cardioviruses in Children with Diarrhea, Thailand

To the Editor: Cardioviruses currently consist of at least 3 viruses: Theiler murine encephalomyocarditis virus, encephalomyocarditis virus, and Saffold virus (SAFV) (*1*–*4*). Saffold cardiovirus in the family *Picornaviridae* was isolated and identified from fecal specimens of a child with fever of unknown origin in the United States (*3*).

Several reports have documented the presence of SAFV in fecal samples and respiratory secretions (*5*–*10*). However, it is not clear whether SAFV is associated with any disease, including gastroenteritis in humans, and epidemiologic data for SAFV are limited. We report an epidemiologic survey of SAFV in children hospitalized with diarrhea in Chiang Mai, Thailand.

A total of 150 fecal specimens were obtained from children hospitalized with acute gastroenteritis in Chiang Mai during January–December 2007. Patient ages ranged from >1 to 5 years. SAFV in fecal specimens was detected by using a nested PCR and primers specific for the virus 5′ untranslated region (*7*). A negative control was also included to monitor any contamination that might have occurred during the PCR.

SAFVs detected were further analyzed by amplification of the viral protein (VP) 1 gene (*6*,*9*,*10*) and direct sequencing of the VP1 PCR amplicon by using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). VP1 sequence was compared with VP1 sequences of reference strains available in the National Center for Biotechnology Information (Bethesda, MD, USA). Phylogenetic and molecular evolutionary analyses were conducted by using MEGA4 (www.megasoftware.net). Nucleotide sequences of SAFV strains described were deposited in GenBank under accession nos. HQ668170–HQ668173.

Four (2.7%) of 150 specimens were positive for SAFV (CMH023/2007, CMH038/2007, CMH045/2007, and CMH143/2007). Two of these specimens (CMH023/2007 and CMH038/2007) were obtained in February 2007, one (CMH045/2007) in March 2007, and 1 (CMH143/2007) in November 2007. Co-infections with other viruses were detected in all 4 samples. Two specimens (CMH023/2007and CMH045/2007), were co-infected with noroviruses GII/16 and GII/4 genotypes, respectively. One SAFV-positive sample (CMH038/2007) was co-infected with a group A rotavirus G1P[8] genotype, and another (CMH143/2007) was co-infected with human parechovirus.

All SAFV-positive specimens were further amplified for the VP1 gene to determine their phylogenetic lineages and genetic relationships with other SAFV reference strains. When we used 3 sets of primers used in other studies (*6*,*9*,*10*) for amplification of the VP1 gene, this gene was amplified only by the primer set reported by Itagaki et al. (*10*).

Analysis of partial VP1 sequences (369 nt) of 4 SAFV strains showed that strains CMH023/2007 and CMH143/2007 were highly conserved (nt sequence identities >97%). These 2 SAFV strains were most closely related to the prototype strain of SAFV1 (EF165067) isolated in the United States (nt sequence identity range 87.6%–88.9%) and SAFV strains from China (LZ50419, BCH895, GL311, and GL377) (Figure). In addition, the other 2 SAFVs identified in the present study (CMH038/2007 and CMH045/2007) were identical to each other and closely related to SAFV2 strains from China (BCHU79, BCHU353)

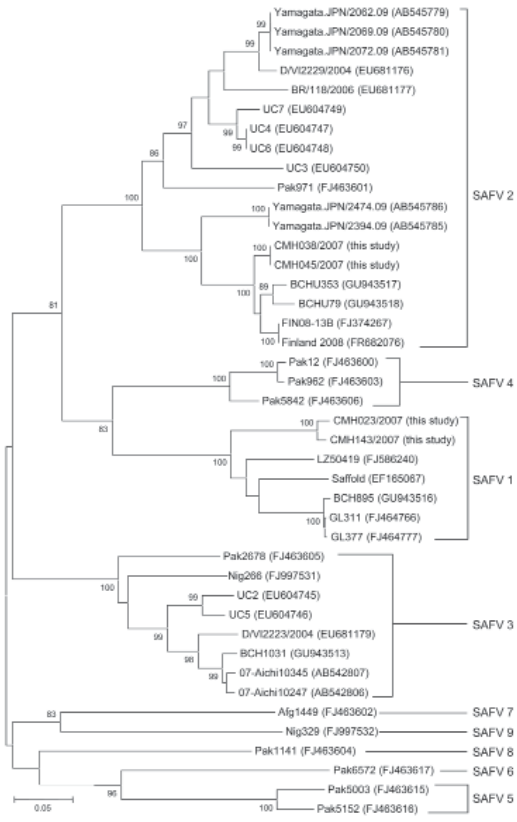

Figure. Phylogenetic analysis of the partial nucleotide sequence (369 nt) encoding the viral protein 1 gene of Saffold virus (SAFV) isolated in this study and other reference strains. The tree was generated by using the neighbor-joining method and MEGA4 (www.megasoftware.net). Bootstrap values >80 are indicated for the corresponding nodes on the basis of a resampling analysis of 1,000 replicates. Scale bar indicates nucleotide substitutions per site.

and Finland (Finland 2008, FIN08–13B) (nt sequence identity range 94.8%–95.6%). Phylogenetic analysis showed that CMH038/2007 and CMH045/2007 were clustered within the SAFV2 lineage (Figure).

The 4 strains of SAFV were isolated from children with acute gastroenteritis who were co-infected with other viral pathogens (norovirus, group A rotavirus, and human parechovirus). Therefore, we could not determine whether SAFVs identified in this study were associated with acute gastroenteritis. The detection rate for SAFV in children with acute gastroenteritis (2.7%) in our study was consistent with that in a study in Beijing, People's Republic of China (3.2%) (*9*).

Phylogenetic analysis of the VP1 region demonstrated that 2 SAFV lineages (SAFV1 and SAFV2) were circulating in Chiang Mai, Thailand. Further extensive epidemiologic surveillance of SAFV in other areas may provide a better understanding of the distribution, heterogeneity, and association of SAFV with enteric diseases in humans.

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Lethal Necrotizing Pneumonia Caused by an ST398 *Staphylococcus aureus* Strain

To the Editor: The prevalent colonization of livestock with methicillin-resistant *Staphylococcus aureus* (MRSA) sequence type (ST) 398 in many countries is a cause for consternation. However, understanding of the emergence of these organisms and their public health implications is embryonic. The perceptions that all MRSA found in livestock are of ST398 lineage or that livestock are the only reservoirs of ST398 oversimplify a complex epidemiology, therefore, prudence is required when attributing human infections with *S. aureus* ST398 to livestock reservoirs. The fatal infection of a young girl with ST398 methicillin-susceptible *S. aureus* (MSSA) is tragic (*1*). However, the conclusion by the authors that "the spread of *S. aureus* ST398 among livestock is a matter of increasing concern because strains of this sequence type were able to acquire PVL [Panton-Valentine leukocidin] genes" is misleading.

The authors report no history of livestock exposure and the *spa* type reported (t571) is relatively rare among livestock isolates (*2*,*3*). The isolate from the fatal case was tetracycline-susceptible and positive for PVL toxin, while livestock ST398 isolates have been almost uniformly tetracycline resistant and PVL negative. Notably, *spa* type t571 ST398 MSSA was detected in 9 families from the Dominican Republic living in Manhattan, New York, without contact with livestock (*4*). Furthermore, t571 was the only *spa* type of MSSA identified in a study in the Netherlands of ST398 isolates, including 3 independent cases of nosocomial bacteremia in Rotterdam with no apparent livestock contact (*5*). *spa* type t571 was the predominant (11%) MSSA type in patients at a Beijing, China, hospital (*6*). More recently, a study of t571 MSSA strains from cases of bloodstream infections in France determined that the isolates differed from pig-borne strains and shared similarities with strains from humans in China and virulent USA300 strains (*7*). These observations concur with a hypothesis that ST398 strains of diverse genotype and geographic origin may also be epidemiologically distinct (*8*), and livestock contact is a notably inconsistent feature of invasive ST398 infections (*5*,*7*–*10*).

The possibility that variants of the ST398 lineage may persist in human populations without livestock contact should not be dismissed. The incidence and severity of clinical infections with ST398 *S. aureus* in livestock workers as yet have been minimal. Understanding the public health implications of ST398 *S. aureus* requires systematic investigation of their epidemiology in animals and humans. Human clinical cases of ST398 *S. aureus* infection should not be indiscriminately attributed to livestock, particularly if isolates are genotypically dissimilar to those occurring commonly in animals.

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In Response: We thank Davies et al. (*1*) for their interest in our report of a lethal case of necrotizing pneumonia caused by a sequence type (ST) 398 *Staphylococcus aureus* strain (*2*). We fully agree with their request that ST398 *S. aureus* infections not be systematically attributed to contact with livestock. They correctly pointed out that several characteristics of the incriminated strain, including methicillin and tetracycline susceptibility, *spa* type, and the presence of genes encoding the Panton-Valentine leukocidin (PVL), differed from the usual genetic features of strains isolated from livestock (*3*,*4*). However, we did not state or suggest in our report that the case originated from livestock contact. Our aim in reporting this case was to warn that *S. aureus* of the ST398 lineage, regardless of its host specificity, is able to acquire PVL genes and provoke severe PVL-related infection in humans. This observation adds support to the need for controlling the increasing animal reservoir of ST398 methicillin-resistant *S. aureus* (MRSA). Indeed, the recent whole-genome analysis of an ST398 strain by Schijffelen et al. (*5*) highlighted several specific features of the ST398 genetic background, including the absence of a type I restriction and modification system. Such features have been proposed to promote horizontal gene transfer and the uptake of mobile genetic elements such as the phage-encoded PVL genes (*5*). Although phage-mediated dissemination of PVL genes into MRSA lineages does not seem to be the preeminent pathway leading to the emergence of highly epidemic PVL-positive MRSA (*6*), this eventuality should not be dismissed with respect to the ST398 lineage, which possesses all the required features to become the next MRSA "superbug" (*7*).

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Extended-Spectrum β-Lactamase–producing *Escherichia coli* in Neonatal Care Unit

To the Editor: Tschudin-Sutter et al. provide convincing evidence of transfer of an extended-spectrum β-lactamase–producing *Escherichia coli* strain from a mother to her vaginally delivered twins, then from the neonates to a health care worker and other neonates in a neonatal care unit (*1*). This finding advances our understanding of how extended-spectrum β-lactamase–positive (and, by extension, other antimicrobial drug–resistant or virulent strains) *E. coli* can spread within the community.

However, the authors' use of the term infection for the asymptomatic colonization that was observed, including in the mother (who had asymptomatic bacteriuria), is

potentially misleading. This term could perpetuate a line of thinking that is all too common among clinicians and leads to unnecessary antimicrobial drug use, thereby ironically aggravating the problem of antimicrobial drug resistance.

Although the first paragraph of their report implicitly acknowledges the distinction between infection and colonization, the rest of the report (including the abstract) uses the terms infection or infected interchangeably with colonization or colonized. Examples include "Subsequently, infection spread by healthcare worker contact with other neonates," "a healthcare worker also was infected," and "a urinary tract infection developed...."

One wonders why, in the absence of genitourinary symptoms, the (postpartum) mother's urine was cultured and why the positive culture prompted antimicrobial drug therapy. This seeming misinterpretation by the mother's providers of what probably was a harmless colonization state as representing acute disease, and their all too typical response (i.e., antimicrobial drug therapy), are to be discouraged (*2*). More cautious use of terminology, to emphasize the distinction between colonization and infection (which have radically different therapeutic implications), may help refine clinicians' thinking and practice in this regard, thereby promoting improved antimicrobial drug stewardship and slowing the antimicrobial drug resistance epidemic.

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In Response: We thank James Johnson for the issue that he has raised in his letter (*1*). We agree that distinction of the terms colonization and infection is crucial to prevent misinterpretation of clinical findings and subsequently unnecessary antimicrobial drug use. The outbreak occurred in the hospital; therefore, definitions of nosocomial infections were used throughout the article (*2*).

Nosocomial urinary tract infection is defined by the Centers for Disease Control and Prevention as asymptomatic bacteriuria or symptomatic infection (urinary tract infection–symptomatic urinary tract infection; www.cdc.gov/nhsn/PDFs/pscManual/17pscNosInfDef_current.pdf). (*3*,*4*). Therefore, the term nosocomial urinary tract infection in our report is correct. However, we agree with the author that the term asymptomatic bacteriuria is less than optimal and it was removed when we submitted our report. We agree that the term infection is misleading for describing spread to health care workers and that colonization should have been used. However, the article clearly states that invasive infection did not occur in any of the neonates or health care workers found to be colonized. In addition, the focus of the article was to describe the mode of transmission rather than the distinction between colonization and infection.

Sarah Tschudin-Sutter, Reno Frei, Manuel Battegay, Irene Hoesli, and Andreas F. Widmer

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DOI: 10.3201/eid1706.110007

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Emerging Infections 9

W. Michael Scheld, M. Lindsay Grayson, and James M. Hughes, editors

ASM Press, Washington, DC, USA, 2010

ISBN: 978-1-55581-525-7

Pages: 380; Price: US $154.95

This book is the ninth in the Emerging Infections series from the American Society for Microbiology. The 18 chapters cover the following diseases and pathogens: influenza in 2009, human adenovirus 14, *Acanthamoeba polyphaga* mimivirus as a cause of pneumonia, hepatitis E, lymphocytic choriomeningitis virus-like arenavirus infections, human T-lymphotropic virus type 1 infections in indigenous populations, cytomegalovirus infection after transplantation, malignancies and HIV infection, *Arcobacter* sp. and food, multidrug-resistant gram-negative bacilli, sepsis in Africa, Buruli ulcer, *Plasmodium knowlesi* malaria, neglected tropical diseases, infections of long-term care, emerging infectious diseases in mobile populations, the One Health concept, and emerging infections of plants.

The book starts with a discussion of pandemic (H1N1) 2009 virus in Australia. In the state of Victoria, a major effort was made to limit the spread of the infection but had little success. Ultimately, 15%–20% of the Victorian population showed evidence of infection, despite a reproduction number computed as ≈1.6. The chapter on Buruli ulcer describes some findings from recent work in Australia on the disease. There is a discussion of a small new epidemic focus where evidence suggests mosquito-borne transmission, including genetic material identified from mosquitoes and evidence of protection from the use of mosquito repellent.

Infections of some special populations are discussed. It struck me that pneumonia and urinary tract infections in residents of long-term care are hardly emerging infections. However, the chapter rightly focuses on demographic shifts and changing effects of antimicrobial drugs. Another group mentioned is mobile populations. The authors discuss hepatitis E that is associated with refugee camps, but it might have been helpful to also emphasize that the risk of communicable diseases in these settings is primarily of outbreaks of diseases of the urban poor. The One Health concept is itself an emerging idea, resting on the interdependence of human and animal health and the implications for disease control.

Overall, this is a worthwhile book. It is not comprehensive but aims to update readers on specific areas in infectious diseases. The text does this quite well, but it has a heavy microbiological and clinical focus, and the public health aspects of the topics could, in general, be expanded. For the reader with an interest in the chapter topics, it is worth perusing.

Robert Hall

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DOI: 10.3201/eid1706.110444

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Max Weber (1881–1961) *Figures* (c. 1914) Pastel on paper (61 cm × 45.7 cm). High Museum of Art, Atlanta. Gift in memory of Louis Regenstein by his wife Helen and sons Lewis and Kent.

The only emperor is the emperor of ice-cream

—Wallace Stevens

Polyxeni Potter

"So many, I had not thought death had undone so many." These words about the precariousness of the early years of the 20th century referred to lives lost in war. T.S. Eliot and other poets and writers, along with many in the arts and sciences, were grappling with growing cities and their beleaguered populations and with technological advancements that allowed the killing of unprecedented numbers of soldiers in battle. At the same time, they were swept in a wave of creativity and change centered in Paris and spreading all over the world. These were the times of Albert Einstein, James Joyce, Diego Rivera, Igor Stravinsky, and many others, who were leading scientific, literary, and artistic trends under the broad umbrella of modernism as they tried to "make it new" with outlandish forms and styles.

In the United States, the effects of modernism also permeated technology, photography, film, and dance. American painters, many of whom had gone to Europe, were familiar with modern styles, which they absorbed and carried to their own continent. Max Weber, along with Marsden Hartley, John Marin, Georgia O'Keefe, and others, was part of this avant garde.

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DOI: 10.3201/eid1706.AC1706

Born in Bialystok, then Russia, Weber immigrated to the United States with his family, which settled in New York City when he was 10. He attended public schools and studied art at the Pratt Institute in Brooklyn. He apprenticed with painter and printer Arthur Wesley Dow, who ahead of his time advocated examining visual relationships between forms rather than working solely with objects and believed in "filling a space in a beautiful way" rather than recreating nature. Weber went off to teach in Virginia and Minnesota to finance travel to Paris, where for a time he studied at the Académie Julian and received classical training under painter Jean-Paul Laurens. While abroad, the artist also traveled to Italy, Holland, and Spain.

In Paris, he made the right connections. He knew Cézanne; Gauguin; and Picasso, who along with Braque pioneered cubism, a movement that quickly spread into sculpture, architecture, literature, and music, moving beyond single point perspective to capture objects from many angles at once, forever changing the way we view the world. He became close friends with Henri Rousseau and was involved in organizing a course led by Matisse, a transformative experience in his handling of color. He exhibited in major Salons.

In cubism objects are broken into parts, analyzed, and reassembled, their form enriched in the process. Surfaces often intersect at unusual angles and with the background,

muddling traditional perception of depth. These radical concepts immediately resonated with poets and writers, many of whom were also in Weber's Parisian circle: Guillaume Apollinaire, Robert Delaunay, Gertrude Stein. Like the art of modernism, its poetry was abstract and multifaceted, complex and demanding. In the United States some of William Faulkner's work has been interpreted in cubist terms, as well as that of Wallace Stevens, who wrote "Thirteen Ways of Looking at a Blackbird" and other poems along these lines.

Back in New York, Weber experimented with cubism as he worked toward his own style. He benefited from a brief association with talented photographer and art publisher Alfred Stieglitz and his gallery 291, which promoted photography as a legitimate method of image making and advocated modernism. In Stieglitz's journal Camera Work, he expanded on his notion of the fourth dimension—"The consciousness of a great and overwhelming sense of space-magnitude in all directions at one time…. It is real and can be perceived and felt."

Cinematic innovations fueled Weber's experiments with movement and time, as seen in *New York at Night* (1915), a painting that conveyed the speed, action, and dynamic energy of the city. His intent was to express "not what I see with my eyes but with my consciousness… mental impressions, not mere literal matter-of-fact copying of line and form. I want to put the abstract into concrete terms." He converted everyday experiences, such as walking into a dark auditorium to attend a lecture, into iconic abstractions: "The late hastening visitor finds himself in an interior of plum-colored darkness… upon which one discerns the focusing spray-like yellowish-white light, the concentric, circular rows of seats, a portion of the screen." This experience of the dark turned into *Slide Lecture at the Metropolitan Museum* (1916).

After the end of his association with Stieglitz, Weber supported himself by teaching art history, appreciation, and design at the Clarence H. White School of Photography and at the Art Students League. Although in the end of his career he turned to less radical subjects and forms, he continued to advance the cause of modernism until his death in Great Neck, Long Island.

One of the first American painters to understand and embrace cubist analysis and restructuring, Weber was able to integrate it into the contemporary American scene: skyscrapers, airplanes, subways, lights, the movies. Initially rejected for this revolutionary work, he is now recognized for expressing the ideals and concerns of his times.

While the beginning of the 20th century was marred by rapid change and the deaths of war, its end was no less shaken by globalization, social and political strife, and on the public health front—brought on by unprecedented industrial growth, ecologic and demographic changes, and explosive travel—one of history's worst pandemics, HIV/AIDS. A modern plague, this one had all the social and economic markers of previous scourges and a death toll of millions.

Figures, on this month's cover, captures both the complexity of the scientific challenge of this unknown and lethal disease and the massive human loss. Oddly reminiscent of the pathetic piles of bodies in carts and public graves during the medieval plague pandemic, Weber's fractured figures, lyrical but lifeless, are frozen in time. Masked and mysterious, they seem neither critical of their demise nor passive and acquiescent. Like Wallace Stevens' poetic characters, whether blackbirds or humans as in "The Emperor of Ice Cream," they simply are. A tangled human web, they array nothing. But their closeness poignantly suggests that we are all in this together, sharing the human condition, the inevitability of death—in this case, an early and cruel one.

In regards to HIV/AIDS, modernism seeped into science. With uncharacteristic speed, the dreaded plague largely found its match in a multidisciplinary but uniquely integrated public health approach combining human rights advocacy and prevention measures and epidemiologic and surveillance data with virology and immunology. And although the puzzle awaits final solution, clinical therapies and rigorous health education have extended the lives of HIV-infected persons, with public health reaching the same conclusion as art and poetry: The only element of value is to "be" alive. Prolong and embrace it. All other considerations only "seem" important. Or as Stevens put it, "Let be be the finale of seem / The only emperor is the emperor of ice cream."

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Article Title

Taenia solium Tapeworm Infection, Oregon, 2006–2009

CME Questions

1. On the basis of the current population surveillance study by Dr. O'Neal and colleagues, which of the following statements about the epidemiology of cysticercosis in Oregon is most likely correct?

A. Cysticercosis is not a significant clinical or public health disease in Oregon
B. Among Hispanics, the annual incidence of cysticercosis is at least 5.8/100,000 population
C. The current incidence of cysticercosis among Hispanics has not changed since the prior estimate for Oregon
D. The observed incidence in this study likely overestimates the true incidence of NCC in Oregon

2. You are a public health official in Oregon planning for needed services related to cysticercosis and NCC. On the basis of O'Neal and colleagues' study, which of the following statements about morbidity and mortality is most likely correct and therefore likely to be a factor during planning?

A. The mortality rate in this study was 10%
B. Hospitalization at time of diagnosis was rare
C. There were no hospitalizations requiring intensive care
D. Surgical complications, shunt failure, and adverse events from prolonged steroid use were noted

3. As the public health official described in question 2, you are now considering appropriate goals of public health interventions for cysticercosis. Which of the following statements is most likely correct on the basis of the current study?

A. Improved selection criteria for household investigations may increase the likelihood of detecting current taeniasis infection
B. Public health intervention should focus on the health of workplace contacts
C. Affected families are already likely to understand how to prevent transmission
D. Clinicians already have a high index of suspicion for this disease

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

Cefepime-Resistant *Pseudomonas aeruginosa*

CME Questions

1. You are seeing a 61-year-old man admitted for pneumonia. His blood culture is now growing *Pseudomonas aeruginosa*, and you are concerned regarding the possibility of antimicrobial resistance of this organism.

What was the approximate rate of resistance to cefepime among isolates of *P. aeruginosa* in the current study?

A. Less than 1%
B. 8%
C. 22%
D. 47%

2. Which of the following variables independently increased the risk for *P. aeruginosa* resistance to cefepime in the current study?

A. Male sex
B. Diagnosis of pneumonia
C. Higher Charlson index score
D. Transfer from another facility

3. The patient was treated with antibiotics as an outpatient prior to hospital admission. Prior treatment with which classes of antibiotics was found to increase the risk for cefepime-resistant *P. aeruginosa* (CRPA) in the current study?

A. Aminoglycosides only
B. Extended-spectrum cephalosporins only
C. Extended-spectrum cephalosporins, extended-spectrum penicillins, and quinolones
D. Aminoglycosides, extended-spectrum penicillins, and macrolides

4. The patient is diagnosed with CRPA. What does the current study suggest regarding the effect of CRPA vs. cefepime-sensitive *P. aeruginosa* on the risk for mortality?

A. CRPA did not confer a higher risk for mortality in any analysis
B. Only older patients with CRPA were at a higher risk for death
C. Only patients with blood isolates for CRPA were at a higher risk for death
D. Any infection with CRPA was associated with a higher risk for death

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

Upcoming Issue

Understanding the Cholera Epidemic, Haiti, 2010

Rickettsia parkeri Rickettsiosis, Argentina

Neurognathostomiasis, a Neglected Parasitosis of the Central Nervous System

Asian Lineage of Peste des Petits Ruminants Virus

Severe *Plasmodium knowlesi* Malaria in Tertiary Hospital, Sabah, Malaysia

Plasmodium knowlesi, *P. falciparum*, and *P. vivax* among Humans and Mosquitoes Vietnam

Influenza-like Illness during Pandemic (H1N1) 2009, New South Wales, Australia

Effectiveness of Seasonal Influenza Vaccine against Pandemic (H1N1) 2009 Virus, Australia, 2010

Transmission of Influenza on International Flights, May 2009

Hansen Disease among Micronesian and Marshallese Persons Living in the United States

Hantavirus Pulmonary Syndrome, United States, 1993–2009

Epidemiology and Control of Legionellosis, Singapore

ESBL Genes of *Escherichia coli* in Chicken Meat and Humans, the Netherlands

Burkholderia pseudomallei in Unchlorinated Domestic Bore Water, Tropical Northern Australia

Melioidosis in Southern Arizona, USA

Melioidosis in Phnom Penh, Cambodia

Viability of *Baylisascaris procyonis* Eggs

Hospitalized Patients with Pandemic (H1N1) 2009, Kenya

Pandemic (H1N1) 2009 and Hajj Pilgrims Who Received Predeparture Vaccination, Egypt

Bartonella spp. Genogroups in Bats, Guatemala

Clonal Genotype of *Geomyces destructans* among Bats with White Nose Syndrome, New York

Complete list of articles in the June issue at http://www.cdc.gov/eid/upcoming.htm

Upcoming Infectious Disease Activities

July 8–10, 2011
International Society for Infectious Diseases Neglected Tropical Diseases Meeting (ISID-NTD)
Boston, MA, USA
http://ntd.isid.org

August 8–19, 2011
12th International Dengue Course
Havana, Cuba
http://www.ipk.sld.cu/cursos/dengue2011/index.htm

August 27–31, 2011
2011 Infectious Disease Board Review Course – 16th Annual Comprehensive Review for Board Preparation
Ritz-Carlton, Tysons Corner
McLean, VA, USA
http://www.IDBoardReview.com

September 17–20, 2011
51st Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC)
McCormick Place Chicago
Chicago, IL, USA
http://www.icaac.org

October 12–15, 2011
The Denver TB Course
Denver, CO, USA
http://www.njhealth.org/TBCourse

October 20–23, 2011
49th Annual Meeting of the Infectious Diseases Society of America
Boston, MA, USA
http://www.idsociety.org/idsa2011.htm

November 16–19, 2011
7th World Congress of the World Society for Pediatric Infectious Diseases (WSPID 2011)
Melbourne, Australia
http://www.kenes.com/wspid2011/mailshot/ms3.htm

Announcements

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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.

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Title Page. Give complete information about each author (i.e., full name, graduate degree(s), affiliation, and the name of the institution in which the work was done). Clearly identify the corresponding author and provide that author's mailing address (include phone number, fax number, and email address). Include separate word counts for abstract and text.

Keywords. 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. Submit figures as separate files, in the native format when possible (e.g., Microsoft Excel, PowerPoint). Photographs should be submitted as high-resolution (600 dpi) .jpg or .tif files. Other file formats may be acceptable; contact fue7@cdc.gov for guidance. Figures should not be embedded in the manuscript file. Use color only as needed. Use Arial font for figure lettering. Figures, symbols, lettering, and numbering should be clear and large enough to remain legible when reduced to print size. Large figures may be made available online only. Place figure keys within the figure; figure legends should be provided at the end of the manuscript file.

Videos. Submit as AVI, MOV, MPG, MPEG, or WMV. Videos should not exceed 5 minutes and should include an audio description and complete captioning. If audio is not available, provide a description of the action in the video as a separate Word file. Published or copyrighted material (e.g., music) is discouraged and must be accompanied by written release. If video is part of a manuscript, files must be uploaded with manuscript submission. When uploading, choose "Video" file. Include a brief video legend in the manuscript file.

Types of Articles

Perspectives. Articles should not exceed 3,500 words and 40 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. Articles should provide insightful analysis and commentary about new and reemerging infectious diseases and related issues. Perspectives may address factors known to influence the emergence of diseases, including microbial adaptation and change, human demographics and behavior, technology and industry, economic development and land use, international travel and commerce, and the breakdown of public health measures.

Synopses. Articles should not exceed 3,500 words and 40 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. This section comprises concise reviews of infectious diseases or closely related topics. Preference is given to reviews of new and emerging diseases; however, timely updates of other diseases or topics are also welcome.

Research. Articles should not exceed 3,500 words and 40 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. Report laboratory and epidemiologic results within a public health perspective. Explain the value of the research in public health terms and place the findings in a larger perspective (i.e., "Here is what we found, and here is what the findings mean").

Policy and Historical Reviews. Articles should not exceed 3,500 words and 40 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. Articles in this section include public health policy or historical reports that are based on research and analysis of emerging disease issues.

Dispatches. Articles should be no more than 1,200 words and need not be divided into sections. If subheadings are used, they should be general, e.g., "The Study" and "Conclusions." Provide a brief abstract (50 words); references (not to exceed 15); figures or illustrations (not to exceed 2); tables (not to exceed 2); and biographical sketch. Dispatches are updates on infectious disease trends and research that include descriptions of new methods for detecting, characterizing, or subtyping new or reemerging pathogens. Developments in antimicrobial drugs, vaccines, or infectious disease prevention or elimination programs are appropriate. Case reports are also welcome.

Photo Quiz. The photo quiz (1,200 words) highlights a person who made notable contributions to public health and medicine. Provide a photo of the subject, a brief clue to the person's identity, and five possible answers, followed by an essay describing the person's life and his or her significance to public health, science, and infectious disease.

Commentaries. Thoughtful discussions (500–1,000 words) of current topics. Commentaries may contain references but no abstract, figures, or tables. Include biographical sketch.

Another Dimension. Thoughtful essays, short stories, or poems on philosophical issues related to science, medical practice, and human health. Topics may include science and the human condition, the unanticipated side of epidemic investigations, or how people perceive and cope with infection and illness. This section is intended to evoke compassion for human suffering and to expand the science reader's literary scope. Manuscripts are selected for publication as much for their content (the experiences they describe) as for their literary merit. Include biographical sketch.

Letters. Letters commenting on recent articles as well as letters reporting cases, outbreaks, or original research, are welcome. Letters commenting on articles should contain no more than 300 words and 5 references; they are more likely to be published if submitted within 4 weeks of the original article's publication. Letters reporting cases, outbreaks, or original research should contain no more than 800 words and 10 references. They may have 1 figure or table and should not be divided into sections. No biographical sketch is needed.

Books, Other Media. Reviews (250–500 words) of new books or other media on emerging disease issues are welcome. Title, author(s), publisher, number of pages, and other pertinent details should be included.

Conference Summaries. Summaries of emerging infectious disease conference activities are published online only and should contain 500–1,000 words. They should focus on content rather than process and may provide illustrations, references, and links to full reports of conference activities.

Online Reports. Reports on consensus group meetings, workshops, and other activities in which suggestions for diagnostic, treatment, or reporting methods related to infectious disease topics are formulated may be published online only. These should not exceed 3,500 words and should be authored by the group. We do not publish official guidelines or policy recommendations.

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. Email to eideditor@cdc.gov.