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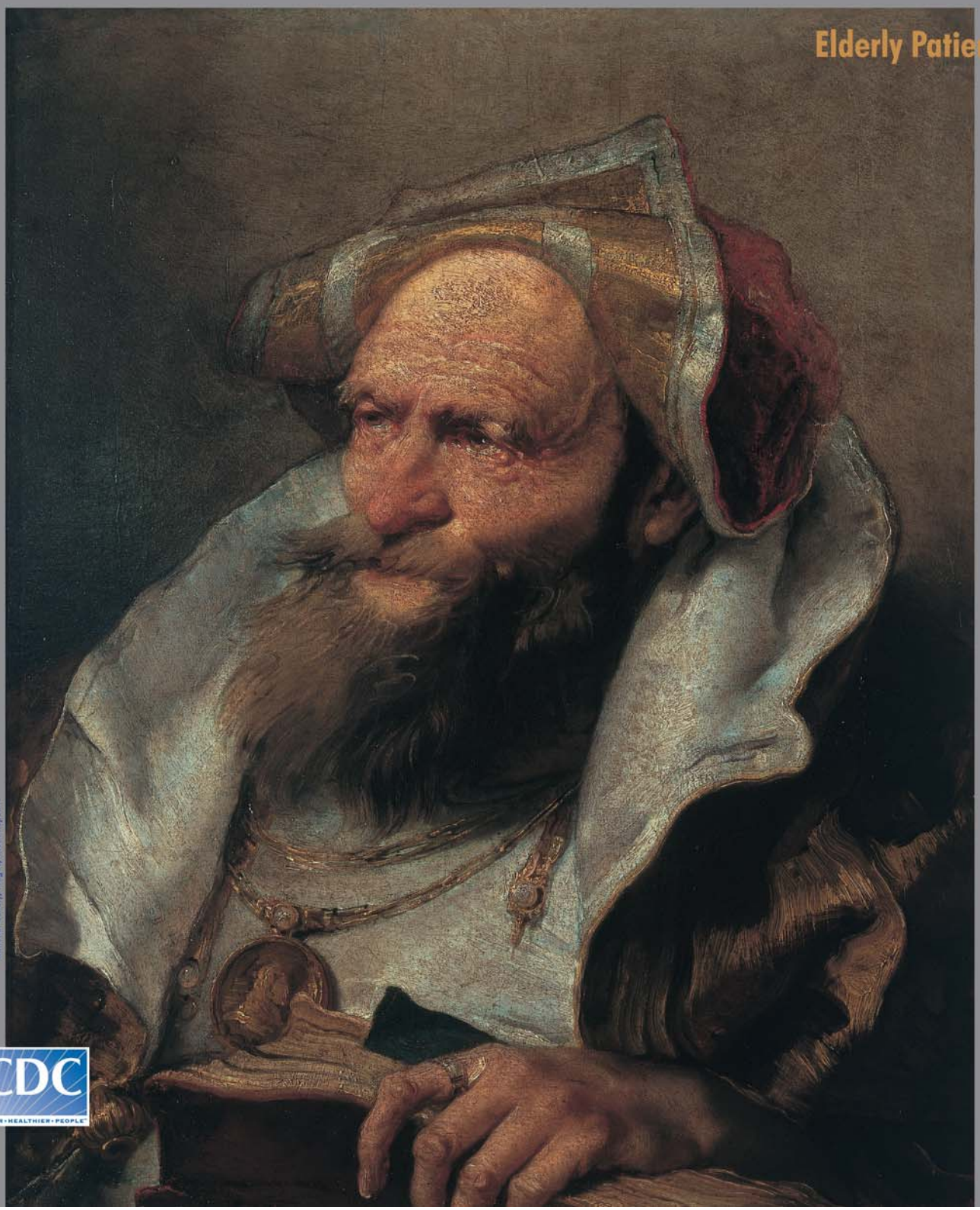

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Giovanni Battista (Giambattista) Tiepolo (1696–1770)
Bust of an Old Man (c. 1751–1755)
Oil on canvas (61 cm × 50.5 cm)
National Gallery, Prague, Czech Republic

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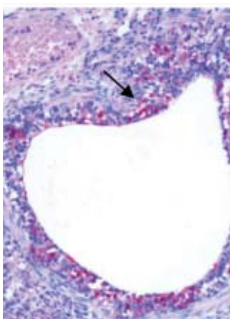

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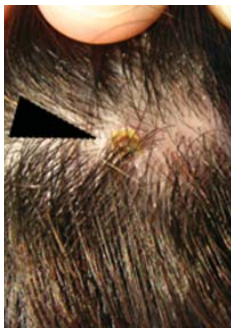

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Scale-up of Multidrug-Resistant Tuberculosis Laboratory Services, Peru

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Over the past 10 years, the Peruvian National Tuberculosis (TB) Program, the National Reference Laboratory (NRL), Socios en Salud, and US partners have worked to strengthen the national TB laboratory network to support treatment of multidrug-resistant TB. We review key lessons of this experience. The preparation phase involved establishing criteria for drug susceptibility testing (DST), selecting appropriate DST methods, projecting the quantity of DST and culture to ensure adequate supplies, creating biosafe laboratory facilities for DST, training laboratory personnel on methods, and validating DST methods at the NRL. Implementation involved training providers on DST indications, validating conventional and rapid first-line DST methods at district laboratories, and eliminating additional delays in specimen transport and result reporting. Monitoring included ongoing quality control and quality assurance procedures. Hurdles included logistics, coordinating with policy, competing interests, changing personnel, communications, and evaluation. Operational research guided laboratory scale-up and identified barriers to effective capacity building.

Heightened awareness of the global threat of tuberculosis (TB) has been spurred, in part, by the widespread prevalence of drug-resistant strains (*1*). Extensively drug-resistant TB (XDR TB) is associated with high death rates among patients co-infected with HIV and has led to renewed efforts to strengthen TB control (*2*,*3*) Program managers and policy makers face the urgent task of quickly scaling-up comprehensive TB programs, often in settings with minimal infrastructure. Although daunting, the task appears feasible in light of favorable early treatment outcomes for multidrug-resistant TB (MDR TB) treatment programs, the growing cadre of technical experts, consensus on TB and MDR TB management (*4*), and availability of global resources to fund programs (*5*,*6*).

From 1996 through 2005 in Peru, a consortium of institutions implemented one of the most comprehensive national MDR TB treatment programs in the world. One component of this effort was the Laboratory Improvement Project, which was charged with scaling-up laboratory services to support MDR TB treatment. We encountered many lessons in expanding laboratory access to quality TB culture and drug susceptibility testing (DST). We summarize the key lessons that may be relevant for other settings where MDR TB treatment is being planned or implemented.

Background

TB incidence in Peru is among the highest in Latin America, at 108.2/100,000 persons in 2005 (Table 1) (*7*). In the densely populated periphery of Lima, where half of all national cases are detected, the risk for infection with *Mycobacterium tuberculosis* may be among the highest recently documented (*8*–*10*). Rates of MDR TB are also high, with a national prevalence of 3% among patients never treated for TB and 12.3% among previously treated patients (*11*). During 1990–2000, Peru implemented a model program based on the World Health Organization (WHO)–endorsed strategy of directly observed treatment, short course (DOTS) (*12*). Massive use of sputum smear microscopy and standardized first-line treatment resulted in effective case detection and cure, with an overall decrease in TB incidence by the end of the decade (*13*). During that period, however, the rates of MDR TB increased (*14*).

Because DOTS alone was insufficient to control ongoing transmission of drug-resistant strains (*15*), Partners in Health (PIH), Harvard University, Massachusetts State

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Table 1. HIV and tuberculosis (TB), Peru, 2005*

Characteristic	Value
Total population	28,300,000
Population in Lima	7,300,000
Average life expectancy, y	69
Infant mortality rate	31/100,000 live births
GDP per capita	$2,500
Population living in poverty	54%
National HIV prevalence	0.6%
Estimated no. HIV positive	60,000–80,000
No. receiving HIV therapy	9,157
TB incidence	108/100,000
MDR TB in new patients	3%
MDR TB in previously treated patients	12.3%
TB in HIV patients	≈30%
HIV in TB patients	≈3%
MDR TB in co-infected patients	30%–47%
Mortality rate among co-infected patients†	≤38%
Mortality rate among MDR TB–HIV patients	≤57%

*GDP, gross domestic product; MDR TB, multidrug-resistant TB.
†Co-infected with HIV and TB but not necessarily MDR-TB.

Laboratory Institute (MSLI), Socios en Salud, the Peruvian National Tuberculosis Control Program (NTP), and the Peruvian National Institute of Health (INS) initiated a collaborative MDR TB treatment effort in 1996 (*16*). Principles included individualized MDR TB treatment and monthly culture to monitor treatment response. Community health promoters provided direct observation of all doses given outside health clinic hours. In 1997, the NTP implemented a standardized MDR TB treatment regimen, which achieved cure rates <50% (*17*). Although protocols changed over time, treatment failures, defaulters, and relapses after first-line treatment were generally referred for standardized MDR TB therapy. Those patients whose standardized treatments failed were, in turn, referred for individualized treatment.

Expansion of Laboratory Capacity, 1996–2000

When we began this project, 1 level III laboratory, the National TB Reference Laboratory, performed DST on first-line drugs; 57 level II laboratories performed mycobacterial culture, and ≈1,000 level I laboratories had smear microscopy capacity (Table 2). Because DST on second-line drugs was not available in Peru, isolates were initially sent to the MSLI until local capacity could be established.

As the MDR TB treatment program expanded in absolute numbers and geographic coverage, so too did demand for laboratory services. From 1996 through 2000, the number of mycobacterial cultures and DSTs performed yearly more than doubled (Figures 1, 2). The process of program scale-up posed additional challenges in patient management, information systems, drug procurement, and regional implementation. Responding to these needs, the Bill & Melinda Gates Foundation awarded a grant for $45 million in 2000 to establish a consortium called PARTNERS, whose principal task was to achieve national coverage of MDR TB treatment in Peru and replicate this project elsewhere. Several key institutions were added to the initial group of collaborators: WHO, the Centers for Diseases Control and Prevention (CDC), and the Task Force for Child Survival and Development. Within the PARTNERS consortium, the Laboratory Improvement Project was established with specialists from MSLI, CDC, Harvard University, PIH, and INS.

Strategy to Scale-up Laboratory Services

NTP norms for DST indications have evolved over the past 10 years. This heterogeneous and dynamic process provided lessons on matching the choice of DST to programmatic strategies (Table 3). Salient aspects guiding laboratory strategies include the choice of standardized versus individualized treatment, criteria for performing DST, rates of HIV and resistance to second-line drugs, and empiric management while awaiting results.

On the basis of projected numbers, DST needs would not be met unless DST on first-line drugs was decentralized to regional laboratories in areas with high rates of TB and MDR TB. In choosing methods for decentralized DST, the INS matched method features with available resources in regional laboratories (Table 4). The need for a rapid DST method was clear. Given that it took an average of almost 5 months to obtain results from a conventional DST performed in Peru (*18*), physicians often had to make treatment decisions empirically. Once results did arrive, they were no longer accurate because patients had been exposed to additional drugs in the interim, to which amplified resistance could have occurred. Rapid DST implemented at the decentralized level would be the most effective way of providing timely results and decompressing the central bottleneck of DST demand.

The INS decided that rapid DST should serve as an initial screening test. By quickly identifying resistance to

Table 2. Baseline laboratory capacity for diagnosis of tuberculosis, Peru, 1996–2000*

Activity	Validation or quality control procedures	No. establishments	No. performed/year
Smear microscopy	Quality control of all AFB+ and 10% of AFB– results each trimester at regional level of laboratories	987	1,164,198
Mycobacterial culture	Once a year, quality control of media culture	57	48,346
Drug susceptibility testing	External quality control in INPPAZ	1	1,045

*AFB, acid-fast bacilli; INPPAZ, Instituto Panamericano de Protección de Alimentos y Zoonosis.

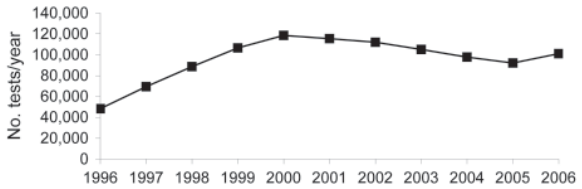

Figure 1. Mycobacterial cultures performed in Peru, by year.

isoniazid and rifampin, isolates with drug resistance could be sent to INS for full DST while standardized MDR TB treatment was started. With input from MSLI, the INS chose the Griess method. This method is a rapid colorimetric method that uses Lowenstein-Jensen (LJ) medium prepared with antimicrobial drugs (Figure 3) (*19*). Previously the method was validated as an indirect method; however, INS opted to implement it as a direct method, i.e., it is performed directly with sputum. INS validation of this method yielded sensitivities and specificities of 99% and 100% to isoniazid and 94% and 100% to rifampin (*20*). Attributes of the Griess method are accuracy, fast turnaround time (21 days), minimal additional equipment needs, inexpensive materials and reagents, and reproducibility in laboratories proficient in mycobacterial culture.

On the basis of this rationale, the following plan was developed. Second-line DST (agar plate proportions method) would be implemented in the INS. Conventional first-line DST (proportions method, indirect variation by LJ medium) would be performed at regional laboratories. Direct Griess method would be performed at regional laboratories; and the indirect BACTEC-460 system (Becton Dickinson, Franklin Lakes, NJ, USA) for first-line drugs would be implemented at INS for high-risk patients, including healthcare workers, HIV-positive patients, and pediatric patients.

Another priority was reducing the overall turnaround time of laboratory data, defined as the time when the patient is first identified at risk for MDR TB to the time that this determination has an effect on patient care. Before DST decentralization, we conducted an assessment of turnaround times in 2 health districts and confirmed that laboratory efficiency, including decentralization of DST and implementation of rapid methods, would have limited effect if pre- and post-DST processing delays were not addressed (*18*). These delays included specimen transport, specimen processing, dissemination of results to the health center, and scheduling of clinical evaluation once results were obtained. Of 924 samples processed over 16 months, the median turnaround time was 147 days; only 81 days were caused by DST processing. On the basis of these data, we worked with leaders at national and regional levels to develop and implement strategies to reduce delays (Table 5).

The overall strategy for laboratory scale-up comprised the following activities. First, establish clear criteria for performing DST. Second, select DST methods for use within the TB program and indications for each method. Third, decentralize first-line DST to 7 regional laboratories. Fourth, project the quantity of DST and cultures and ensure adequate supplies. Fifth, create biosafe laboratory facilities for DST. Sixth, train laboratory personnel on new methods. Seventh, train healthcare providers and level I laboratory personnel on DST indications. Eighth, validate DST methods, first in the INS and then at each implementing site. Ninth, establish and enact quality control and quality assurance protocols. Tenth, eliminate additional delays in specimen transport and result reporting. These strategies were used and modified in 3 phases of scale-up: preparation, implementation, and monitoring.

Preparation Phase

Key elements of the preparation phase were mobilizing political commitment (i.e., agreeing upon the strategic plan, obtaining adequate financial and human resources, and formalizing collaborations and the respective roles of different, competing and cooperating, institutions); establishing adequate laboratory infrastructure; and forming a skilled workforce. A needs assessment performed early in the project identified the need for documented biologic safety cabinet (BSC) certification and maintenance and repair of BSCs throughout the TB laboratory network. Because Peru had no trained personnel who could certify BSCs, a training program was developed and delivered with the help of MSLI and the Eagleson Institute in Sanford, Maine. The trained certifiers then certified and repaired BSCs for the TB laboratory network.

To proceed with decentralization efforts, INS contacted directors of regional laboratories. Only 1 of the laboratories met minimal space and biologic requirements to safely perform DST. The remaining 6 laboratories were asked to submit a proposal for laboratory renovations; only 3 were able to respond in a timely fashion. We explored why the other 3 laboratories did not respond and found that the administrative time and technical expertise required to elaborate a proposal was often not within the capacity of district and laboratory leaders.

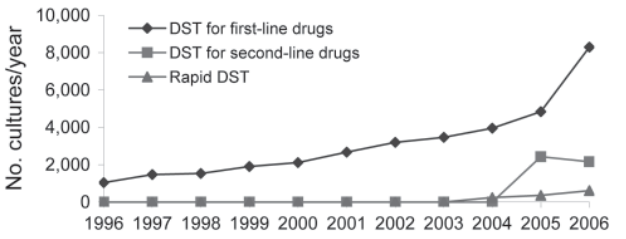

Figure 2. Drug susceptibility testing (DST) performed in Peru, by method and year.

Table 3. Optimal DST characteristics depending on MDR TB management strategy, Peru*

Programmatic and epidemiologic features	Optimal DST characteristics
Standardized versus individualized regimens	
Standardized regimens for MDR based on regional resistance patterns	Centralized, complete DST (i.e., first- and second-line drugs) of representative samples to guide standardized treatment regimen; turnaround time less important
Individualized regimens	Rapid, point-of-care DST optimal to accommodate high demand and minimize turnaround time. Semi-individualized regimens may be constructed if only DST to first-line drugs performed.
Who is tested for DST?	
Narrow DST indications (e.g., treatment failures only)	High pretest probability for MDR TB; therefore, optimal to perform DST to first- and second-line drugs to guide regimen design
Moderate DST indications (e.g., healthcare worker, smear-positive in second month of DOTS)	Rapid DST to first-line drugs to screen MDR TB versus non–MDR TB. If individualized treatment, drug-resistant samples may be referred for complete DST. Sensitivity may be more important than specificity because of greatest illness from failing to start appropriate treatment in patients with drug resistance.
Universal DST	Rapid DST to first-line drugs to screen MDR TB versus non–MDR TB. Rapid point-of-care testing (decentralized) optimal. If individualized treatment, drug-resistant samples may be referred for complete DST. Sensitivity may be more important than specificity.
Epidemiologic features	
Patients with smear-negative disease (e.g., HIV, children)	Direct DST by using liquid medium or indirect DST after culture by liquid medium. Rapid turnaround time important given high illness rates in these risk groups.
High rates of resistance to second-line drugs (XDR TB)	Complete DST if high rates of resistance to second-line drugs, including XDR. If limited resources, DST to first-line drugs plus key second-line drugs (e.g., quinolone, kanamycin) to enable identification of XDR TB cases.
Management while awaiting DST results	
Empiric first-line regimen	Greater risk for inadequate treatment of MDR TB cases; rapid testing more important
Empiric MDR TB regimen	Less risk for inadequate treatment of MDR TB cases, excess cost and toxicity for non–MDR TB cases. Complete DST results permit adjustment of empiric MDR TB therapy.

*DST, drug susceptibility testing; MDR TB, multidrug-resistant tuberculosis; XDR TB, extensively drug-resistant TB; DOTS, directly observed treatment, short course.

We supported 2 laboratory renovations and discovered that substantial time and resources were required to complete this process. Producing detailed and thorough technical proposals required substantial input from a range of experts, including architects; building, sanitary and electrical engineers; and construction companies. We identified experts with interest and competence in designing TB health facilities and encouraged collaboration by team, with technical assistance from an engineer experienced in TB infection control at CDC. Cultivating such a team with specialized knowledge in TB infrastructure has proven to be an asset for Peru. This team has since worked on other projects to renovate TB clinics and laboratories.

Once elaborated, the proposals then required approval by the governmental institution responsible for approving renovations and construction of public health facilities. Construction for both projects was delayed by an average of 6 months because of these administrative requirements. District and laboratory leaders played an important role by making frequent inquiries into the status of the approval process. In the meantime, we purchased necessary equipment, materials, and supplies.

Another step to expand DST capacity was the training and validation process for each DST method. MSLI trained INS in DST to second-line drugs by the agar plate proportion method; validation was completed in 2005. Concomi-

Table 4. Considerations for decentralized drug susceptibility testing (DST) capacity for first-line drugs, Peru

Criterion	Ideal situation
Drugs to test	First-line DST; isoniazid and rifampin most important because empiric treatment regimen and further DST may follow
Reproducibility	Because drug-resistant samples identified by regional DST, then referred to National Reference Laboratory for DST to second-line drugs, sensitivity most important
Sample source	Direct method optimal for processing at local health clinic to minimize turnaround time
Cost per sample	Low cost
Time to obtain result	Rapid
Technical demand	Less technically demanding, less processing time
Biologic safety risk	Low biosecurity risk
Required equipment	Limited additional equipment (refridgerated centrifuge) procured and maintained in local site
Reagents and supplies	Commonly used reagents and supplies available through local vendors is preferable

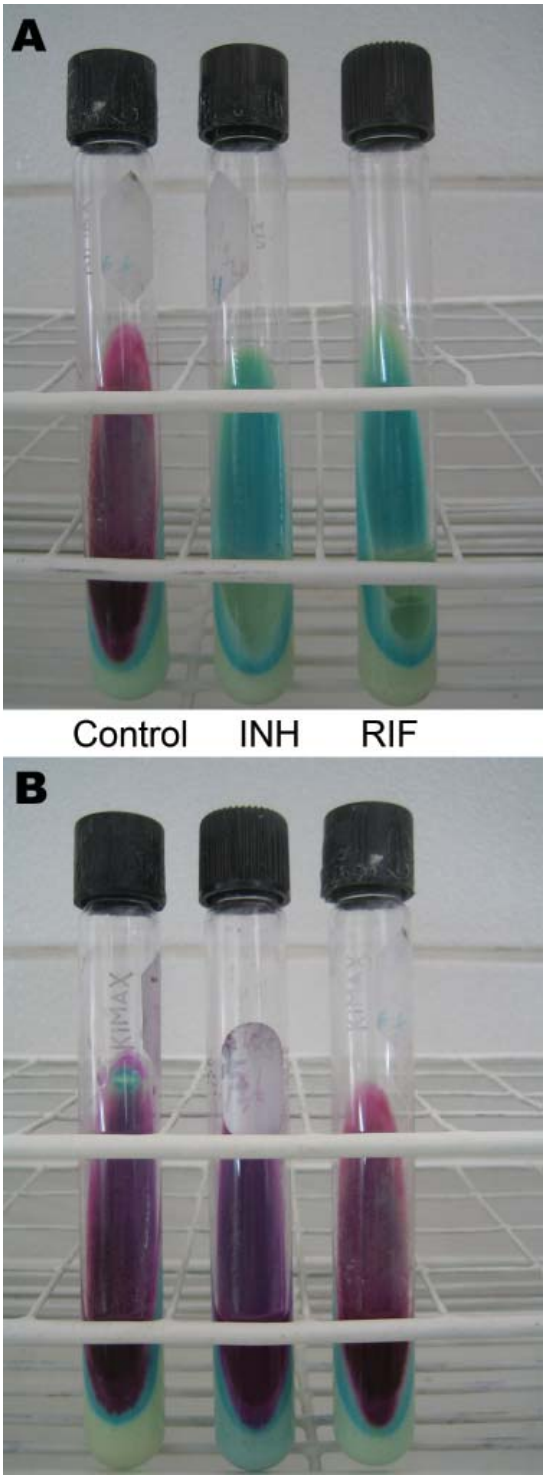

Figure 3. Description and costs of the direct Griess method in Peru. A) Pan-susceptible *Mycobacterium tuberculosis* isolate. B) *M. tuberculosis* isolate resistant to isoniazid (INH) and rifampin (RIF). The left (control) tube in panel A and all tubes in panel B indicate mycobacterial growth. The costs of the test are US $5.30 per sample, including personnel, materials (items that can be reused), and supplies (reagents and consumable items), and US $4.80 per sample, including materials and supplies.

tantly, INS trained regional laboratory personnel in DST of first-line line drugs, by the LJ medium proportions method. To initiate rapid DST, the Griess method was validated first at INS; then personnel from each implementing laboratory were trained in the method. Both conventional DST and rapid DST were validated at the regional laboratories. Samples were collected under program conditions. DST was performed by trained personnel in the regional laboratories. These same strains were then sent to INS for validation.

INS also validated BACTEC against LJ medium proportions and sped the process by performing BACTEC culture followed by indirect BACTEC DST on first-line line drugs. Validation was done for the AccuProbe method (Gen-Probe, Inc., San Diego, CA, USA) to identify *M. tuberculosis* and *M. avium* complex. Finally, INS leaders developed standard operating procedures, including protocols for all laboratory methods, biosafety and equipment standards, and quality assurance and quality control procedures.

Other activities during the preparation stage were aimed at reducing turnaround time. We developed and piloted an electronic laboratory information system connecting INS, regional laboratories, and health centers to provide health personnel (physicians, nurses, and laboratory technicians) with real-time access to culture and DST results. To support the system, we worked with health district leaders to provide Internet access, computers, and Web access points at health centers (*21*). We also purchased 2 automobiles to aid in specimen transport. At the administrative level, NTP increased the frequency of MDR TB treatment–approval meetings to reduce the bottleneck of cases pending approval for initiation of MDR TB treatment.

Implementation Phase

After successful completion of validation procedures in regional laboratories, DST was incorporated into programmatic services. Aggregate data on DST results were reviewed by each laboratory on a monthly basis to monitor rates of contamination, culture growth, and drug resistance. INS supervisors made frequent visits to these laboratories to monitor performance and troubleshoot any challenges. For instance, when low rates of culture growth were observed among acid-fast bacilli smear-positive samples, smear microscopy slides from these samples were reviewed by a biologist and decontamination protocols were reviewed. During this period, we simultaneously trained healthcare personnel in workshops and one-on-one interactions. Laboratory and TB program directors led workshops to review programmatic norms for soliciting each DST method and to explain the performance and characteristics of each method. Health workers were also trained to use the laboratory information system. Regional administrators trained providers in patient confidentiality and established

Table 5. Strategies to reduce turnaround time of culture and DST, Peru*

Step	Median baseline turnaround time, d	Strategies used	Goal turnaround time, d
From time DST processed to DST result at INS	81	Decentralize conventional and rapid DST methods	21
From receipt of DST result at intermediate laboratory to receipt of DST result at health establishment	6	Implement laboratory information system linking health centers, regional and national laboratories; improve transport of samples from health centers to regional laboratories	1
From receipt of DST result at health establishment to patient reevaluation with DST result	33	Train local providers to improve identification and referral of patients in need of MDR TB treatment; increase frequency of MDR TB treatment approval meetings; create new national culture/DST request form with DST indicators	7

*DST, drug susceptibility testing; INS, Instituto Nacional de Salud; MDR TB, multidrug-resistant tuberculosis.

a plan for sustained Internet access and computer maintenance after the pilot phase of the information system. We secured the commitment of health center directors to guarantee that TB personnel would have access to the computers during designated hours because computers were rarely placed in the TB services areas to reduce the risk for theft and vandalism.

Monitoring Phase

Sustainable laboratory infrastructure depends on administrative commitment and monitoring laboratory performance quality. Throughout the entire planning and implementation stages, MSLI provided training to INS and regional laboratories in basic and method-specific quality control/quality assurance.

The appropriate use of DSTs and culture data by healthcare workers also required ongoing evaluation. Preliminary data demonstrate that despite the reinforcement of NTP norms, health personnel often failed to adhere to NTP norms for DST (*22*). Approximately 50% of DSTs in 2005 in Lima were requested for patients without an indication for testing by NTP norms. Of DSTs not meeting NTP norms, ≈28% of these were for patients who had MDR TB compared with 32.5% among those with NTP criteria. These findings support the need for broadened indications for DST. Monitoring laboratory and programmatic performance was not effective unless these data were fed back to healthcare personnel. An example is a series of reports generated by the information system and provided to laboratory and regional TB program directors (Table 6) (*23*,*24*).

TB management protocols, such as DST indications and optimal DST methods, are dynamic; they must respond to changes in regional epidemiology as well as the availability of resources. For example, decentralization of DST

Table 6. Automated reports generated by tuberculosis (TB) laboratory information system, Peru*

Report	Informed	Purpose	Type of access†
Frequency of information system access by healthcare center personnel	Regional laboratory and TB director	Maintain frequent use of information system to access real-time laboratory data	Monthly report prepared by data administrator
No. laboratory results entered at regional laboratory	Regional laboratory and TB director	Identify delays in data entry	Monthly report prepared by data administrator
No. laboratory results verified and released to providers	Regional laboratory and TB director	Identify delays lags in result verification	Monthly report prepared by data administrator
DST results for any specified period grouped by every variable in request form	Regional and INS laboratory director	Report and identify trends in laboratory performance	Constant
Culture results for any specified period grouped by every variable in request form	Regional and INS laboratory director	Report and identify trends in laboratory performance	Constant
DST and cultures in process too long, DST missing reception date, DSTs needed to be entered into system, duplicate tests	Regional and INS laboratory director	Quality control	Constant
Rate of culture contamination; rate of negative culture growth from smear-positive specimens	Regional and INS laboratory director	Identify trends in laboratory performance	Constant
Persons with a positive culture for any specified date	Regional and INS laboratory director	Reporting to regional TB program	Constant
Persons with new DST or culture results	Healthcare center personnel	Minimize turnaround time of laboratory results	Constant and email notification
Tests that are in process and the number of days in process	Healthcare center personnel	Inform personnel of when to expect results	Constant

*DST, drug susceptibility testing; INS, Instituto Nacional de Salud.
†Constant access indicates that laboratory users could view this information in the system at any time. Some reports let the user specify the start and end dates.

resulted in an increased demand for DST because of increased awareness of MDR TB and availability of testing. Additionally, health professionals and patients perceived the benefit of rapid, real-time laboratory data. This increase in demand is an example of how our ongoing monitoring and evaluation could be applied to reassess the use and capacity of laboratory services. Our preliminary data of adherence to NTP indications for DST (*22*) and rates of MDR TB among risk groups (*25*) have helped inform modifications of NTP policy. The experience thus far in matching the appropriate DST methods to NTP norms should enable a rational application and operational assessment of promising new DST methods (*26*). Without adequately quantifying and responding to an increase in DST demand, laboratory operations may become bottlenecked, and excessive demand on limited personnel could result in deviations from laboratory protocols and a decrease in laboratory performance. Figures 1 and 2 reflect the level of laboratory expansion in Peru as of 2006, which demonstrates the trajectory of scale-up, not only in terms of DST, but for culture as well.

Lessons Learned

TB programs faced with incorporating MDR TB treatment must often expand laboratory infrastructure far beyond existing capacity. Although laboratory improvement efforts in Peru have taken a decade to accomplish and are still evolving, several key lessons can be distilled from our experience.

Responding in Time and Stepwise, Overlapping Efforts to Prevent Delays

The introduction and decentralization of DST and culture capacity can involve a wide range of activities, ranging from obtaining permits from national authorities to purchasing automobiles to streamline specimen transport. Attention to detail, the dedication of human resources to push these activities along, and parallel planning and coordination of activities can receive inadequate priority among program planners. Although these logistics can be painfully mundane, they are often the greatest obstacles, thus indirectly causing the most serious illness due to excessive delays. The recent outbreak of XDR TB among HIV-positive populations in KwaZulu-Natal, South Africa, demonstrates the need to scale-up laboratory services in a timely but correct manner (*27*).

Coordination of National Reference Laboratory and National TB Programs

Political commitment must include stable leadership; a strong central, coordinating unit; and a working relationship between TB laboratories and a TB program (*28*). The importance of coordinating laboratory and programmatic efforts may seem obvious but cannot be overstated. Within the DOTS model, smear microscopy can be performed at health centers with local coordination with TB services. In contrast, MDR TB treatment requires more complex methods (culture, DST) and is usually performed and overseen at a central site. Strategies must be informed by NTP policy and vice versa. Coordination must persist because the needs of a TB program will likely change over time.

Importance of Operational Research

Our experience in Peru was informed by our operational research. The profile of a DST method and its characteristics, when first validated in a local laboratory, may be different from its performance, strengths, and weakness when it is operating under actual program conditions. Operational assessment of a laboratory method or strategy is the sole means of understanding its effectiveness when considered within the larger context of how the method is used, associated complexities or challenges in its implementation, the mitigation of its effect caused by other system delays, and other factors. If tools to monitor laboratory performance are incorporated into information and reporting systems at the outset, effective operational research can be conducted with minimal additional resources, coupled with ongoing feedback, to create a sustainable laboratory system.

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References

1. Glusker A. Global tuberculosis levels plateau while extensively drug resistant strains increase. BMJ. 2007;334:659.
2. Raviglione MC, Smith IM. XDR tuberculosis—implications for global public health. N Engl J Med. 2007;356:656–9.
3. Ridderhof JC, van Deun A, Nam K, Nayayanan P, Abdul Aziz M. Roles of laboratories and laboratory systems in effective tuberculosis programmes. Bull World Health Organ. 2007;85:354–9.
4. World Health Organization. Guidelines for the programmatic management of drug-resistant tuberculosis. 2006. Geneva: The Organization. [cited 2008 Feb 8]. Available from http://whqlibdoc.who.int/publications/2006/9241546956_eng.pdf

5. World Health Organization. The global fund to fight AIDS, tuberculosis and malaria. 2004. Geneva: The Organization [cited 2008 Feb 8]. Available from http://www.who.int/hdp/publications/13h.pdf
6. Gupta R, Cegielski JP, Espinal MA, Henkens M, Kim JY, Lambregts-Van Weezenbeek CS, et al. Increasing transparency in partnerships for health—introducing the Green Light Committee. Trop Med Int Health. 2002;7:970–6.
7. Estrategia sanitaria nacional de control de tuberculosis—Perù. Lima: Informe Operacional; 2005.
8. Dye C, Scheele S, Dolin P, Pathania V, Raviglione MC. Consensus statement. Global burden of tuberculosis: estimated incidence, prevalence, and mortality by country. WHO Global Surveillance and Monitoring Project. JAMA. 1999;282:677–86.
9. Getchell WS, Davis CE, Gilman J, Urueta G, Ruiz-Huidobro E, Gilman RH. Basic epidemiology of tuberculosis in Peru: a prevalence study of tuberculin sensitivity in a pueblo joven. Am J Trop Med Hyg. 1992;47:721–9.
10. Madico G, Gilman RH, Checkley W, Cabrera L, Kolstadt I, Kacena K, et al. Community infection ratio as an indicator for tuberculosis control. Lancet. 1995;345:416–9.
11. Espinal MA, Laszlo A, Simonsen L, Boulahbal F, Kim S, Reniero A, et al. Global trends in resistance to antituberculosis drugs. N Engl J Med. 2001;344:1294–303.
12. Netto EM, Dye C, Raviglione MC. Progress in global tuberculosis control 1995–1996, with emphasis on 22 high-incidence countries. Global Monitoring and Surveillance Project. Int J Tuberc Lung Dis. 1999;3:310–20.
13. World Health Organization. Peru set to halve new TB cases every 10 years. Geneva: The Organization. [cited 2008 Feb 8]. Available from http://www.who.int/inf-new/tuber1.htm
14. World Health Organization. Anti-tuberculosis drug resistance in the world report no. 3. Geneva: The Organization. 2004 [cited 2008 Feb 8]. Available from http://www.who.int/tb/publications/who_htm_tb_2004_343/en/index.html
15. Farmer P, Kim JY. Community based approaches to the control of multidrug resistant tuberculosis: introducing "DOTS-plus." BMJ. 1998;317:671–4.
16. Mitnick C, Bayona J, Palacios E, Shin S, Furin J, Alcantara F, et al. Community-based therapy for multidrug-resistant tuberculosis in Lima, Peru. N Engl J Med. 2003;348:119–28.
17. Suarez PG, Floyd K, Portocarrero J, Alarcon E, Rapiti E, Ramos G, et al. Feasibility and cost-effectiveness of standardised second-line drug treatment for chronic tuberculosis patients: a national cohort study in Peru. Lancet. 2002;359:1980–9.
18. Yagui M, Perales MT, Asencios L, Vergara L, Suarez C, Yale G, et al. Timely diagnosis of MDR-TB under program conditions: is rapid drug susceptibility testing sufficient? Int J Tuberc Lung Dis. 2006;10:838–43.
19. Coban AY, Birinci A, Ekinci B, Durupinar B. Drug susceptibility testing of *Mycobacterium tuberculosis* with nitrate reductase assay. Int J Antimicrob Agents. 2004;24:304–6.
20. Solis LA, Shin SS, Han LL, Llanos F, Stowell M, Sloutsky A. Validation of a rapid method for detection of *M. tuberculosis* resistance to isoniazid and rifampin in Lima, Peru. Int J Tuberc Lung Dis. 2005;9:760–4.
21. Douglas GP, Killam WP, Hochgesang MS, Deula RA, Limbe W, Davis MK. Improving completeness, accuracy and timeliness of HIV voluntary counseling and testing client data in Malawi using touchscreen computers. AMIA Annu Symp Proc 2005:942.
22. Yale G, Bonilla C, Perales M, Contreras C, Yagui M, Shin S, et al. Evaluation of drug susceptibility testing requests under program conditions in Lima, Peru. Presented at International Union Against Tuberculosis and Lung Disease; Paris, France; 2006 Oct 31–Nov 4.
23. Blaya J, Shin S, Yagui M, Yale G, Suarez C, Asencios L, et al. Implementing and evaluating a laboratory information system to optimize the treatment of tuberculosis patients in Peru. Int J Tuber Lung Dis. 2006;10(Suppl. 1):S58–9.
24. Fraser H, Blaya J, Choi S, Bonilla C, Jazayeri D. Evaluating the impact and costs of deploying an electronic medical record system to support TB treatment in Peru. AMIA Annu Symp Proc. 2006:264–8.
25. Bonilla C, Yale G, Perales M, Contreras C, Yagui M, Cegielski P, et al. Distribution of risk factors for MDR-TB in a health district of Lima, Peru (preliminary report). Presented at International Union Against Tuberculosis and Lung Disease; Paris, France; 2006 Oct 31–Nov 4.
26. Moore DA, Evans CA, Gilman RH, Caviedes L, Coronel J, Vivar A, et al. Microscopic-observation drug-susceptibility assay for the diagnosis of TB. N Engl J Med. 2006;355:1539–50.
27. Gandhi NR, Moll A, Sturm AW, Pawinski R, Govender T, Lalloo U, et al. Extensively drug-resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa. Lancet. 2006;368:1575–80.
28. Cegielski JP, Chin DP, Espinal MA, Frieden TR, Rodriquez Cruz R, Talbot EA, et al. The global tuberculosis situation. Progress and problems in the 20th century, prospects for the 21st century. Infect Dis Clin North Am. 2002;16:1–58.

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Pandemic Influenza Planning in the United States from a Health Disparities Perspective

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We explored how different socioeconomic and racial/ethnic groups in the United States might fare in an influenza pandemic on the basis of social factors that shape exposure, vulnerability to influenza virus, and timeliness and adequacy of treatment. We discuss policies that might differentially affect social groups' risk for illness or death. Our purpose is not to establish the precise magnitude of disparities likely to occur; rather, it is to call attention to avoidable disparities that can be expected in the absence of systematic attention to differential social risks in pandemic preparedness plans. Policy makers at the federal, state, and local levels should consider potential sources of socioeconomic and racial/ethnic disparities during a pandemic and formulate specific plans to minimize these disparities.

The threat of pandemic influenza has generated concern among politicians, policy makers, healthcare professionals, and the general public. For the past several centuries, major influenza pandemics have occurred every 10 to 30 years (*1*); it is widely believed that a new pandemic is "inevitable" (*2*). The possibility of an imminent influenza pandemic has been heightened by the appearance and spread of avian influenza A (H5N1), which has a case-fatality ratio of >50% (*3*). Although the assumption has been that avian influenza viruses could not directly infect humans, the transmission of influenza virus (H5N1) directly from chickens to humans in 1997 caused experts to reconsider that assumption (*4*). Genetic changes in influenza virus subtype H5N1 in 2003 resulted in a new strain of the virus, which spread to multiple countries in East and Southeast Asia (*5*), as well as Europe and Africa. Whether the avian influenza virus (H5N1) develops human pandemic potential, its spread from birds to humans and the severity of the resulting disease have heightened concerns about a possible future influenza pandemic.

Considerable financial resources have been devoted to pandemic influenza preparedness planning at the federal and state levels (*6,7*); however, resources at state and local levels may be inadequate to implement a robust preparedness plan (*8,9*). Past experience with natural disasters and current socioeconomic and racial/ethnic disparities in healthcare in the United States (*10,11*) raise questions about the adequacy of plans to address the needs of disadvantaged populations. For example, in responding to Hurricane Katrina, planners apparently failed to consider that many low-income persons might lack private modes of transportation and would depend on institutional help for evacuation. Although the evacuation was successful overall (*12*), deaths, injuries, and illness occurred disproportionately among low-income persons in New Orleans because of economic and logistic constraints on their ability to respond to government recommendations to leave the city. Low-income and disadvantaged persons often suffer disproportionately during natural disasters and epidemics, and historical evidence demonstrates that low-income persons fared considerably worse than high-income persons during the 1918 pandemic in the United States (*13*).

In this article, we describe ways in which different socioeconomic and racial/ethnic groups might fare differently in an influenza pandemic, on the basis of current knowledge of social factors that shape exposure and vulnerability to influenza virus and that influence the timeliness and adequacy of treatment among those who become ill. We also discuss policy decisions, made either before or

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during a pandemic, which might differentially affect risk for illness or death for those of low income and of specific racial/ethnic groups. Our purposes are to 1) call attention to potentially major and avoidable social disparities in suffering and death during an influenza pandemic and 2) highlight the importance of including in pandemic preparedness plans targeted strategies for minimizing or avoiding these social disparities. The following discussion is not meant to be exhaustive; rather, it is meant to provoke reflection about how potential disparities in the effects of an influenza pandemic might be reduced or eliminated through appropriate planning and implementation of clinical and public health activities.

Conceptual Framework

Using a conceptual framework adapted from Diderichsen et al. (*14*), we systematically considered possible sources of disparities during an influenza pandemic by examining the following 3 levels at which underlying socioeconomic or racial/ethnic differences could lead to disparities in illness or death: 1) likelihood of being exposed to the influenza virus; 2) likelihood of contracting influenza disease, if exposed; and 3) likelihood of receiving timely and effective treatment after influenza disease has developed. To explore socioeconomic and racial/ethnic disparities at each level, we searched the literature for relevant findings based on population-based national data (Figure, Table).

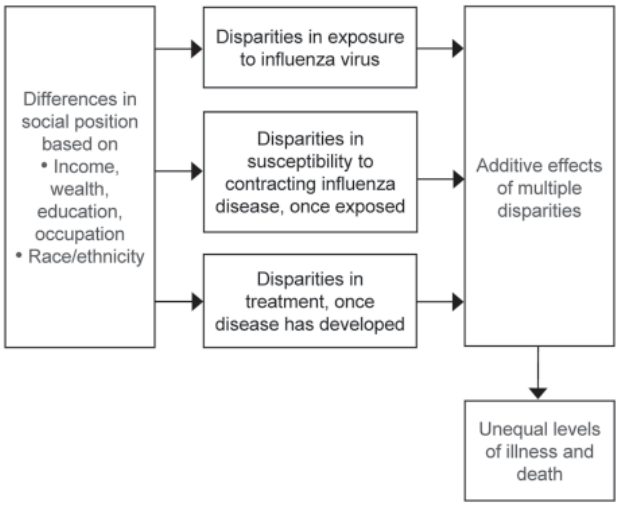

Figure. Possible sources of disparities during a pandemic influenza outbreak.

How Could Disparities Arise?

Differences in Exposure

Regardless of which strain of influenza virus causes the next pandemic, it will be highly transmissible between humans. Transmission of influenza is primarily airborne, through aerosolized respiratory tract secretions expelled during coughing and sneezing, although transmission by direct and possibly indirect contact may occur. Transmission can be expected to occur in various settings, including homes, healthcare facilities, schools, work sites, public transportation, and other settings at which people gather for social, commercial, or entertainment purposes. Higher exposure risk among particular population groups as a result of factors such as crowding and occupation could contribute to health disparities among socioeconomic and racial/ethnic groups during an influenza pandemic.

Crowding, an established risk factor for many infectious diseases, can increase the likelihood of pathogen transmission. In the United States, urban poverty and Hispanic and Asian ethnicity are correlated with domestic crowding; even at higher income levels, Hispanic and Asian households are relatively more crowded than white and African-American households (*15*). In addition, in the United States, low-income persons, African Americans, and nonwhite Hispanics are more likely than persons in other groups to obtain regular medical care at emergency departments and publicly funded clinics (*10*), where airborne transmission of infectious agents has been documented. Because these locations typically do not segregate sick and well patients and are becoming increasingly crowded (*16*), patients waiting for care in these settings are likely to have greater exposure to influenza viruses and other respiratory pathogens. Another source of increased exposure to infected persons is public transportation, where persons from low-income and minority households account for 63% of users (*17*).

Occupational factors are also likely to lead to differential exposure risk during an influenza pandemic, particularly in terms of adherence to strategies that aim to limit case-patient contact with others (*18*). Staying home may not be economically feasible for persons in lower wage occupations; these persons are less able to afford losing income as a result of missed work and often lack the job flexibility that would permit them to work at home. In addition, their jobs may be necessary because they provide essential goods and services. For these reasons, parents in lower wage/lower status occupations may be more likely to keep their children in communal childcare settings—where exposure risks are relatively high—during an influenza pandemic, placing everyone in the family at greater risk for exposure.

Differences in Susceptibility

Among persons who have been exposed to influenza virus, the likelihood of contracting disease may be modified by underlying host factors and medical conditions, such as age, smoking status, nutritional status, stress levels, and cardiopulmonary disease. The influence that most host

Table. Factors that could contribute to health disparities among socioeconomic and racial/ethnic groups during an influenza pandemic

Differences in exposure to influenza virus
Crowding in households, medical facilities, public transportation
Occupational factors such as inability to work from home, dependence on childcare outside of the home
Differences in susceptibility to influenza disease, once exposed to the virus
Host factors, including preexisting immunity, age, other underlying diseases or conditions, smoking, nutritional status, stress
Vaccination status, reflecting differences in vaccine seeking and acceptance and in vaccine availability
Differences in timely effective treatment, once influenza disease has developed
Access to outpatient and inpatient medical care
Care-seeking attitudes and behavior
Financial obstacles, including lack of adequate insurance coverage
Logistic obstacles, including transportation, language
Quality of care
Availability of antiviral treatments
Appropriate inpatient treatment

factors will have on the development of influenza during a future pandemic is uncertain; some evidence suggests that the factors affecting disease severity and death may differ from those typically observed during annual influenza epidemics (*19*). However, given overwhelming evidence that low-income persons are generally more susceptible to infectious diseases, it is reasonable to plan on the basis of well-documented annual epidemic patterns, in which influenza disease development is influenced by factors that are differentially distributed across socioeconomic and racial/ethnic groups. These patterns, as well as patterns of many other diseases, indicate that socially disadvantaged groups are likely to be at higher risk for influenza disease, particularly severe disease.

The inability to predict which influenza virus will cause a future pandemic, together with the very limited national and global capacity to produce influenza vaccine in massive quantities in a short time, almost ensures that an effective vaccine will be unavailable to most or all of the population during the early stages of a pandemic and in very short supply thereafter. Even so, current plans assume that local and state public health agencies will have a primary role for distributing pandemic influenza vaccine. In general, however, these plans do not adequately address preventing or minimizing socioeconomic or racial/ethnic disparities in vaccine distribution and acceptance, despite evidence that such disparities have been the rule for the annual influenza vaccine, even among persons ≥65 years of age (*20*). In the United States, routine use of annual influenza vaccine in preschool children has only recently been introduced; information focusing on school-age children is limited (*21*). Nevertheless, African American/black children and children from lower income families, who are at higher risk of contracting influenza (*22*) in this country, are less likely to be up to date with other routine immunizations (*23*). It is possible that, in the context of an influenza pandemic, vaccine-seeking and acceptance behavior and resultant coverage patterns may differ from those observed during routine vaccination efforts; however, the weight of available evidence indicates that social disparities in vaccine coverage are likely to occur in the absence of careful planning to prevent them.

Differences in Treatment

Among those who contract influenza, subsequent illness and death may be influenced by underlying factors and conditions and by the timeliness and effectiveness of various treatment modalities. Most influenza illnesses are self-limiting, and most infected persons during both annual influenza epidemics and influenza pandemics (including that of 1918–19) recover with only supportive care in the community. Even so, current planning efforts recognize the potential importance of reducing disease during a pandemic, through early treatment with antiviral drugs and through other forms of treatment such as respiratory support and antimicrobial agents to treat secondary bacterial pneumonia, among those with more severe disease.

In the United States, the likelihood of substantial disparities in access to timely and appropriate care under influenza pandemic conditions is high, given long-standing and persistent disparities in access to medical care. For example, persons with low income are ≈2× as likely as those with higher incomes to lack a usual source of healthcare (*24*). Similarly, non-Hispanic black and Hispanic persons are significantly less likely than non-Hispanic white persons to report having a usual primary care provider (*10*). Among persons who do report having a usual source of care, those who are poor or near poor and those who are non-Hispanic black or Hispanic are 2.5–4× as likely as their relatively higher income and white counterparts to rely on a hospital-based source of primary care (*24*). These same groups are also more likely to report having difficulty obtaining timely appointments for illness or injury, which suggests problems with access to care even among those with a usual source of healthcare (*10*). Language and cultural barriers to seeking and receiving medical care also may contribute to disparities. In emergency departments, for example, interpreters are frequently unavailable or underused, which has potentially adverse implications for patients' understanding of their disease or treatment and for clinical decision making

and quality of care (*25*). In addition, the large numbers of persons who lack health insurance, as well as those who lack documentation of US citizenship, often delay seeking care because they are concerned about paying for the care or encountering legal difficulties.

Evidence from previous outbreaks suggests that antiviral drugs may be effective for treatment (*26*) and prevention (*27*) of pandemic influenza, and current antiviral drugs seem to be biologically effective against 1918 and 1918-like viruses (*28*). Because vaccine may not be available when a pandemic begins, experts have suggested that the antiviral drug oseltamivir should be stockpiled for use during a pandemic influenza outbreak. Recent models suggest that early use of oseltamivir may contain outbreaks if certain criteria regarding transmissibility and compliance are met (*29*). However, experience with nonpandemic influenza indicates that oseltamivir must be given early during symptom development for it to have any substantial biological effect (*30*); modest delays may vitiate the treatment effectiveness (*31*). Although plans for release and distribution of antiviral drugs are still being finalized, overcoming long-standing disparities in access to timely treatment by socioeconomic status, race/ethnicity, ability to speak English, and legal status will present numerous challenges to ensuring equal access to such drugs during a pandemic.

Reasons for concern about disparities in the timeliness and appropriateness of the care received by influenza patients who might benefit from in-hospital care are similar. Given the predicted insufficient supply of hospital beds and staff during a pandemic (*32*), a person's access to potentially lifesaving therapies such as respiratory support and antimicrobial treatment of secondary bacterial pneumonias in an inpatient setting is likely to depend on factors that include usual source of care, citizenship status, and ability to speak English. Disparities may also occur in the quality of care received by persons who are hospitalized. Earlier US studies of persons hospitalized for pneumonia have found that blacks and "other minorities" are 71% and 79% as likely, respectively, as non-Hispanic whites to receive antimicrobial agents within 8 hours of arrival at the hospital (*33*) and significantly less likely to have blood cultures obtained before receiving antimicrobial therapy (*10*). Such disparities in quality of care would likely persist during an influenza pandemic.

Discussion

Although reducing or eliminating socioeconomic and racial/ethnic disparities in health and healthcare has been an official federal and state policy priority for 2 decades (*34*), such disparities remain prevalent and may inadvertently become wider when not explicitly addressed by policies designed to improve the health of the population as a whole and of disadvantaged persons in particular (*35*). Given the current limitations of our public health infrastructure and the disparities in healthcare, a pandemic influenza outbreak in the United States is likely to disproportionately affect persons from socially disadvantaged groups. Explicit, systematic, and detailed plans are essential for overcoming the social barriers that are predicted to result in socioeconomic and racial/ethnic disparities in pandemic influenza illness and death. Saunders and Monet also have called for pandemic influenza planning that appropriately considers the needs of disadvantaged populations (*36*).

The Pandemic Influenza Plan of the US Department of Health and Human Services (HHS) (*37*) does not adequately address potential social disparities in exposure, vaccination, or treatment; the possible effects of such disparities; or strategies for minimizing or eliminating them. The HHS plan (*37*), the federal guidance on vaccine allocation (*38*), and the recent Centers for Disease Control and Prevention (CDC) guidelines for community-level mitigation strategies (*18*) should be credited for calling for community engagement and inclusion of a wide variety of stakeholders in planning at the local level. Outreach to providers, community leaders, and organizations, particularly in disadvantaged communities, will be an important component of any strategy for addressing disparities during a pandemic. However, the available versions of official plans do not call attention to the need for special efforts to overcome the greater barriers likely to be faced by socially disadvantaged groups.

On a US government website for pandemic influenza (www.pandemicflu.gov), a question asks which groups would be especially vulnerable during an influenza pandemic. The answer notes that people may be vulnerable for a variety of reasons, including limited access to healthcare; limited proficiency in English; or being disabled, homeless, economically disadvantaged, or a single parent. The response calls for faith-based and community-based organizations to develop plans "to care for dependent populations" and to "provide financial aid to the poor who are unable to work and are in need of emergency income for housing, medicine, or other essential needs" (www.pandemicflu.gov/faq/pandemicinfluenza/pi-0001.html), which implies that attention to the needs of economically or socially vulnerable persons is not primarily a public-sector responsibility but is more a matter for private charity. The 2005 HHS plan (*37*) itself acknowledges that some groups may need financial assistance if they are unable to work but does not indicate how that assistance would be provided or who would provide it.

Those who are still formulating plans should consider likely differences in influenza exposure and identify potential strategies for mitigating such disparities. Mathematical models have demonstrated that community-based interventions, such as quarantine and individual isolation, may

be important for reducing influenza attack rates and overall incidence (*29*). Most pandemic plans call for limiting public gatherings and closing schools to slow the spread of influenza, without adequately taking into account how implementing these strategies could differentially affect disadvantaged groups. Recent recommendations from CDC go further in recognizing the differential effect of social-distancing measures on vulnerable communities (*18*). Although CDC advocates flexible work arrangements, income replacement, and job security to minimize the negative effects of social-distancing measures, it pays inadequate attention to those whose jobs will not accommodate these interventions. More specific solutions should be outlined in pandemic preparedness plans to address the economic effects of quarantine on low-income persons, who by staying home may be at risk wage loss, job termination, or both. Job security and income replacement are key components to limiting the effects of potential quarantine measures on disadvantaged persons (*39*) and should be extended to all persons, regardless of their type of work.

Important decisions also will need to be made concerning access to vaccination and treatment in the event of a pandemic. The federal government's Draft Guidance on Allocating and Targeting Pandemic Influenza Vaccine (*38*) provides a basic framework for allocating vaccine during the pandemic. An appendix to that document mentions (on p.17) that the principles of "fairness and equity (recognizing that all persons have equal value, and providing equal opportunity for vaccination among all persons in a priority group)" were considered when drafting the guidelines. Although the proposed schema very reasonably first defines groups of different priority levels according to occupation and then, within the general population, according to age and pregnancy status, it does not provide explicit attention to groups who are vulnerable because of social disadvantage. Nor does it note the need for explicit attention to vulnerable social subgroups, for example, low-wage workers in prioritized occupational fields and low-income and minority pregnant women, infants, and toddlers. We are not questioning the rationality of defining major priority groups according to occupation or of using biological criteria to further prioritize within the general population. Rather, our concern is with the absence of attention to both biological and social risk factors, which must be addressed to overcome the many social barriers to equal opportunity for vaccination.

Well-documented evidence of existing healthcare disparities suggests that during a pandemic shortages of influenza vaccine, antiviral drugs, inpatient services, and healthcare staff will disproportionately affect persons in socially disadvantaged groups. To limit the crowds that might occur at hospitals and clinics, plans for the release of stockpiles of vaccines, medications, or both could include distribution from private pharmacies or doctors' offices. However, because private pharmacies and private practitioners are less likely to be located in lower income neighborhoods, plans to make access to potentially lifesaving vaccines and drugs speedier and more equitable might, in fact, exacerbate disparities. Distribution plans may need to include mobile community health centers (staffed by nurses and nurse practitioners) that can travel to low-income areas, along with a variety of community medical and other service providers and nontraditional sites like soup kitchens, sheltered workshops, and transit points, which have become popular places for administering yearly influenza vaccine (*40*). Other factors, such as the availability of transportation to a hospital, might also become more important during a pandemic. Access to a private car may be a major determinant of who is able to obtain care, presenting constraints like those that led to disparities in evacuation from New Orleans before Hurricane Katrina. To ensure that disadvantaged communities are reached and that resources are equitably allocated during an influenza pandemic, preparedness plans can and should involve community-based providers and organizations that are familiar with vulnerable groups.

Conclusions

Social group disparities in exposure, susceptibility, and access to timely and effective treatment for a variety of diseases have been well documented in the United States. Influenza pandemic preparedness plans that fail to explicitly provide guidelines on how to mitigate these issues could lead to decisions that may, on the surface, seem reasonable, but that are likely to exacerbate social group disparities in health outcomes. Given the existence of major disparities in health and healthcare, we cannot expect pandemic preparedness and response planning to eliminate the deep divides that exist between socioeconomic and racial/ethnic groups. These disparities can, however, be minimized through careful planning that considers and proactively addresses vulnerability at each level: exposure to disease, susceptibility to disease if exposed, and treatment of disease. Public officials should systematically consider the additional barriers faced by socially disadvantaged groups at each of these levels and then actively seek ways to address those barriers. Local service providers, leaders of community-based organizations and other organizations working with socially vulnerable groups, and leaders of labor unions representing low-wage service workers are likely to have valuable insights and should be included in the planning process. Plans calling for stakeholder involvement without explicitly emphasizing the need to involve representatives of socially disadvantaged groups are unlikely to be effective at minimizing social disparities during an influenza pandemic.

We have focused here on the United States, but similar

fundamental principles—the need for systematic and concrete planning to minimize the social disparities that can be expected to occur in the face of natural disasters such as an influenza pandemic—apply worldwide. Countries with universal financial access to healthcare and strong social safety nets will be best positioned to minimize such disparities. Countries in which large proportions of the population are impoverished or otherwise socially excluded and countries that have more limited resources and weaker public health and social welfare infrastructures will face the greatest challenges. The framework used here—considering and proactively addressing social vulnerability in exposure to pathogens, susceptibility to disease once exposed, and consequences of illness—should be applicable across national and subnational settings.

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References

1. Patterson KD. Pandemic influenza, 1700–1900: a study in historical epidemiology. Totowa (NJ): Rowan & Littlefield; 1986.
2. World Health Organization. World is ill-prepared for "inevitable" flu pandemic. Geneva: The Organization; 2004.
3. World Health Organization. Epidemiology of WHO-confirmed human cases of avian influenza A (H5N1) infection. Wkly Epidemiol Rec. 2006;81:249–60.
4. Monto AS. The threat of an avian influenza pandemic. N Engl J Med. 2005;352:323–5.
5. Li KS, Guan Y, Wang J, Smith GJ, Xu KM, Duan L, et al. Genesis of a highly pathogenic and potentially pandemic H5N1 influenza virus in eastern Asia. Nature. 2004;430:209–13.
6. Garfield R. State preparedness for bioterrorism and public health emergencies. Issue Brief (Commonw Fund). 2005;829:1–12.
7. Centers for Disease Control and Prevention. Public health preparedness: mobilizing state by state. Atlanta: The Centers; 2008 [cited 2008 Mar 11]. Available from http://emergency.cdc.gov/publications/feb08phprep
8. Mitka M. National plan for pandemic flu unveiled. JAMA. 2006;295:2707–8.
9. Levi J, Inglesby T. Working Group on Pandemic Influenza Preparedness: joint statement in response to Department of Health and Human Services Pandemic Influenza Plan. Clin Infect Dis. 2006;42:92–4.
10. Agency for Healthcare Research and Quality. National healthcare disparities report, 2004. Rockville (MD): US Department of Health and Human Services; 2004.
11. Institute of Medicine. Unequal treatment: what healthcare providers need to know about racial and ethnic disparities in healthcare. Washington (DC): The Institute; 2002.
12. Senate Committee on Homeland Security and Governmental Affairs. Testimony of Ray C. Nagin, Mayor, City of New Orleans, February 1, 2006 [cited 2008 Mar 11]. Available from http://hsgac.senate.gov/public/_files/020106Nagin.pdf
13. Sydenstricker E. The incidence of influenza among persons of different economic status during the epidemic of 1918. 1931. Public Health Rep. 2006;121(Suppl 1):191–204.
14. Diderichsen F, Evans T, Whitehead M. The social basis of disparities in health. In: Evans T, Whitehead M, Diderichsen F, Bhuiya A, Wirth M, editors. Challenging inequities in health: from ethics to action. New York: Oxford University Press; 2001. p. 13–23.
15. Myers D, Baer WC, Choi SY. The changing problem of overcrowded housing. J Am Plann Assoc. 1996;62:66–84.
16. Trzeciak S, Rivers EP. Emergency department overcrowding in the United States: an emerging threat to patient safety and public health. Emerg Med J. 2003;20:402–5.
17. Pucher J, Renne JL. Socioeconomics of urban travel: evidence from the 2001 NHTS. Transportation Quarterly. 2003;57:49–77.
18. Centers for Disease Control and Prevention. Interim pre-pandemic planning guidance: community strategy for pandemic infuenza mitgation in the United States—early, targeted, layered use of nonpharmaceutical interventions. Atlanta: The Centers; 2007.
19. Simonsen L, Clarke MJ, Schonberger LB, Arden NH, Cox NJ, Fukuda K. Pandemic versus epidemic influenza mortality: a pattern of changing age distribution. J Infect Dis. 1998;178:53–60.
20. Centers for Disease Control and Prevention. Influenza vaccination levels among persons aged ≥65 years and among persons aged 18–64 years with high-risk conditions—United States, 2003. MMWR Morb Mortal Wkly Rep. 2005;54:1045–9.
21. King JC Jr, Cummings GE, Stoddard J, Readmond BX, Magder LS, Stong M, et al. A pilot study of the effectiveness of a school-based influenza vaccination program. Pediatrics. 2005;116:e868–73.
22. Erhart LM, Rangel MC, Lu PJ, Singleton JA. Prevalence and characteristics of children at increased risk for complications from influenza, United States, 2000. J Pediatr. 2004;144:191–5.
23. Santoli JM, Huet NJ, Smith PJ, Barker LE, Rodewald LE, Inkelas M, et al. Insurance status and vaccination coverage among US preschool children. Pediatrics. 2004;113(6 Suppl):1959–64.
24. Lewis CT, Mathews TJ, Heuser RL. Prenatal care in the United States, 1980–94. National Center for Health Statistics. Vital Health Stat Series No. 21(54); 1966 [cited 2008 Mar 11]. Available from http://www.cdc.gov/nchs/products/pubs/pubd/series/sr21/pre-31/sr21_54.htm
25. Baker DW, Parker RM, Williams MV, Coates WC, Pitkin K. Use and effectiveness of interpreters in an emergency department. JAMA. 1996;275:783–8.
26. Van Voris LP, Betts RF, Hayden FG, Christmas WA, Douglas RG Jr. Successful treatment of naturally occurring influenza A/USSR/77 H1N1. JAMA. 1981;245:1128–31.
27. Monto AS, Gunn RA, Bandyk MG, King CL. Prevention of Russian influenza by amantadine. JAMA. 1979;241:1003–7.
28. Tumpey TM, Garcia-Sastre A, Mikulasova A, Taubenberger JK, Swayne DE, Palese P, et al. Existing antivirals are effective against influenza viruses with genes from the 1918 pandemic virus. Proc Natl Acad Sci U S A. 2002;99:13849–54.
29. Ferguson NM, Cummings DA, Fraser C, Cajka JC, Cooley PC, Burke DS. Strategies for mitigating an influenza pandemic. Nature. 2006;442:448–52.
30. Hayden FG, Atmar RL, Schilling M, Johnson C, Poretz D, Paar D, et al. Use of the selective oral neuraminidase inhibitor oseltamivir to prevent influenza. N Engl J Med. 1999;341:1336–43.
31. Aoki FY, Macleod MD, Paggiaro P, Carewicz O, El Sawy A, Wat C, et al. Early administration of oral oseltamivir increases the benefits of influenza treatment. J Antimicrob Chemother. 2003;51:123–9.

32. Schull MJ, Stukel TA, Vermeulen MJ, Guttmann A, Zwarenstein M. Surge capacity associated with restrictions on nonurgent hospital utilization and expected admissions during an influenza pandemic: lessons from the Toronto severe acute respiratory syndrome outbreak. Acad Emerg Med. 2006;13:1228–31.
33. Fine JM, Fine MJ, Galusha D, Petrillo M, Meehan TP. Patient and hospital characteristics associated with recommended processes of care for elderly patients hospitalized with pneumonia: results from the medicare quality indicator system pneumonia module. Arch Intern Med. 2002;162:827–33.
34. Agency for Healthcare Research and Quality. National healthcare disparities report, 2005. Rockville (MD): US Department of Health and Human Services; 2005.
35. Mechanic D. Disadvantage, inequality, and social policy. Health Aff (Millwood). 2002;21:48–59.
36. Saunders GL III, Monet T. Eliminating injustice toward disadvantaged populations during an influenza pandemic. N C Med J. 2007;68:46–8.
37. US Department of Health and Human Services. HHS Pandemic Influenza Plan. Washington (DC): The Department; 2005.
38. Federal Interagency Working Group DGoAaTPIV. Draft guidance on allocating and targeting pandemic influenza vaccine. 2007 [cited 2008 Mar 11]. Available from http://www.pandemicflu.gov/vaccine/prioritization.pdf
39. Rothstein MA, Talbott MK. Encouraging compliance with quarantine: a proposal to provide job security and income replacement. Am J Public Health. 2007;97(Suppl 1):S49–56.
40. Centers for Disease Control and Prevention. Adult immunization programs in nontraditional settings: quality standards and guidance for program evaluation. MMWR Morb Mortal Wkly Rep. 2000;49:1–39.

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Declining Artesunate-Mefloquine Efficacy against Falciparum Malaria on the Cambodia–Thailand Border

Chansuda Wongsrichanalai* and Steven R. Meshnick†

Resistance to many antimalaria drugs developed on the Cambodia–Thailand border long before developing elsewhere. Because antimalaria resistance is now a global problem, artemisinin-based combination therapies (ACTs) are the first-line therapies in most malaria-endemic countries. However, recent clinical and molecular studies suggest the emergence of ACT-resistant *Plasmodium falciparum* infections in the Cambodia–Thailand border area, where standard ACT is artesunate and mefloquine. These ACT failures might be caused by high-level mefloquine resistance because mefloquine was used for monotherapy long before the introduction of ACT. This observation raises 2 questions. First, how can existing *P. falciparum*–resistant strains be controlled? Second, how can the evolution of new ACT- resistant strains be avoided elsewhere, e.g., in Africa? Enforcement of rational drug use and improved diagnostic capacity are among the measures needed to avoid and contain ACT resistance.

Artemisinin-based combination therapies (ACTs), considered the best current treatment for falciparum malaria (*1*), have energized worldwide programs to control malaria. Combination therapies, in general, tend to delay the development of microbial resistance. However, several ACT regimens are combinations of artesunate and older antimalarial drugs against which resistance already exists. Preexisting resistance to these older partner drugs could lead to drug failure. This may have already happened on the Cambodia–Thailand border (*2*).

Historical Perspective

The western Cambodia–southeastern Thailand border, which comprises the areas around the town of Pailin in Cambodia and the provinces of Trat and Chanthaburi in Thailand, has been an epicenter of drug-resistant malaria (*3*) (Figure). Resistance to chloroquine and sulfadoxine-pyrimethamine occurred there in the late 1950s and 1960s, respectively. Mefloquine was introduced there in 1983, first on the Thai side and in the form of mefloquine in combination with sulfadoxine-pyrimethamine. Mefloquine resistance led to replacement by artesunate-mefloquine in Thailand in 1995. In Chanthaburi and Trat Provinces, considered by the Thai national malaria control program to be the areas with the highest level of mefloquine resistance, the dosages were 12 mg/kg artesunate and 25 mg/kg mefloquine or a maximum adult dose of 600 mg artesunate and 1,250 mg mefloquine, given for 2 days. This regimen was 99% efficacious when field tested in the same border areas in 1993 (*4*). It also ensured better compliance than an extended (3-day) regimen. With the exception of the southeastern border of Thailand with Cambodia, the regimen remained effective throughout Thailand even when the control program switched to a 3-day treatment course in 2007 in accordance with the World Health Organization (WHO) recommendation.

In Cambodia in 2000, artesunate-mefloquine became the first-line drug for the treatment of falciparum malaria. The dosages were 12 mg/kg artesunate and 20 mg/kg mefloquine, or a maximum adult dose of 600 mg artesunate and 1,000 mg mefloquine, given for 3 days, in accordance with the WHO recommendation. The lower mefloquine dose was based on the perception that Cambodians were slightly smaller than Thais in body build. Malaria control programs of each country regularly monitor antimalarial therapeutic efficacy at selected sentinel sites.

Resistance to Artesunate-Mefloquine

Emerging resistance is supported by 3 independently conducted studies. In Pailin in 2002, clinical monitoring

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Figure. Map of the Cambodia–Thailand border showing the town of Pailin, Cambodia, and the provinces of Chanthaburi and Trat, Thailand; the areas are collectively known as the epicenter of drug-resistant malaria.

of response to combination artesunate (≈12 mg/kg) and mefloquine (≈20 mg/kg) co-blister packs (artesunate for 3 days and mefloquine for 1 day) showed 85.7% efficacy at day 28 follow-up (*5*) (Table). A repeat study in Pailin in 2004, which used the same drug combination but more precise dosing and follow-up at 42 days, found efficacy to be 79.3% (*5*). To exclude cases of reinfection from analysis, parasite variants were identified by using nested PCR amplification of 3 polymorphic genes for merozoite surface protein 1 (*msp1*), *msp2*, and glutamate-rich protein (*7*). In Thailand's Trat Province in 2003, efficacy of 78.6% (95% confidence interval 66.4%–91.1%) was reported from a 28-day follow-up study of 44 patients who received the same total dosage of this combination in a 2-day regimen (*6*).

Each of the 3 studies used directly observed therapy, followed the WHO standard in vivo study protocol, and obtained from their respective national control programs reliable drugs that worked effectively at other sentinel sites. Although each of the 3 studies was small, in aggregate they attest to a worrisome level of in vivo artesunate-mefloquine resistance.

The above clinical observations are supported by molecular evidence. High copy numbers of the *Plasmodium falciparum* multidrug resistance 1 (*pfmdr1*) gene, a marker of multidrug resistance (*8*), predicted recrudescence in the 2004 Pailin study, even after PCR correction and adjustment for age and parasite density (*9*). Thus, clinical and molecular evidence indicate that artesunate-mefloquine failures are occurring on the Cambodia–Thailand border. In contrast, artesunate-mefloquine remains effective in eastern Cambodia and elsewhere in Thailand.

In field situations, artesunate-mefloquine failure is likely to be worse than in controlled studies because of poorer compliance and variations in drug quality. Some of the results presented above might have overestimated artesunate-mefloquine therapeutic efficacy because 1) 28-day follow-up is inadequate time to evaluate drugs with a long elimination half-life, such as mefloquine for which up to 60% of recrudescence may occur between day 28 and day 42 (*9*), and 2) the current genotyping method is likely to misclassify some recrudescence as reinfection because of the method's limited ability to detect minor subpopulations of parasites that carry drug resistance mutations (J. J. Juliano, pers. comm.). In our opinion, PCR correction to classify recurrent infections is not necessary for a low-transmission area such as the Cambodia–Thailand border, where infection frequently results from occupational exposure in the jungles and patients usually remain at low risk for reinfection while in the village during study follow-up.

These treatment failures most likely result from mefloquine resistance rather than artemisinin resistance because increased *pfmdr1* copy number has been linked most closely with mefloquine resistance in vivo (*10,11*). Monitoring of in vitro antimalaria drug susceptibility by the Pasteur Institute of Cambodia since 2001 also suggests progressive loss of mefloquine sensitivity in western Cambodia (*2*). Most likely, mefloquine resistance in this area had already reached a level too extreme for the drug to be further protected by artesunate. To some extent, the development of resistance to artesunate-mefloquine in areas of preexist-

Table. Studies that demonstrated poor artesunate-mefloquine efficacy, Cambodia–Thailand border*

Reference	Study site, country, y	ACT	No. patients	Follow-up duration, d	Efficacy, %
Denis et al., 2006 (*5*)	Pailin, Cambodia, 2002	ATS ≈12 mg/kg in 2 doses on days 0, 1, and 2 + MFQ ≈20 mg/kg in 2 doses on day 0	70 children and adults	28	85.7 (PCR-corrected)
Vijaykadga et al., 2006 (*6*)	Trat, Thailand, 2003	ATS 12 mg/kg (maximum 600 mg) in 2 doses on days 0 and 1 + MFQ 25 mg/kg (maximum 1,250 mg) in 2 doses on day 0	44, age ≥10 y, mostly adults	28	78.6
Denis et al., 2006 (*5*)†	Pailin, Cambodia, 2004	ATS 12 mg/kg in 2 doses on days 0, 1, and 2 + MFQ 25 mg/kg in 2 doses on day 0	58 children and adults	42	79.3 (PCR-corrected)

*ACT, artemisinin-based combination therapy; ATS, artesunate; MFQ, mefloquine; day 0, first 24 h of enrollment and start of therapy.
†Also in this study, increased copy numbers of *Plasmodium falciparum* multidrug resistance 1 gene were found to be associated with parasite recrudescence, and as many as 44% of patients did not clear parasites until after 48 hours.

ing mefloquine resistance mirrors the experience with the mefloquine-sulfadoxine-pyrimethamine combination that was introduced to deal with widespread preexisting sulfadoxine-pyrimethamine resistance (*3*).

Fortunately, no clear evidence points to artesunate failure, although it is possible. No mutations thought to be associated with artemisinin resistance have been detected in Cambodia, and no isolates resistant to artesunate in vitro have been found (*12*). However, the increased parasite clearance times recorded in Pailin are worrisome. In the Pailin 2004 study, as many as 34% of patients cleared parasites between 48 and 72 hours, and 10% after 72 hours (*9*). When artesunate was first introduced in the early 1990s, failure to clear parasites by 48 hours was rare among patients with uncomplicated falciparum malaria in southeastern Thailand. Additionally, increased copy numbers of *pfmdr1* gene have recently been shown to be associated with in vitro resistance to artemisinin (and to lumefantrine and quinine) (*13*).

Likely Resistance Factors

Several reasons may explain the emergence of artesunate-mefloquine resistance on the Cambodia–Thailand border. First, the concept of rational therapy is poorly reinforced. Improper use of antimalaria drugs based on clinical diagnosis alone or on misdiagnosis as a result of poor microscopy technique or interpretation could have accelerated the onset of resistance. In Cambodia, because of poor transportation and public health infrastructure, artesunate and mefloquine are made available in the private sector to increase patients' access to the drug. This access, in turn, increases the risks that drug quality will be substandard and drug use will be uncontrolled (*5*). Because adherence and indication are not adequately emphasized, drugs are consumed in incomplete dosages or for prophylaxis such as before a jungle trip. Social marketing helps to control drug quality but cannot ensure adherence.

Although ACT use has been restricted and prescription is based on microscopic confirmation at government-run malaria clinics in Thailand, ACT is less well-controlled in Cambodia. Unreliable services and poor diagnostic capabilities at peripheral health facilities further discourage patients from seeking malaria treatment from the public sector and encourage self-purchase of drugs. A recent study of malaria treatment–seeking behavior in Cambodia showed that >80% of the patients initially sought treatment from private providers and pharmacies or consumed drugs on their own (*14*).

Second, the short half-life of artesunate relative to that of mefloquine means that tolerance to mefloquine could develop when treated patients are reinfected (*15*). Third, the malaria parasites in this region could have a unique ability to develop resistance to any antimalaria drugs (*16*); their genetics need to be further studied.

Possible Development of New Foci of Resistance

As of 2007, reduced efficacy of artesunate-mefloquine was noted in Kampot, a province southwest of Phnom Penh (*17*). Thus, resistant parasites may be spreading. Although no other ACT is immediately ready for field use, the Greater Mekong subregion will soon need alternatives to artesunate-mefloquine.

In sub-Saharan Africa, presumptive treatment with ACT may soon become the norm; this drug-use practice may similarly promote evolution of resistance. ACT resistance in Africa could be devastating. This concern has been raised repeatedly (*18*,*19*). Although artesunate-mefloquine is not recommended for African countries, 1 of the ACTs now used is the combination of artemether and lumefantrine (Coartem; Novartis, Basel, Switzerland); lumefantrine is chemically related to mefloquine. One way to delay the emergence of resistance would be to enforce ACT prescription based on accurate biological diagnosis.

Conclusions

Studies are now under way to replicate these initial findings on the Cambodia–Thailand border (*20*). Nevertheless, existing data strongly suggest that artesunate-mefloquine resistance exists in this area. This finding is a warning message for Africa, where ACT has been used in a large scale but not with parallel effort to enhance rational therapy. Continued surveillance for ACT resistance should be an integral part of any malaria control program that uses these drugs.

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References

1. World Health Organization. Guidelines for the treatment of malaria. WHO/HTM/MAL 2006.1108 [cited 2008 Mar 5]. Geneva: The Organization; 2006. Available from http://www.who.int/malaria/docs/TreatmentGuidelines2006.pdf
2. World Health Organization. Containment of malaria multi-drug resistance on the Cambodia-Thailand border. Report of an informal consultation, Phnom Penh, 29–30 January 2007. Report no. SEA-MAL-246 [cited 2008 Mar 5]. Available from http://www.who.int/malaria/docs/drugresistance/ReportThaiCam.pdf
3. Wongsrichanalai C, Prajakwong S, Meshnick SR, Shanks GD, Thimasarn K. Mefloquine—its 20 years in the Thai Malaria Control Program. Southeast Asian J Trop Med Public Health. 2004;35:300–8.

4. Thimasarn K, Sirichaisinthop J, Chanyakhun P, Palananth C, Rooney W. A comparative study of artesunate and artemether in combination with mefloquine on multidrug resistant falciparum malaria in eastern Thailand. Southeast Asian J Trop Med Public Health. 1997;28: 465–71.
5. Denis MB, Tsuyuoka R, Poravuth Y, Narann TS, Seila S, Lim C, et al. Surveillance of the efficacy of artesunate and mefloquine combination for the treatment of uncomplicated falciparum malaria in Cambodia. Trop Med Int Health. 2006;11:1360–6.
6. Vijaykadga S, Rojanawatsirivej C, Cholpol S, Phoungmanee D, Nakavej A, Wongsrichanalai C. In vivo sensitivity monitoring of mefloquine monotherapy and artesunate-mefloquine combinations for the treatment of uncomplicated falciparum malaria in Thailand in 2003. Trop Med Int Health. 2006;11:211–9.
7. Snounou G, Beck HP. The use of PCR genotyping in the assessment of recrudescence or reinfection after antimalarial drug treatment. Parasitol Today. 1998;14:462–7.
8. Reed MB, Saliba KJ, Caruana SR, Kirk K, Cowman AF. Pgh1 modulates sensitivity and resistance to multiple antimalarials in *Plasmodium falciparum.* Nature. 2000;403:906–9.
9. Alker AP, Lim P, Sem R, Shah NK, Yi P, Bouth DM, et al. *pfmdr1* and in vivo resistance to artesunate-mefloquine in falciparum malaria on the Cambodian–Thai border. Am J Trop Med Hyg. 2007;76:641–7.
10. Nelson AL, Purfield A, McDaniel P, Uthaimongkol N, Buathong N, Sriwichai S, et al. *pfmdr1* genotyping and in vivo mefloquine resistance on the Thai-Myanmar border. Am J Trop Med Hyg. 2005;72:586–92.
11. Price RN, Uhlemann AC, Brockman A, McGready R, Ashley E, Phaipun L, et al. Mefloquine resistance in *Plasmodium falciparum* and increased *pfmdr1* gene copy number. Lancet. 2004;364: 438–47.
12. Jambou R, Legrand E, Niang M, Khim N, Lim P, Volney B, et al. Resistance of *Plasmodium falciparum* field isolates to in-vitro artemether and point mutations of the SERCA-type *PfATPase6.* Lancet. 2005;366:1960–3.
13. Sidhu AB, Uhlemann AC, Valderramos SG, Valderramos JC, Krishna S, Fidock DA. Decreasing *pfmdr1* copy number in *Plasmodium falciparum* malaria heightens susceptibility to mefloquine, lumefantrine, halofantrine, quinine, and artemisinin. J Infect Dis. 2006;194:528–35.
14. Duong S, Chanthap L, Babu VVRS. Piloting of a strategy for collection of malaria information from the private sector, Cambodia, Final Report, March 2005. National Centre for Parasitology, Entomology and Malaria Control GTZ BACKUP Initiative. Phnom Penh: The Initiative; 2005
15. Hastings IM, Ward SA. Coartem (artemether-lumefantrine) in Africa: the beginning of the end? J Infect Dis. 2005;192:1303–4.
16. Rathod PK, McErlean T, Lee PC. Variations in frequencies of drug resistance in *Plasmodium falciparum.* Proc Natl Acad Sci U S A. 1997;94:9389–93.
17. Sem R, Lim P, Muth S, Kim S, Chim P, Duong S, et al. Efficacy of current standard therapy for uncomplicated *P. falciparum* and *P. vivax* malaria in central Cambodia. Abstracts of the Joint International Tropical Medicine Meeting 2007 "Health Security in the Tropics"; 2007 Nov 29–30; Bangkok. Abstract No. 47.
18. Wongsrichanalai C, Thimasarn K, Sirichaisinthop J. Antimalarial drug combination policy: a caveat. Lancet. 2000;355:2245–7.
19. Bloland PB, Ettling M, Meek S. Combination therapy for malaria in Africa: hype or hope? Bull World Health Organ. 2000;78:1378–88.
20. World Health Organization. Resistance to artemisinin derivatives along the Thai-Cambodian border. Wkly Epidemiol Rec. 2007;82:360.

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Increasing Hospitalizations and General Practice Prescriptions for Community-onset Staphylococcal Disease, England

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Rates of hospital-acquired staphylococcal infection increased throughout the 1990s; however, information is limited on trends in community-onset staphylococcal disease in the United Kingdom. We used Hospital Episode Statistics to describe trends in hospital admissions for community-onset staphylococcal disease and national general practice data to describe trends in community prescribing for staphylococcal disease. Hospital admission rates for staphyloccocal septicemia, staphylococcal pneumonia, staphylococcal scalded-skin syndrome, and impetigo increased >5-fold. Admission rates increased 3-fold for abscesses and cellulitis and 1.5-fold for bone and joint infections. In primary care settings during 1991–2006, floxacillin prescriptions increased 1.8-fold and fusidic acid prescriptions 2.5-fold. The increases were not matched by increases in admission rates for control conditions. We identified a previously undescribed but major increase in pathogenic community-onset staphylococcal disease over the past 15 years. These trends are of concern given the international emergence of invasive community-onset staphylococcal infections.

Harmless colonization of the nasal membranes and skin with *Staphylococcus aureus* is common in the community (*1*), but the organism can also cause a variety of infections (*2*). *S. aureus* is the most common cause of skin and soft tissue infections, including wound infections, abscesses, furuncles, carbuncles (*3*), and impetigo (*4*) and is a major cause of cellulitis (*5*). It can also cause deep-seated infections such as osteomyelitis (*6*), endocarditis, pneumonia, and septicemia (*2*). Toxigenic strains can cause toxic shock, staphylococcal scalded-skin syndrome (SSSS), and staphylococcal food poisoning (*2*).

More than 70 different potential virulence factors have been identified in *S. aureus,* including adhesins, exoenzymes, and exotoxins (*7*). Exfoliative toxins are associated with impetigo and SSSS (*8*), and production of Panton-Valentine leukocidin (PVL) toxin has been associated with invasive strains that cause abscesses, bone and joint infection, and pneumonia (*9,10*). In the 1950s, PVL production was associated with particularly invasive infections caused by the penicillin-resistant phage-type 80/81 strain (*11,12*). More recently, in 2006, a review of data on isolates referred to the Health Protection Agency's Staphylococcal Reference Unit identified 27 deaths in 27 months from infections with invasive PVL-positive *S. aureus*. These deaths were in previously healthy people with community-onset pneumonia, bacteremia, or severe skin and soft tissue infection (*13*). Most were caused by methicillin-sensitive *S. aureus* (MSSA) strains. In view of these data and the wider concern about emerging community-onset methicillin-resistant *S. aureus* (MRSA) (*14–16*), we sought to determine whether there had been a generalized increase in 1) community-onset staphylococcal disease severe enough to merit hospitalization and 2) general practice (GP) antimicrobial drug prescribing for skin and soft tissue infections putatively caused by staphylococci.

Methods

We analyzed Hospital Episode Statistics (HES) to identify trends in admissions for community-onset disease that were likely to be caused by pathogenic staphylococci.

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HES has been operating nationally, recording all admissions to NHS hospitals, since 1989. Inpatient admissions are coded by professional coders who used the International Classification of Diseases (ICD) version 9 before 1995 and version 10 subsequently. The main reason for admission is recorded as the "primary diagnostic code." HES data have been widely used to examine time trends in disease and variations in practice and to make international comparisons (www.hesonline.nhs.uk).

We developed ICD-9 and ICD-10 code lists for the following infections: staphylococcal septicemia, staphylococcal pneumonia, abscess, furuncle, carbuncle, cellulitis, impetigo, bone and joint infections, and SSSS (Table 1). These infections were chosen because *S. aureus* is the identified or most likely causative organism (or, in the case of cellulitis, 1 of the most important organisms). We then obtained HES data extracts on admissions to hospitals where the primary diagnosis was 1 of these codes. These data covered all NHS hospitals in England from the 1989–1990 financial year to the 2003–2004 financial year (April 1 to the following March 31). Abscess and cellulitis codes were not well discriminated in ICD-9; many codes both identified abscess and cellulitis and thus precluded separate analysis before 1995. In ICD-10, the codes for abscess and cellulitis are separated. There was no code for SSSS in ICD-9, precluding analysis of trends for this condition before 1995.

We obtained annual population denominator data from the Office for National Statistics (www.statistics.gov.uk) and used these to calculate 1) age-specific annual rates of admission for the different infections and 2) age-specific rate ratios comparing rates in 2003–04 with those in 1989–90 or, where data before 1995 were not available because of coding changes (abscesses, cellulitis, and SSSS), comparing rates in 2003–04 with those in 1995–96. We calculated 95% confidence intervals (CIs) by the delta method. We calculated annual age-standardized admission rates by using indirect standardization; the population of England in the earliest year of the trend was used as the baseline population (1989–90 for most staphylococcal conditions but 1995–96 for abscess, cellulitis, and SSSS). Standardized admission ratios and 95% CIs comparing 2003–04 rates with those in the earliest year of the trend were calculated by using indirect standardization to the baseline population (*17*). Age standardization was used to account for the fact that the age structure of the population had changed over the study period and to allow valid comparison of rates in different years.

Table 1. Comparison of trends in hospital admissions, England, 1989–90 to 2003–04*

Type of infection and ICD codes	No. admissions (age-standardized admission rate per 100,000 population)		Standardized admission ratios (95% CI)
Acute community-onset infections likely to be caused by staphylococci (ICD-9: ICD-10 codes)	1989–90	2003–04	2003–04 vs. 1989–90
Abscess, carbuncle, and furuncle and/or cellulitis (6800–6811, 6819–6829: L02.0–L03.9)	23,884 (50.0)	74,447(148.8)	2.98 (2.96–3.00)
Bone and joint infection (7300–7309, 7110: M86.0–M86.6, M86.8, M86.9, M00.0–M00.2,M00.8,M00.9)	4,104 (8.9)	6,700 (13.4)	1.57 (1.53–1.60)
Staphylococcal septicemia (381: A41.0–A41.2)	249 (0.5)	1,681 (3.3)	6.39 (6.10–6.71)
Impetigo (684: L01.0,L01.1)	199 (0.4)	1,108 (2.5)	5.92 (5.58–6.28)
Staphylococcal pneumonia (4824: J15.2)	109 (0.2)	568 (1.2)	5.04 (4.64–5.47)
	1995–96	2003–04	2003–04 vs. 1995–96
Cellulitis (NA: L03.0–L03.9)	24,388 (50.3)	49,980 (98.9)	1.97 (1.95–1.98)
Abscess, carbuncle, and furuncle (NA: L02.0–L02.9)	12,675 (26.1)	24,467 (49.3)	1.89 (1.86–1.91)
Staphylococcal scalded-skin syndrome (NA: L00.X)	149 (0.3)	747 (1.6)	5.27 (4.91–5.63)
Acute community-onset control conditions (ICD-9: ICD-10 codes)	1989–90	2003–04	2003–04 vs. 1989–90
Forearm fracture (8130–8135:S520–S529)	30,272 (63.2)	54,089(106.9)	1.69 (1.01–1.68)
Acute appendicitis (5400–5409, K35.0–K35.9)	30,946 (64.6)	30,324 (61.5)	0.95 (0.94–0.96)
Ingrown toenail (7030:L60.0)	16,606 (34.7)	11,182 (22.7)	0.65 (0.64–0.67)
Other septicemias (380–384, 388,389: A400–A409, A414, A415, A418, A419	3,793 (7.9)	12,873 (24.9)	3.15 (3.10–3.20)
Gastroenteritis/diarrhea of presumed infectious origin (91: A09.X)	8,416 (17.6)	6,528 (14.3)	0.81 (0.79–0.83)
Cholecystitis (5750: K81.0)	3,171 (6.6)	4,264 (8.3)	1.25 (1.21–1.28)
Conjunctivitis (3720–3724:H10.0–H10.9)	639 (1.3)	193 (0.3)	0.33 (0.29–0.38)
Viral pneumonia (4800–4809:J12.0–J12.9)	585 (1.2)	501 (1.1)	0.88 (0.81–0.96)
Erysipelas (35:A46.X)	373 (0.8)	357 (0.7)	0.89 (0.80–0.99)

*Years are financial years, April 1 to March 31. CI, confidence interval; ICD, International Classification of Diseases; NA, not available.

To verify that observed trends were not part of a generalized increase in hospital admissions, we used HES data to examine trends in the following control conditions: appendicitis, cholecystitis, conjunctivitis, fractured forearm, gastroenteritis/diarrhea of presumed infectious origin, ingrown toenail, and erysipelas. These conditions were chosen as examples of acute community-onset conditions that are not normally caused by staphylococci. We also examined trends in hospitalizations for septicemia in which staphylococci were not identified as the causative organism.

We analyzed data from the Prescription Prescribing Authority, which collects information on all prescriptions issued by general practitioners and dispensed by community pharmacists and dispensing general practitioners (PACT data). The information collected includes the name of the drug and the number of items dispensed (an item is defined as each preparation on the prescription). The data cannot be linked routinely to patient demographic or clinical data. Hence, they cannot be used to calculate age- and sex-specific prescribing rates or to look at prescribing rates for specific conditions (*18*). We obtained data on all GP prescriptions of floxacillin (including co-fluampicil) and fusidic acid (excluding Fucidin eyedrops but including combined steroid and fucidic acid preparations) for England from 1991 through 2006. We focused on prescriptions of floxacillin and fusidic acid because staphylococcal infection is the only indication given for these conditions in the British National Formulary. We used national population data to calculate crude annual prescribing rates. Analyses used anonymous aggregate data and did not require ethical approval.

Results

For all staphylococcal diseases from 1990–2001 to 2003–04, we found increasing admission trends. These trends are illustrated in Figure 1, panel A, which shows age-standardized hospital admission ratios for suspected staphylococcal disease, and in Table 1, which compares the number of admissions and standardized admission rates in baseline periods and in 2003–04 and shows the standardized admission ratios. For staphylococcal septicemia, staphylococcal pneumonia, impetigo, and SSSS, increases in admission rates were >5-fold over the study period; for abscesses, furuncles, carbuncles, and cellulitis, the increases were nearly 3-fold; for bone and joint infections, the increase was >50%. There were no similar increases in admission rates for the control conditions in general, although there was a 3-fold increase in admissions for septicemia not attributed to staphylococci (Table 1; Figure 1, panel B). However, most septicemias recorded in HES have no causative organism specified (68% in 1989–90 vs. 65% in 2003–04), and many of these will in fact have been caused

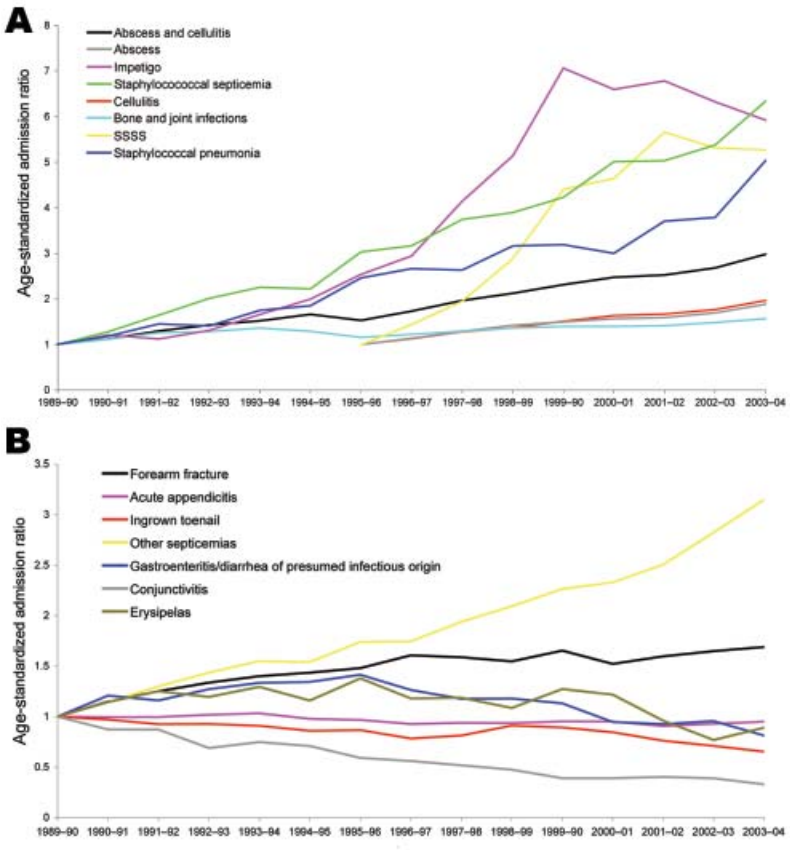

Figure 1. A) Age-standardized admission ratios for community-onset infections identified as or likely to be caused by staphylococci. B) Age-standardized admission ratios for community-onset control conditions. SSSS, staphylococcal scalded-skin syndrome.

by staphylococci. This lack of data on the organisms that cause septicemia may have masked an even greater increase in staphylococcal septicemias.

For staphylococcal septicemia, staphylococcal pneumonia, abscess, furuncles and carbuncles, cellulitis, and bone and joint infections, both the admission rates and the extent of their rise increased with patient age. Among those ≥85 years of age, there was a 15-fold increase in admission rates for staphylococcal septicemia, an 8-fold increase in staphylococcal pneumonia, a 4-fold increase in abscesses and cellulitis, and a 2.7-fold increase in bone and joint infections. Nevertheless, major statistically significant increases in admissions for community-onset staphylococcal septicemia, abscess, furuncles and carbuncles, and cellulitis were seen for all age groups. For staphylococcal pneumonia, statistically significant increases were seen for those ≥16 years of age, whereas for bone and joint infections, they were seen in all age groups except 5- to 15-year-olds. Predictably, impetigo and SSSS were seen primarily in those <16 years of age, and statistically significant increases were confined to children (Table 2).

On the basis of PACT data, a major increase in prescribing for staphylococcal disease by general practitioners was evident (Figure 2). The floxacillin prescribing rate per 100

Table 2. Age-specific hospital admission rates (2003–04, per 100,000) and rate ratios (2003–04 vs. baseline) for invasive community-onset staphylococcal infections, England*

Infection	Age group, y							
	All	≤4	5–14	15–44	45–64	65–74	75–84	≥85
Baseline data 1989–90								
Abscess/cellulitis								
Admission rate	149.3	110.1	45.1	123.2	149.5	216.1	349.5	634.3
Rate ratio (95% CI)	3.0 (2.9–3.0)	1.8 (1.7–1.9)	1.8 (1.7–1.9)	2.7 (2.6–2.8)	3.3 (3.2–3.4)	3.8 (3.6–3.9)	3.5 (3.4–3.7)	4.0 (3.8–4.3)
Staphylococcal septicemia								
Admission rate	3.4	2.2	0.7	1.3	2.7	7.4	14.1	28.7
Rate ratio (95% CI)	6.5 (5.7–7.4)	3.8 (2.2–6.3)	5.5 (2.5–12.2)	6.4 (4.7–8.9)	4.6 (3.5–6.1)	5.4 (4.1–7.2)	7.7 (5.7–10.4)	15.2 (8.9–25.9)
Staphylococcal pneumonia								
Admission rate	1.1	0.7	0.2	0.4	0.7	2.9	5.5	10.0
Rate ratio (95% CI)	5.0 (4.1–6–1)	1.2 (0.6–2.3)	1.3 (0.5–3.2)	4.2 (2.5–6.9)	4.2 (2.5–7.0)	6.0 (3.8–9.7)	9.0 (5.4–15.1)	8.2 (4.2–16.3)
Impetigo								
Admission rate	2.2	24.9	4.2	0.5	0.2	0.1	0.3	0.4
Rate ratio (95% CI)	5.3 (4.6–6.2)	6.1 (5.0–7.3)	11.8 (7.6–18.4)	3.6 (2.3–5.4)	2.1 (0.9–4.7)	1.3 (0.3–4.7)	1.3 (0.4–4.1)	3.2 (0.4–28.2)
Bone and joint infections								
Admission rate	13.4	18.3	9.2	8.5	14.0	22.8	28.6	40.5
Rate ratio (95% CI)	1.6 (1.5–1.6)	1.2 (1.1–1.4)	1.0 (0.9–1.2)	1.4 (1.3 –1.5)	1.7 (1.5–1.8)	2.0 (1.8–2.3)	1.9 (1.7–2.2)	2.7 (2.2–3.4)
Baseline data 1995–96								
Staphylococcal scalded skin syndrome								
Admission rate	1.5	21.3	2.0	0.03	0.03	0.1	0.1	0.1
Rate ratio (95% CI)	4.9 (4.1–5.8)	6.4 (5.2–7.9)	3.7 (2.5–5.4)	1.7 (0.5–5.8)	1.9 (0.3–10.2)	3.0 (0.3–29.3)	1.8 (0.2–20.4)	0.2 (0.0–1.6)
Cellulitis								
Admission rate	100.3	50.5	22.9	57.4	108.4	181.6	316.5	595.5
Rate ratio (95% CI)	2.0 (2.0–2.0)	2.2 (2.0–2.4)	1.5 (1.4–1.7)	2.0 (1.9–2.0)	2.0 (1.9–2.1)	2.0 (1.9–2.0)	2.0 (1.9–2.1)	2.0 (1.9–2.1)
Abscess								
Admission rate	491.0	59.6	22.2	65.8	41.2	34.5	33.0	38.8
Rate ratio (95% CI)	1.9 (1.8–1.9)	1.6 (1.5–1.7)	1.5 (1.4–1.7)	2.1 (2.0–2.1)	1.8 (1.7–1.9)	1.7 (1.5–1.8)	1.7 (1.5–1.8)	1.5 (1.3–1.8)

* Years are financial years, April 1 to March 31. CI, confidence interval.

population was 4.0 prescriptions in 1991 and 7.3 in 2006 (a 1.8-fold increase). The fusidic acid prescription rate per 100 persons was 2.0 in 1991 and 5.0 in 2006 (a 2.5-fold increase).

Discussion

We have identified a major and previously undocumented increase in community-onset staphylococcal disease. This increase has been ongoing for at least 15 years, since collection of HES data began in 1989, preceding the major (and largely separate) nosocomial increase in MRSA by several years (*19*). The increase has included a wide variety of staphylococcal diseases, has affected all age groups, included both GP prescribing and hospital admissions, and was not matched by similar increases in admission rates for control conditions. There were increasing trends in admissions for septicemia in general, but this increase may have been influenced by the rising incidence of staphylococcal septicemia. Although the increase in admissions has been marked, community-onset staphylococcal infections severe enough to merit admission remain comparatively rare. GP prescriptions for staphylococcal infections have increased to a lesser extent than admissions, but such prescriptions are very common.

Although we cannot be certain that *S. aureus* was the causative organism for all admissions identified here in the HES data, the degree of certainty for those recorded as staphylococcal septicemia, staphylococcal pneumonia, and SSSS is high. *S. aureus* is also the most likely etiologic agent for abscesses, furuncles, and carbuncles (*3*), and bone and joint infections (*6*) and is a very common cause of cellulitis (although β-hemolytic streptococci predominate) (*5*). *S. aureus* is the sole cause of bullous impetigo and the main agent of nonbullous impetigo, where, however, *Streptococcus pyogenes* is sometimes isolated (*20*). In moderate climates such as in the United Kingdom, staphylococcal impetigo is more common, whereas the streptococcal form predominates in warmer and more humid climates (*21*).

National data on voluntary reporting of *S. aureus* bacteremia show a 2.5-fold increase from 1990 to 2004 (an equivalent period to our study) (*22*). This included a 2.1-fold increase in reports of methicillin-sensitive *S. aureus* (MSSA). Our hospitalization data show a much more marked increase in community-onset staphylococcal bacteremia. However, the voluntary reporting system could not distinguish between hospital- and community-acquired bacteremias during this period and therefore cannot give insight into trends in community-onset infections. This means that national surveillance of bacteremia could easily have missed a major increase in community-onset staphylococcal bacteremia. A study from an Oxford hospital laboratory with an estimated referral population of 600,000 identified 697 cases of *S. aureus* bacteremia when the sample was taken within 48 h of admission between 1997 and 2003 but found no evidence of an increase in the numbers of these cases during the study period (*23*). Other research has shown that ≈50% of *S. aureus* bacteremia cases identified at the time of admission are not associated with clinical evidence of septicemia and are thus likely to result from contaminants (*24*). By contrast, our study includes >13,000 admissions in which staphylococcal septicemia was identified as the main clinical reason for the admission and identifies a major increase in these admissions over a longer period. Our study also covers a very much larger population (all of England, ≈50 million persons). The trends in community-onset staphylococcal bacteremia are, in fact, only a small part of a wider trend of increasing hospitalizations for other more common staphylococcal infections and a marked increase in community prescribing for skin and soft tissue infections. Laboratory surveillance, however, tells us very little about trends in these conditions because most are not laboratory confirmed.

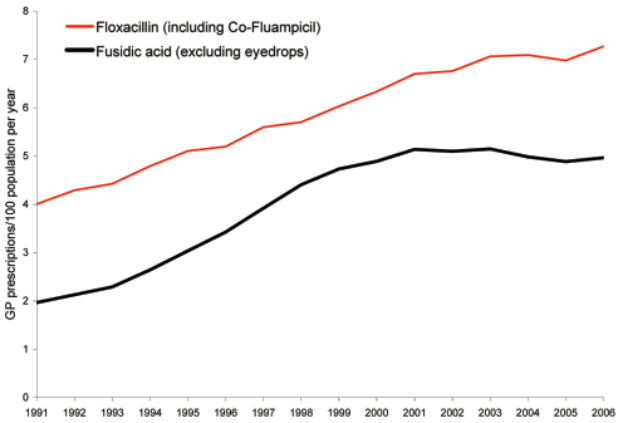

Figure 2. Crude general practitioner (GP) prescription rates (per 100 population), England, 1991–2006.

Our hospital admission data relate to the primary reason for admission, and the trends identified are therefore for community-onset invasive disease rather than disease arising during periods of hospitalization. This does not necessarily mean that infection was community acquired because infection may only become manifest after the patient is discharged into the community (*15*). We were unable to assess whether the present diagnoses related to previous episodes of hospitalization. However, *S. aureus* is a common cause of infection, and it is unlikely that most of this increase relates to healthcare activity. Previous research has shown that most patients with community-onset MRSA and MSSA bacteremia had a previous history of hospitalization. (However, for MSSA, the average time since last discharge was >100 days, so most patients probably acquired the infection in the community [*23*]). Similarly, trends in community-onset osteomyelitis or abscesses may relate to hospital acqui-

sition, for example, as complications of surgery, but most are not likely to be nosocomial. The trends in admissions for furuncles and carbuncles, cellulitis, impetigo, SSSS, and staphylococcal pneumonia are more likely to reflect real increases in community-acquired infections. Changes in the likelihood of specifying an organism for septicemia are not a probable reason for the increase in staphylococcal septicemia because the proportion of septicemias with a recorded organism (68% in 1989–90 and 65% in 2003–04) did not increase significantly. Ascertainment bias resulting from increased awareness of MRSA may conceivably have led to increased recording of staphylococcal septicemia but is unlikely to have affected recording of community-onset abscesses and boils, cellulitis, impetigo, SSSS, or osteomyelitis because the doctors attending patients and the clerks who code the reasons for admission would be unlikely to associate these with MRSA. Similarly, increased awareness of MRSA cannot account for increases in GP prescribing of floxacillin and fusidic acid.

Our analysis showed a marked increase in admissions of adults for staphylococcal pneumonia. This condition has been linked to the production of PVL toxin (*25*), although nearly 90% of staphylococcal pneumonia cases are not associated with PVL production (*26*). Several outbreaks of PVL-associated infection in the United Kingdom have been well-publicized recently (*27*), but PVL-producing strains of staphylococci are rarely identified in samples other than those from skin and soft tissue infections (*26*). Most skin and soft tissue infections, however, are not microbiologically investigated, and in those for which staphylococci are identified, tests for PVL production are not usually conducted. Testing occurs at the national staphylococcal reference laboratory after referral of isolates. However, referral is usually based on suspicion about the isolate or made because of a local outbreak. Thus, surveillance can miss important increases in the occurrence of PVL-associated disease.

Analysis of routinely collected data on hospital admissions and GP prescriptions has given a unique insight into the macroepidemiology of community-onset staphylococcal disease, impossible through any other means. The large numbers of cases exclude chance as an explanation for the trends. Moreover, the data are representative of England because HES data cover all admissions in the country and PACT data cover all NHS GP prescriptions that are dispensed. Although changes from ICD-9 to ICD-10 complicated the analysis, the underlying trends in pathogenic staphylococcal infections remain clear, and the fact that no equivalent increases in admission rates for unrelated acute community-onset conditions occurred suggests that the increases in staphylococcal disease represent a real shift. The system for collecting national data on hospitalizations and GP prescriptions has remained essentially unchanged throughout the study period. The fact that all NHS hospitals in the country and all community pharmacists and dispensing practices are obligated to use the systems means that the denominator can be accurately determined from census data, which allow measurement of rates. By contrast, in the national voluntary bacteremia reporting system, the size of the denominator is unknown and varies from year to year, depending on which laboratories participate. There are likely to be inconsistencies between hospitals in how diagnostic codes are applied in HES data and how conditions are chosen as the main reason for admission. The absence of major changes in the data collection method throughout the study period, however, means that the large increase in admissions for staphylococcal infections is highly unlikely to be an artifact.

Floxacillin has been the treatment of choice for suspected staphylococcal infections in general practice since the 1970s, so a switch in prescribing from penicillin is not a credible explanation for increasing floxacillin-prescribing rates (*12*). We also note that the increases are in marked contrast to previously reported declines in consultation and antimicrobial prescribing rates for respiratory tract infection over this period (*28*). The increase in GP prescribing mirrors, but is greater than, a similar increase in *S. aureus*–associated skin and soft tissues infections in outpatients in the United States; that increase is hypothesized to be caused by the emergence of community-acquired MRSA, but trends in prescribing in ambulatory care were not examined (*29*). Our data show admissions for skin and soft tissue infections have increased more sharply than GP prescribing for these conditions. This finding could reflect an increase in the average severity of infections.

The yearly increases were relatively modest, but the cumulative effect is a major and previously unrecognized shift in the epidemiology of *S. aureus*. This trend may result from altered virulence or transmissibility of *S. aureus* in general or of particular strains; changes in the host that affect vulnerability (e.g., increasing levels of obesity and diabetes or of intravenous drug use) or transmission dynamics (e.g., increasing use of preschool child care); or changes in the environment, such as widespread use of antimicrobial agents or changes in hygiene behavior. However, these explanations are speculative, and our ignorance of the factors driving such a major change is worrisome, particularly in view of the international concerns about the emergence of community-acquired MRSA and serious PVL-related disease.

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References

1. Abudu L, Blair I, Fraise A, Cheng K. Methicillin-resistant *Staphylococcus aureus* (MRSA): a community-based prevalence survey. Epidemiol Infect. 2001;126:351–6.
2. Projan SJ, Novick RP. The molecular basis of pathogenicity. In: Crossley KB, Archer GL, editors. The staphylococci in human diseases. New York: Churchill Livingstone; 1997. p. 55–81.
3. Lee PK, Weinberg AN, Swartz MN, Johnson AR. Pyodermas: *Staphylococcus aureus*, *Streptococcus*, and other gram-positive bacteria. In: Freedberg IM, Eisen AZ, Wolff K, Austen KF, Goldsmith LA, Katz S, editors. Fitzpatrick's dermatology in general medicine. London: McGraw-Hill; 2003. p. 2182–207.
4. Brown J, Shriner DL, Schwartz RA, Janniger CK. Impetigo: an update. Int J Dermatol. 2003;42:251–5.
5. Baddour LM. Cellulitis syndromes: an update. Int J Antimicrob Agents. 2000;14:113–6.
6. Lew DP, Waldvogel FA. Osteomyelitis. Lancet. 2004;364:369–79.
7. Kuroda M, Ohta T, Uchiyama I, Baba T, Yuzawa H, Kobayashi I, et al. Whole genome sequencing of methicillin-resistant *Staphylococcus aureus*. Lancet. 2001;357:1225–40.
8. Hanakawa Y, Schechter NM, Lin C, Garza L, Li H, Yamaguchi T, et al. Molecular mechanisms of blister formation in bullous impetigo and staphylococcal scalded skin syndrome. J Clin Invest. 2002;110:53–60.
9. Lina G, Piémont Y, Godail-Gamot F, Bes M, Peter M, Gauduchon V, et al. Involvement of Panton-Valentine leukocidin–producing *Staphylococcus aureus* in primary skin infections and pneumonia. Clin Infect Dis. 1999;29:1128–32.
10. Gillet Y, Issartel B, Vanhems P, Fournet J, Lina G, Bes M, et al. Association between *Staphylococcus aureus* strains carrying gene for Panton-Valentine leukocidin and highly lethal necrotising pneumonia in young immunocompetent patients. Lancet. 2002;359:753–9.
11. Donahue JA, Baldwin JN. Hemolysin and leukocidin production by 80/81 strains of *Staphylococcus aureus*. J Infect Dis. 1966;116:324–8.
12. Robinson DA, Kearns AM, Holmes A, Morrison D, Grundmann H, Edwards G, et al. Re-emergence of early pandemic *Staphylococcus aureus* as a community-acquired methicillin-resistant clone. Lancet. 2005;365:1256–8.
13. Kearns A. Review of 27 cases of fatal PVL disease in England: clinical presentation and strain diversity. Health Protection Agency Annual Conference, Warwick, England. 2006 Nov 9 [cited 2006 Sep 28]. Available from http://www.eventsforce.net/hpa/frontend/absViewDocument.csp?documentID=154&eventID=5&CSPCHD=00200000000d3wgqfSle000009908984
14. Chambers HF. The changing epidemiology of *Staphylococcus aureus*? Emerg Infect Dis. 2001;7:178–82.
15. Zetola N, Francis JS, Nuermberger EL, Bishai WR. Community-acquired methicillin-resistant *Staphylococcus aureus*: an emerging threat. Lancet Infect Dis. 2005;5:275–86.
16. Vandenesch F, Naimi T, Enright MC, Lina G, Nimmo GR, Heffernan H, et al. Community-acquired methicillin-resistant *Staphylococcus aureus* carrying Panton-Valentine leukocidin genes: worldwide emergence. Emerg Infect Dis. 2003;9:978–84.
17. Kirkwood BR, Sterne JAC. Essential medical statistics. 2nd ed. London: Blackwell Publishing Company; 2003. p. 263–71.
18. Majeed A, Evans N, Head P. What can PACT tell us about prescribing in general practice? BMJ. 1997;315:1515–9.
19. Johnson AP, Pearson A, Duckworth G. Surveillance and epidemiology of MRSA bacteraemia in the UK. J Antimicrob Chemother. 2005;56:455–62.
20. Koning S, Verhagen AP, van Suijlekom-Smit LWA, Morris A, Butler CC, van der Wouden JC. Interventions for impetigo. Cochrane Database Syst Rev. 2004;(2):CD003261.
21. Dagan R. Impetigo in childhood: changing epidemiology and new treatments. Pediatr Ann. 1993;22:235–40.
22. Health Protection Agency. Voluntary reporting of staphylococcal bacteraemias [cited 2007 May 18]. Available from http://www.hpa.org.uk/infections/topics_az/staphylo/staphylo_total_reports.htm
23. Wyllie DH, Peto TE, Crook D. MRSA bacteraemia in patients on arrival in hospital: a cohort study in Oxfordshire 1997–2003. BMJ. 2005;331:992.
24. Neish B, Gillespie T, Liddell KG. What is the significance of MRSA isolated from blood cultures taken in acute hospital admissions? BMJ Rapid Response. 2005 [cited 2005 Oct 5]. Available from http://www.bmj.com/cgi/eletters/331/7523/992#top
25. Morgan M. *Staphylococcus aureus,* Panton-Valentine leukocidin, and necrotising pneumonia. BMJ. 2005;331:793–4.
26. Holmes A, Ganner M, McGuane S, Pitt TL, Cookson BD, Kearns AM. *Staphylococcus aureus* isolates carrying Panton-Valentine leucocidin genes in England and Wales: frequency, characterization, and association with clinical disease. J Clin Microbiol. 2005;43:2384–90.
27. Dyer O. New MRSA strain is not at epidemic level, expert says. BMJ. 2007;334:10.
28. Frischer M, Heatlie H, Norwood J, Bashford J, Millson D, Chapman S. Trends in antibiotic prescribing and associated indications in primary care from 1993 to 1997. J Public Health Med. 2001;23:69–73.
29. McCaig LF, McDonald LC, Mandal S, Jernigan DB. *Staphylococcus aureus*–associated skin and soft tissue infections in ambulatory care. Emerg Infect Dis. 2006;12:1715–23.

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Increasing Hospital Admissions for Pneumonia, England

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Pneumonia is an important cause of illness and death in England. To describe trends in pneumonia hospitalizations, we extracted information on all episodes of pneumonia that occurred from April 1997 through March 2005 recorded in the Hospital Episode Statistics (HES) database by searching for International Classification of Diseases 10th revision codes J12–J18 in any diagnostic field. The age-standardized incidence of hospitalization with a primary diagnosis of pneumonia increased by 34% from 1.48 to 1.98 per 1,000 population between 1997–98 and 2004–05. The increase was more marked in older adults, in whom the mortality rate was also highest. The proportion of patients with recorded coexisting conditions (defined by using the Charlson Comorbidity Index score) increased over the study period. The rise in pneumonia hospital admissions was not fully explained by demographic change or increasing coexisting conditions. It may be attributable to other population factors, changes in HES coding, changes to health service organization, other biologic phenomenon, or a combination of these effects.

Community-acquired pneumonia is an important cause of illness and death in the United Kingdom, particularly for elderly adults. In recent years, increases in hospital admissions for pneumonia have been noted in the United States (*1*,*2*), Denmark (*3*), and the Netherlands (*4*). It has been suggested that this rise is due to an aging population and an increased prevalence of coexisting conditions (such as diabetes and chronic obstructive pulmonary disease); however, these factors seem to only partially explain the observed increase in pneumonia hospitalizations (*2*). Furthermore, these increases have occurred despite widespread influenza and pneumococcal vaccination programs that target the elderly. *Streptococcus pneumoniae* is a leading cause of community-acquired pneumonia (*5*,*6*), but the 23-valent pneumococcal polysaccharide vaccine currently recommended for the elderly in the United Kingdom has little efficacy against nonbacteremic pneumonia (*7*). A 7-valent pneumococcal conjugate vaccine (PCV7) was introduced into the United Kingdom infant immunization schedule in September 2006 (*8*). Experience with PCV7 in the United States suggests that, by reducing carriage and thus the opportunity for transmission of vaccine types, vaccination can lead to a reduction in invasive pneumococcal disease (*9*) and pneumonia (*10*) in unvaccinated cohorts. We report on the epidemiology of pneumonia before PCV7 vaccination was introduced by examining trends in hospital admissions for pneumonia in England during an 8-year period (April 1997 through March 2005).

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Materials and Methods

The Hospital Episode Statistics (HES) from the National Health Service (NHS) Information Centre for Health and Social Care contains details of all admissions to NHS hospitals in England (www.hesonline.org.uk). The database holds information on patient characteristics and clinical diagnoses and procedures, in addition to geographic and administrative data. Diagnoses in HES are recorded in up to 14 diagnostic fields by using the International Classification of Diseases, 10th revision (ICD-10), coding system.

Each record in the HES database relates to 1 "finished consultant episode." This is the period a person spends under the care of 1 NHS consultant during a single hospital admission; multiple episodes may occur within 1 admission. We identified all episodes of pneumonia from April 1997 through March 2005 by searching the HES database for ICD-10 codes J12–J18 in any of the 14 diagnostic fields. Episodes were classified into those with pneumonia as a primary diagnosis and those with pneumonia listed in

any diagnostic field. A patient identifier—based on date of birth, postal code, and sex—was created for each episode and encrypted to ensure anonymity. This patient identifier was then used to identify and order the number of episodes for each patient within 1 HES year, which runs from April through March. For our analyses, we retained only the first episode for each patient because the main purpose of this analysis was to identify the number of persons admitted with pneumonia at least once a year, rather than to identify multiple episodes or admissions for the same patient. Because deaths may occur in episodes subsequent to the first, we identified patients who died in hospital with an ICD-10 code for pneumonia in any diagnostic field within 30 days of the first admission with pneumonia. (Note that in-hospital deaths that were not associated with pneumonia and deaths that occurred outside of hospital were not identified.)

To adjust for coexisting conditions, we computed the Charlson Comorbidity Index score for each patient and grouped this into 4 levels: no coexisting conditions and mild, moderate, and severe coexisting conditions (*11*,*12*). This index includes 19 major disease categories and has been adapted and validated for use with hospital discharge data in ICD databases (*13*). Excess alcohol consumption is also a risk factor for pneumonia, but the Charlson index does not specifically include codes for alcohol use or alcohol-related illness. To address this, we also searched for the presence of ≥1 of the following alcohol-related ICD-10 codes within each episode: F10*, G31.2, G62.1, I42.6, K29.2, K70*, K860, T51*, X45*, X65*, Y15*, Y919, Z721 (* indicates that either a 3-digit code is valid or that all 4th digits are valid).

Length of stay is recorded in HES and is equal to the difference between admission date and discharge date (where both are recorded). Differences between the median length of stay by year and age group were assessed by using a nonparametric equality-of-medians test.

Mid-year population estimates for England for 1997 to 2004, stratified by 5-year age groups and sex, were obtained from the Office for National Statistics (ONS). The annual incidence rates of hospitalization for pneumonia were calculated overall and stratified by age by using ONS population statistics as a denominator. Age-standardized incidence rates were calculated by using the 1997 English population as the reference standard. To identify seasonal patterns, the weekly number of admissions was summarized, and a graph was created that showed the 4-week moving average number of pneumonia admissions (stratified by patients <65 years and ≥65 years). We used multivariable logistic regression to examine changes in the odds of death (within 30 days after hospital admission for pneumonia) over time (years), controlling for known risk factors, e.g., age group, sex, and coexisting conditions. All analyses were performed by using Stata version 9.2 (StataCorp LP, College Station, TX, USA).

Results

Incidence of Pneumonia (Primary Diagnosis)

The number of patients admitted to an NHS hospital in England at least once per year with a primary diagnosis of pneumonia increased from ≈72,060 in 1997–98 to 101,381 in 2004–05. Overall, the age-standardized incidence rate rose by 34% over the study period from 1.48 per 1,000 population to 1.98 per 1,000 population (Table 1). This increase was noted in all age groups but was most marked in older adults. The age-specific incidence of hospitalization with pneumonia as a primary diagnosis was 7% higher overall for male patients than for female patients over the study period. The percentage of total admissions that were due to pneumonia increased over the study period (online Appendix Figure 1, available from www.cdc.gov/EID/content/14/5/727-appG1.htm). Figure 1 shows the trends in specific diagnoses of pneumonia. Most of the increase over the study period was observed after 2000–2001 in just 2 codes, J181 (lobar pneumonia, unspecified) and J189 (pneumonia, unspecified). Diagnoses of J180 (bronchopneumonia, unspecified) decreased over the study period. Only 6% of episodes with pneumonia as a primary diagnosis had a causative pathogen within that primary diagnostic code. Of these, the most common organisms specified were *S. pneumoniae* (37%), *Mycoplasma pneumoniae* (26%), Streptococci other than group B and *S. pneumoniae* (10%), *Staphylococcus* spp. (8%), and *Haemophilus influenzae* (8%).

Table 1. Age-standardized incidence of hospital admission for pneumonia (primary diagnosis) by age group per 1,000 population, England*

HES year (April through March)	All ages	<65 y	65–74 y	75–84 y	≥85 y
1997–98	1.48	0.70	2.63	6.84	15.99
1998–99	1.67	0.72	3.02	8.08	18.59
1999–2000	1.62	0.65	3.04	8.05	18.80
2000–01	1.50	0.65	2.67	7.08	16.75
2001–02	1.67	0.71	3.02	7.73	18.89
2002–03	1.77	0.75	3.20	8.14	19.62
2003–04	1.91	0.78	3.46	8.79	22.41
2004–05	1.98	0.84	3.55	8.77	22.18
% change from 1997–98 to 2004–05	34	20	35	28	39

*HES, Hospital Episode Statistics.

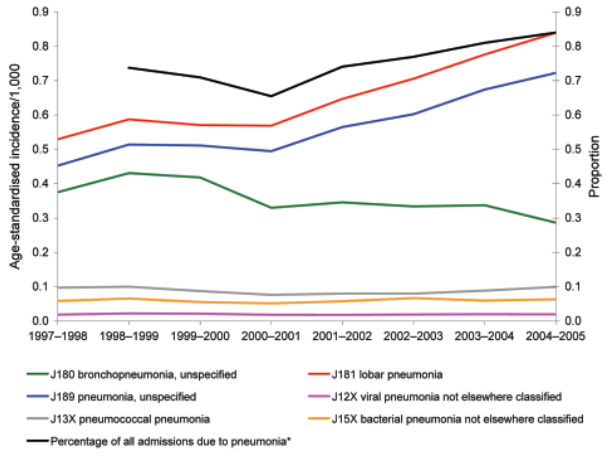

Figure 1. Trends in age-standardized incidence of hospital admission with a primary diagnosis of pneumonia-specific International Classification of Diseases (10th revision) codes, by Hospital Episode Statistics year (April to March). *Additional data on percentage of all admissions due to pneumonia published by the Information Centre for Health and Social Care (www.ic.nhs.uk). Data not available for 1997–98.

Pneumonia in Any Diagnosis

The number of hospitalizations for which pneumonia was listed in any diagnostic field increased from 110,143 to 153,312; the equivalent age-standardized rate increased 32%, from 2.26 per 1,000 to 2.98 per 1,000 population over the same period. The trends in the rate of hospitalization with at least 1 pneumonia episode per year were very similar when we compared pneumonia as a primary diagnosis with any listed diagnosis (data not shown).

Coexisting Conditions

The proportion of patients with a primary diagnosis of pneumonia and coexisting conditions (defined by Charlson Comorbidity Index score) varied over time and by age (Figure 2). In all age groups, the proportion of patients with coexisting conditions increased between 1997–98 and 2004–05. The median number of ICD-10 diagnoses recorded increased from 2 in 1997–98 to 3 in 2004–05 in patients with a primary diagnosis of pneumonia, and from 3 to 4 in those with pneumonia in any diagnostic field. In each year, <1% of patients overall (range 0.5%–1%) had an additional alcohol-related code recorded. These codes were slightly more common (range 0.9%–1.9%) in those <65 years of age compared to those >65 years of age (range 0.3%–0.5%).

Length of Stay

Length of stay was recorded in HES for 73% of admissions. Where reported, the median duration of stay (all ages) for those with a primary diagnosis of pneumonia was 5 days for all years apart from 1999–2000 (6 days, $p<0.001$ compared to 1997–98) and 2004–05 (4 days, $p<0.001$ compared to 1997–98). For all-cause admissions, the median length of stay was 2 days each year in the study period. The median duration of stay in hospital increased with age. For those with a primary diagnosis of pneumonia, length of stay was 3 days for patients <65 years of age, 6 days for those 65–74 years, 8 days for those 75–84 years, and 9 days for those ≥85 years ($p<0.001$).

Admission Patterns

To assess any possible changes in admission practices and patterns, we analyzed the proportion of weekend admissions compared to those at midweek. Overall, the proportion of admissions with a primary diagnosis of pneumonia that occurred on a Saturday or Sunday only changed from 23% to 24% between 1997–98 and 2004–05. In adults ≥85 years of age, the increase in weekend admissions was

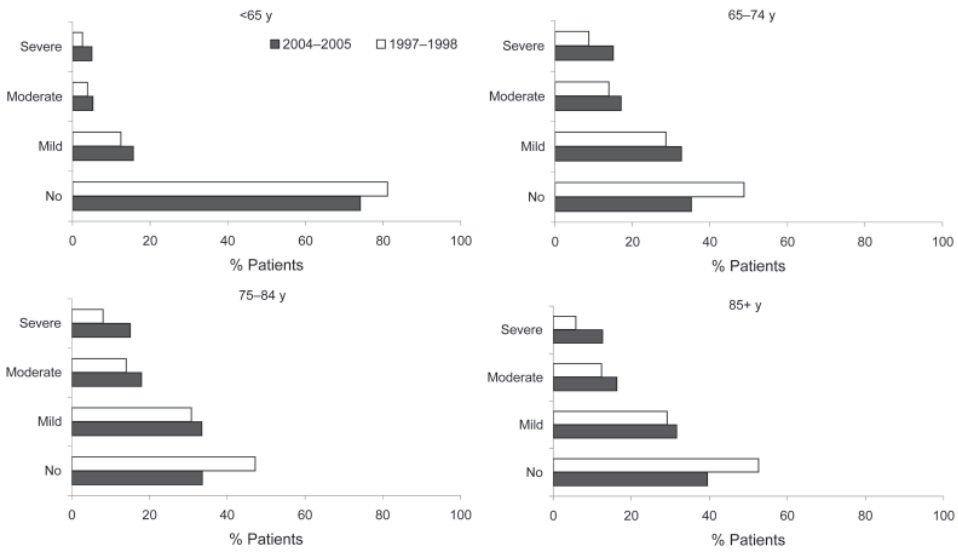

Figure 2. Percentage of patients admitted to hospital with a primary diagnosis of pneumonia with coexisting conditions, as defined by using the Charlson Comorbidity Index, by age group.

slightly greater, increasing from 22.5% in 1997–98 to 25.2% in 2004–05 (p<0.001).

A clear seasonal variation in admissions for pneumonia occurred; incidence was highest in the winter months. Seasonal patterns were similar for those <65 of age and those ≥65 years of age (online Appendix Figure 2, available from www.cdc.gov/EID/content/14/5/727-appG2.htm).

Mortality Rates

The crude 30-day in-hospital mortality rates for all ages were fairly stable over the study period, declining slightly in older adults (Figure 3). Mortality rates were higher in the older age groups; in adults ≥85 years of age; 47% were reported to have died in the hospital within 30 days of admission with a primary diagnosis of pneumonia over the study period.

After age, sex, and Charlson Comorbidity Index score were controlled for, the odds of death within 30 days were significantly higher in 1998–99 and 1999–2000 compared to 1997–98, and significantly lower from 2000–01 onwards (Table 2). The odds of death were lowest in 2004–05, although this finding may partly reflect censoring of the data at the end of the study period. The odds of death rose with severity of the Charlson Comorbidity Index score and with increasing age (Table 2).

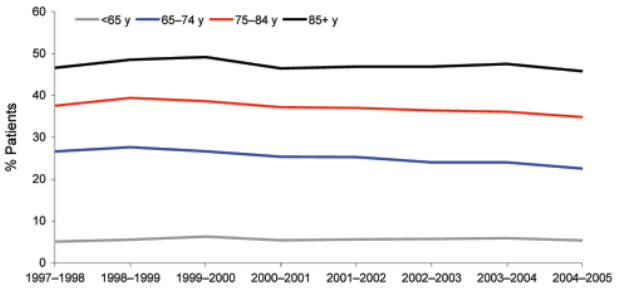

Figure 3. Percentage of patients admitted with a primary diagnosis of pneumonia who died in hospital with pneumonia within 30 days of their first pneumonia admission, by Hospital Episode Statistics year (April to March).

Discussion

The age-standardized incidence of hospital admissions for pneumonia in England increased 34% between 1997–98 and 2004–05. The increase occurred particularly in nonspecific codes—lobar pneumonia, unspecified; and pneumonia, unspecified—and was more marked among older age groups. The proportion of patients with recorded coexisting conditions increased over the study period; alcohol-related codes were recorded infrequently. In-hospital deaths among patients admitted with pneumonia were high, particularly in the most elderly and those with severe coexisting conditions. After adjustment, the odds of death were lower in more recent years compared to 1997–98.

The magnitude of the increase in hospital admission is similar to that reported in the United States (*1*) and Denmark (*3*). Our findings also correspond with the reported rise in the extent of pneumonia in adult intensive care units in the United Kingdom, where community-acquired pneumonia admissions rose by 128% between 1996 and 2004, compared to a rise in total admissions of 24% (*14*). In contrast to hospital admissions, reports from primary care show that consultations for respiratory tract infections as a whole have markedly decreased (*15*,*16*) and that the overall mean weekly incidence of "pneumonia and pneumonitis" decreased from 3.69 per 100,000 in 1997 to 1.39 per 100,000 in 2005 (data from the Birmingham Research Unit of the Royal College of General Practitioners, www.rcgp.org.uk/bru_/bru_home.aspx). In adults ≥75 years of age, the mean weekly incidence declined from 16.9 and 14.6 per 100,000 (in men and women, respectively) in 1999 to 6.5 and 5.5 per 100,000 in 2005.

Whether the decline in the case-fatality rate over the study period is due to improved care leading to better outcomes, or to patients with less severe pneumonia being admitted, is not clear. The number of ONS-registered deaths with pneumonia as an underlying cause in England has fallen since 2001, but this is thought to be primarily due to changes in the rules for classifying deaths and the switch from ICD-9 to ICD-10 coding in 2001 (*17*). A reduction in the length of stay in hospital may also indicate that less severe case-patients are being admitted to hospital, but we found only slight variation in the median length of stay over time (i.e., 6 days vs. 4 days).

The rise in pneumonia hospitalizations may be attributable to population factors, changes in HES coding, changes to health service organization, other biologic phenomenon, or a combination of these effects. We assessed some of these factors with our database, and we discuss possible explanations for the observed trends in pneumonia below.

Age, coexisting conditions, and residence in nursing homes are among the established risk factors for community-acquired pneumonia (*18*). The United Kingdom has an aging population; ONS population statistics for 1997 through 2004 show a 13% increase in the number of adults in England >80 years of age and a 25% increase in those >90 years. To control for this fact, we present our results as age-standardized incidence, although we cannot take into account other secular trends, such as increasing numbers of elderly persons living alone with little support. The proportion of patients with reported coexisting conditions increased over the study period in all age groups. This may reflect a true increase in the prevalence of heart disease, diabetes, and other conditions in the population (*19*), but it may also be a reflection of improved coding in HES, as the median number of diagnoses per patient increased over

Table 2. Odds of death within 30 days of admission for hospitalized patients with a primary diagnosis of pneumonia*

Variable	No. deaths (%)	Adjusted odds ratio† (95% CI)
Year (April to March)		
1997–98	17,743 (24.8)	1.00
1998–99	22,152 (27.2)	1.07 (1.05–1.10)
1999–2000	22,425 (28.2)	1.07 (1.04–1.10)
2000–01	19,392 (26.1)	0.94 (0.92–0.97)
2001–02	22,198 (26.5)	0.93 (0.90–0.95)
2002–03	23,352 (26.2)	0.89 (0.87–0.91)
2003–04	25,946 (26.7)	0.88 (0.86–0.90)
2004–05	25,379 (25.0)	0.80 (0.78–0.82)
Age, y		
<65	13,576 (5.6)	1.00
65–74	25,349 (25.1)	4.34 (4.25–4.44)
75–84	67,526 (37.0)	7.77 (7.61–7.93)
≥85	72,136 (47.2)	12.81 (12.55–13.08)
Sex		
M	87,132 (25.2)	1.00
F	91,455 (27.6)	0.97 (0.96–0.98)
Comorbidity index		
None	63,254 (16.9)	1.00
Mild	54,030 (31.9)	1.59 (1.56–1.61)
Moderate	33,598 (42.9)	2.47 (2.42–2.51)
Severe	27,705 (50.1)	3.72 (3.65–3.80)

*Results from a multivariable logistic regression. CI, confidence interval.
†Model includes year, age, sex, and coexisting conditions.

the study period. Because the residential status of patients is not recorded in HES, we could not analyze the impact of place of residence directly, but there has been little recent change in the numbers and proportions of elderly persons living in residential care (*20*).

HES is essentially an administrative database; nevertheless, it has been widely used for epidemiologic studies. Diagnoses are recorded by coding clerks who review patient case notes, rather than by the attending physician, and some variability in the quality and consistency of coding is likely. We attempted to control for this variability by principally reporting on case-patients with pneumonia as a primary diagnosis. We also demonstrated that the proportion of admissions attributed to pneumonia increased over the period. Nevertheless, we have not attempted to verify the accuracy of the diagnoses recorded in HES. Since our analyses are based on the first admission for pneumonia in a year, most cases are likely to be community-acquired, but we have not excluded some potential sources of hospital-acquired pneumonia, e.g., patients recently hospitalized for reasons other than pneumonia before their pneumonia episode.

Changes in healthcare organization in England may have contributed to some of the increase in admissions and may help to explain the contrasting trends in hospital admission and primary care consultations. General practitioners are no longer obliged to provide after-hours care, so patients with pneumonia may go directly to hospital, or be seen by an unfamiliar doctor who may be more likely than the patient's usual general practitioner to admit him or her to hospital. The proportion of admissions occurring on a weekend (a proxy for after-hours admissions because HES only contains information on the date of admission and not the time) changed little overall, and only increased slightly, from 22.5% to 25.2%, between 1997–98 and 2004–05 for adults ≥85 years. The high in-hospital death rate observed in this age group may also reflect people's unwillingness to have a patient die at home; data on place of death suggest that the percentage of deaths (from all causes) in England and Wales that occurs outside a hospital or other medical establishment fell slightly, from 30.6% in 1998 to 25.9% in 2004 (*21*). The level of care available to elderly patients has also improved, as evidenced by the introduction of the National Service Framework for older people in 2001 and an increasing number of specialists in geriatric medicine (*22*). Expectations of patients and families may also be changing. Organizational changes may be contributing to the observed trends; however, similar trends have been observed in other countries, so this is unlikely to be the sole explanation.

A range of organisms are implicated in the etiology of community-acquired pneumonia. These include *S. pneumoniae*, *M. pneumoniae*, *H. influenzae*, *Chlamydia* species, *Legionella* species, *Staphylococcus aureus,* and respiratory viruses (influenza, respiratory syncytial virus [RSV], adenovirus, parainfluenza) (*6*,*23*,*24*). In this study, only 6% of hospital admissions had a specific pathogen identified in the primary diagnostic code. The absence of microbiologic data in these cases means that indirect methods must be used to investigate the underlying etiology. For example, Muller-Pebody et al. (*25*) used seasonal regression models to estimate that 42% of hospital admissions for unspecified pneumonia were attributable to *S. pneumoniae*, 10% to influenza, 9% to *H. influenzae*, 7% to *Bordetella pertussis,* and 5% to RSV. Further analysis is required to investigate whether such model estimates are similar when more recent HES data are used.

Another factor to consider is that community prescribing of antimicrobial agents has decreased substantially in the United Kingdom in recent years (*26*). Reduced usage of antimicrobial agents in general practice may be related to increased pneumonia deaths (*27*). The cause of this reduction in usage of antimicrobial agents, either as a result of higher prescribing thresholds or fewer consultations for respiratory illness, has been debated (*16*,*27–29*). In either case, a plausible argument can be made that reduced use of these agents may result in increased or prolonged (asymptomatic) carriage and thus transmission of common respiratory pathogens that cause pneumonia in the population. This ecologic effect is difficult to substantiate, and reductions in antimicrobial agents seem to predate the rise in pneumonia hospitalizations. Nonetheless, international

comparisons of pneumonia hospitalizations and drug-prescribing trends may be enlightening.

The increase in pneumonia admissions has occurred despite increasing coverage for influenza and pneumococcal vaccinations in the elderly (*30*,*31*). Since 1992, a 23-valent pneumococcal polysaccharide vaccine (PPV23) has been recommended in England for persons at high risk, and in 2003, this recommendation was extended to include all persons ≥65 years of age. This universal program was phased in over 3 years, targeting those ≥80 years of age from August 2003, ≥75 years of age from April 2004, and ≥65 years of age from April 2005. Although PPV23 offers a modest level of protection against invasive disease, the vaccine appears to have little benefit against pneumonia (*7*). A person's vaccination status is not recorded in HES so we could only explore the association between PPV23 and pneumonia at an ecologic level. We did not observe any associations between the trends of PPV23 coverage and overall pneumonia incidence (ICD-10 codes J12–J18). PPV23 coverage increased steadily from 1995 onwards (*30*); from 2003 onwards, coverage increased in the age groups targeted in the universal program. By contrast, the incidence of pneumonia increased in all age groups from 2001–02 onwards. The trend in pneumococcal pneumonia (ICD10 code J13X), which is most likely to represent confirmed pneumococcal pneumonia, was stable, suggesting that PPV23 had little influence. The experience of the United States leads us to expect that the introduction of the 7-valent pneumococcal conjugate vaccine (PCV7) into the United Kingdom infant immunization schedule in September 2006 will result in a reduction in the transmission of pneumococcal vaccine serotypes and subsequent reductions in invasive (*32–34*), and to a lesser extent noninvasive, pneumococcal disease (including pneumonia) (*10*) even in unvaccinated persons. Analysis of temporal trends in *Pneumococcus*-attributable illnesses pre- and post-PCV7 introduction will help to quantify these effects in England.

Conclusion

We have observed a 34% increase in the incidence of hospital admissions for pneumonia in England in recent years, particularly in older adults. We believe this increase is real; however, the trends are not fully explained by an aging population, rising prevalence of coexisting conditions, or coding changes. Further research is required to understand the reasons for the increase in pneumonia hospitalizations so that the most appropriate interventions can be determined.

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References

1. Fry AM, Shay DK, Holman RC, Curns AT, Anderson LJ. Trends in hospitalizations for pneumonia among persons aged 65 years or older in the United States, 1988–2002. JAMA. 2005;294:2712–9.
2. Hebert PL, McBean AM, Kane RL. Explaining trends in hospitalizations for pneumonia and influenza in the elderly. Med Care Res Rev. 2005;62:560–82.
3. Thomsen RW, Riis A, Norgaard M, Jacobsen J, Christensen S, McDonald CJ, et al. Rising incidence and persistently high mortality of hospitalized pneumonia: a 10-year population-based study in Denmark. J Intern Med. 2006;259:410–7.
4. Oosterheert JJ, Bonten MJ, Hak E, Lammers JW, Schneider MM, Hoepelman IM. The increase in pneumonia-related morbidity and mortality among adults in the Netherlands and possible explanations for it [in Dutch]. Ned Tijdschr Geneeskd. 2004;148:1765–9.
5. Brown PD, Lerner SA. Community-acquired pneumonia. Lancet. 1998;352:1295–302.
6. Woodhead M. Community-acquired pneumonia in Europe: causative pathogens and resistance patterns. Eur Respir J Suppl. 2002;36:20s–7s.
7. Mangtani P, Cutts F, Hall AJ. Efficacy of polysaccharide pneumococcal vaccine in adults in more developed countries: the state of the evidence. Lancet Infect Dis. 2003;3:71–8.
8. Donaldson L, Beasley C, Howe J. Planned changes to the routine childhood immunisation programme. 2006 Feb 8 [cited 2008 Feb 27]. Available from http://www.dh.gov.uk/assetRoot/04/12/81/21/04128121.pdf
9. Centers for Disease Control and Prevention. Direct and indirect effects of routine vaccination of children with 7-valent pneumococcal conjugate vaccine on incidence of invasive pneumococcal disease—United States, 1998–2003. MMWR Morb Mortal Wkly Rep. 2005;54:893–7.
10. Grijalva CG, Nuorti JP, Arbogast PG, Martin SW, Edwards KM, Griffin MR. Decline in pneumonia admissions after routine childhood immunisation with pneumococcal conjugate vaccine in the USA: a time-series analysis. Lancet. 2007;369:1179–86.
11. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis. 1987;40:373–83.
12. Charlson M, Szatrowski TP, Peterson J, Gold J. Validation of a combined comorbidity index. J Clin Epidemiol. 1994;47:1245–51.
13. de Groot V, Beckerman H, Lankhorst GJ, Bouter LM. How to measure comorbidity. a critical review of available methods. J Clin Epidemiol. 2003;56:221–9.
14. Woodhead M, Welch CA, Harrison DA, Bellingan G, Ayres JG. Community-acquired pneumonia on the intensive care unit: secondary analysis of 17,869 cases in the ICNARC Case Mix Programme Database. Crit Care. 2006;10(Suppl 2):S1.

15. Fleming DM, Ross AM, Cross KW, Kendall H. The reducing incidence of respiratory tract infection and its relation to antibiotic prescribing. Br J Gen Pract. 2003;53:778–83.
16. Health Protection Agency. A winter's tale: coming to terms with winter respiratory illnesses. London: Health Protection Agency; 2005 [cited 2008 Feb 27]. Available from http://www.hpa.org.uk/publications/2005/winters_tale/winters_tale_revised_may05.pdf
17. Brock A, Griffiths C, Rooney C. The impact of introducing ICD-10 on analysis of respiratory mortality trends in England and Wales. Health Stat Q. 2006;29:9–17.
18. Loeb M. Pneumonia in older persons. Clin Infect Dis. 2003;37:1335–9.
19. National Centre for Social Research, Department of Epidemiology and Public Health at the Royal Free and University College Medical School. Health Survey for England 2003. Sproston K, Primatesta P, editors. London: Department of Health; 2004 [cited 2008 Feb 27]. Available from http://www.dh.gov.uk/en/Publicationsandstatistics/Publications/PublicationsStatistics/DH_4098712
20. Department of Health. Community care statistics. Supported residents (adults), England. London: The Department; 2004 [cited 2008 Feb 27]. Available from http://www.dh.gov.uk/PublicationsAndStatistics/Statistics/StatisticalWorkAreas/StatisticalSocialCare/StatisticalSocialCareArticle/fs/en?CONTENT_ID=4096247&chk=F5cFKe
21. Mortality Statistics Series DH. 1 – annual review of the Registrar General on deaths in England and Wales. London: Office for National Statistics; 2007 [cited 2008 Feb 27]. Available from http://www.statistics.gov.uk/statbase/explorer.asp?CTG=3&SL=&E=4250#4250
22. Department of Health. Better health in old age. Report from Professor Ian Philp, National Director for Older People's Health to Secretary of State for Health. London: The Department; 2004 [cited 2008 Feb 27]. Available from http://www.dh.gov.uk/en/Publicationsandstatistics/Publications/PublicationsPolicyAndGuidance/DH_4092957
23. Jokinen C, Heiskanen L, Juvonen H, Kallinen S, Kleemola M, Koskela M, et al. Microbial etiology of community-acquired pneumonia in the adult population of 4 municipalities in eastern Finland. Clin Infect Dis. 2001;32:1141–54.
24. Ruiz M, Ewig S, Marcos MA, Martinez JA, Arancibia F, Mensa J, et al. Etiology of community-acquired pneumonia: impact of age, comorbidity, and severity. Am J Respir Crit Care Med. 1999;160:397–405.
25. Muller-Pebody B, Crowcroft NS, Zambon MC, Edmunds WJ. Modelling hospital admissions for lower respiratory tract infections in the elderly in England. Epidemiol Infect. 2006;134:1150–7.
26. Sharland M, Kendall H, Yeates D, Randall A, Hughes G, Glasziou P, et al. Antibiotic prescribing in general practice and hospital admissions for peritonsillar abscess, mastoiditis, and rheumatic fever in children: time trend analysis. BMJ. 2005;331:328–9.
27. Price DB, Honeybourne D, Little P, Mayon-White RT, Read RC, Thomas M, et al. Community-acquired pneumonia mortality: a potential link to antibiotic prescribing trends in general practice. Respir Med. 2004;98:17–24.
28. Ashworth M, Latinovic R, Charlton J, Cox K, Rowlands G, Gulliford M. Why has antibiotic prescribing for respiratory illness declined in primary care? A longitudinal study using the General Practice Research Database. J Public Health (Oxf). 2004;26:268–74.
29. Ashworth M, Charlton J, Latinovic R, Gulliford M. Age-related changes in consultations and antibiotic prescribing for acute respiratory infections, 1995–2000. Data from the UK General Practice Research Database. J Clin Pharm Ther. 2006;31:461–7.
30. Noakes K, Pebody RG, Gungabissoon U, Stowe J, Miller E. Pneumococcal polysaccharide vaccine uptake in England, 1989–2003, prior to the introduction of a vaccination programme for older adults. J Public Health (Oxf). 2006;28:242–7.
31. Joseph C, Goddard N, Gelb D. Influenza vaccine uptake and distribution in England and Wales using data from the General Practice Research Database, 1989/90–2003/04. J Public Health (Oxf). 2005;27:371–7.
32. Whitney CG, Farley MM, Hadler J, Harrison LH, Bennett NM, Lynfield R, et al. Decline in invasive pneumococcal disease after the introduction of protein-polysaccharide conjugate vaccine. N Engl J Med. 2003;348:1737–46.
33. Poehling KA, Talbot TR, Griffin MR, Craig AS, Whitney CG, Zell E, et al. Invasive pneumococcal disease among infants before and after introduction of pneumococcal conjugate vaccine. JAMA. 2006;295:1668–74.
34. Lexau CA, Lynfield R, Danila R, Pilishvili T, Facklam R, Farley MM, et al. Changing epidemiology of invasive pneumococcal disease among older adults in the era of pediatric pneumococcal conjugate vaccine. JAMA. 2005;294:2043–51.

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Increasing Incidence of Listeriosis in France and Other European Countries

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From 1999 through 2005, the incidence of listeriosis in France declined from 4.5 to 3.5 cases/million persons. In 2006, it increased to 4.7 cases/million persons. Extensive epidemiologic investigations of clusters in France have ruled out the occurrence of large foodborne disease outbreaks. In addition, no increase has occurred in pregnancy-associated cases or among persons <60 years of age who have no underlying disease. Increases have occurred mainly among persons ≥60 years of age and appear to be most pronounced for persons ≥70 years of age. In 8 other European countries, the incidence of listeriosis has increased, or remained relatively high, since 2000. As in France, these increases cannot be attributed to foodborne outbreaks, and no increase has been observed in pregnancy-associated cases. European countries appear to be experiencing an increased incidence of listeriosis among persons ≥60 years of age. The cause of this selective increased incidence is unknown.

Surveillance methods for listeriosis vary across Europe (*1*). Reported incidence of listeriosis ranged from 0 to 7.5 cases/million persons in 2002; the highest rates were reported from countries with statutory reporting of cases and surveillance through a national reference laboratory (*1*). In recent years, interest in developing a European surveillance network for listeriosis has led to enhanced surveillance activities in several countries and has generally heightened awareness of the public health importance of *Listeria monocytogenes*. The epidemiologic picture that has emerged from recent national surveillance reports suggests that rates of listeriosis across Europe have been increasing or have remained stable at relatively high levels (*2*). In contrast, the incidence of listeriosis in France declined from 4.5 cases/million persons during 1999–2000 to ≈3.5 cases/million during 2001–2003 (*3*).

In 2006, however, France reported an increase in the incidence of listeriosis. This increase appears to share many of the epidemiologic features of recent increases in other European countries. We describe the emerging epidemiology of listeriosis in France and discuss it in the context of recent increases in other European countries.

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Methods

Data Collection from France

Surveillance of human listeriosis in France has been conducted with consistent methods since 1999 (*4*). These methods include mandatory reporting of cases (monitored by Institut de Veille Sanitaire [InVS]) and voluntary submission of *L. monocytogenes* strains to the National Reference Center for Listeria, Institut Pasteur, Paris (NRC). By using these complementary approaches, information about clinical data, demographic data, and food consumption can be collected for each patient. In addition, temporal trends in disease occurrence can be tracked, and possible common sources of exposure can be identified among cases in clusters detected by NRC.

Definitions

A case of listeriosis was defined by isolation of *L. monocytogenes* from a patient with a clinically compatible illness. A case is considered maternal/neonatal when it involves a pregnant woman, a miscarriage, a stillbirth, or a newborn <1 month of age. *L. monocytogenes* isolated from both a pregnant woman and her newborn child is counted as a single case. If a case fits none of these groups, it is considered not maternal/neonatal. Patients are considered to be at risk if they have an underlying pathologic condition

that weakens their immune system, such as cancer, blood malignancy, organ transplant, chronic hemodialysis, liver failure, diabetes, HIV, or treatment with cytolytic or corticosteroid immunosuppressants. A cluster was defined as the occurrence of at least 3 listeriosis cases that involved strains of the same pulsovar over a period of 1) 14 weeks during January 2000–July 2006 or 2) 6 weeks after July 2006. Cases not belonging to a cluster were defined as sporadic cases.

Mandatory reporting includes submission of case-related information on a standard report form. This form includes information such as the patient's district of residence, patient's age, the clinical form of disease, the possible existence of an underlying illness, and whether the patient died before follow-up. A standard questionnaire is administered in person or by telephone to ascertain food items consumed in the 2 months before onset of illness, including food items previously identified as vehicles in outbreaks and foods that have been previously found to be contaminated by *L. monocytogenes* and typically consumed uncooked. Answers are entered into a food-exposure database.

Analysis of Strains by NRC

Listeria isolates from patients and foods referred to NRC were confirmed with API Listeria (bioMérieux, Marcy l'Etoile, France) and serotyped by the classic technique until January 2005 (*5*) and by multiplex PCR (*6*) since February 2005. According to our experience, PCR group fully corresponds to the 4 major serovars that cause human disease. Ongoing subtyping was conducted by DNA macrorestriction profiles analysis (pulsed-field gel electrophoresis) according to standard protocols (*7*). Isolates with indistinguishable Apa1 and Asc1 DNA macrorestriction profiles, first based on visual comparison of banding patterns (since 2006 by using BioNumerics 4.5 software [Applied Maths Saint-Martens-Latem, Belgium]), were considered to be the same pulsovar. Detected clusters were reported to InVS for investigation (*3*).

Data Analysis

Data analysis was performed with Epi-Info version 6.04 (Centers for Diseases Control and Prevention, Atlanta, GA, USA). Incidence rate ratios (RRs) were calculated with Stata version 8.2 (StatCorp, College Station, TX, USA). For the data from France, we compared incidence rates in 2006 and 2007 with the mean incidence over the precedent 5-year period (2001–2005). To estimate the incidence data for 2007 by extrapolation of the incidence observed from January to June 2007, we multiplied the incidence from January to June by a factor of 1.2, which represents the mean annual multiplier (annual cases/cases January–June) observed from 2001 through 2006.

Data from Europe

We conducted an Internet search for surveillance data from the institutes in charge of infectious disease surveillance in Western European countries. We also reviewed articles published on listeriosis trends in European countries with data for 2000–2006.

Results

Data from France

Epidemiologic Characteristics

The annual incidence of listeriosis in France decreased in 2001 (Table 1) and stabilized until 2005 at ≈3.5 cases/million persons. In 2006, the incidence increased to 4.7 cases/million persons. In 2007, 159 cases were reported from January through June, which corresponds to an estimated incidence of 5.6 cases/million persons. For the 6-month period from January through June, the incidence of listeriosis increased by 46% in 2006 and 2007 compared with incidence during 2001–2005 (RR 1.4; 95% confidence interval [CI] 1.2–1.6; p<0.001).

The increased incidence of listeriosis in 2006–2007 over that of 2001–2005 was mainly due to a rise in cases in persons ≥60 years of age (+51%; RR 1.6; 95% CI 1.4–1.8; p<0.001) and was most pronounced in those ≥75 years of age (+58%; RR 1.7; 95% CI 1.4–2.1; p<0.001) (Figure 1). This increase was observed in persons ≥60 years of age, regardless of whether they had a recognized underlying condition. The mortality rates among these cases did not increase. From 2001–2005 to 2006 and 2007, there was a larger overall increase in bacteremia cases (+67%) than in central nervous system cases (+35%).

Table 1. Incidence and characteristics of cases of listeriosis by year, France

Characteristic	1999	2000	2001	2002	2003	2004	2005	2006
No. cases reported	269	263	188	220	209	236	221	290
Incidence/1 million inhabitants	4.5	4.4	3.1	3.6	3.4	3.8	3.5	4.6
Clinical form								
Maternal/neonatal	67	64	44	55	47	49	39	36
Not maternal/neonatal	202	199	144	165	162	187	182	254
Bacteriema	122	110	85	89	100	124	115	171
Central nervous system infection	65	73	51	67	54	53	60	65
Focal infection	15	16	8	9	8	10	7	18

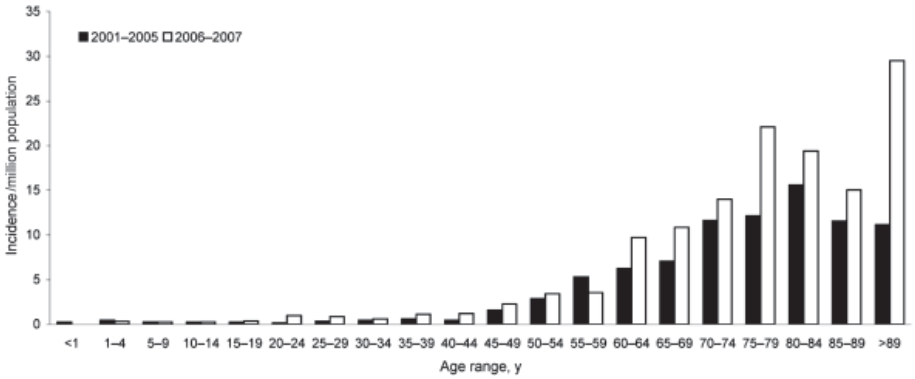

Figure 1. Trends of non–maternal/neonatal listeriosis by age, France, January 1, 2006–June 30, 2007, versus January 1, 2001–December 31, 2005.

The regional distribution of cases did not differ significantly from 1 region to another during 2006 and 2007 versus during 2001–2005. The increase was similar for sporadic cases and cluster-associated cases (Table 2). A seasonal effect, with an increase of cases during summer, was observed in 2006, as in preceding years. However, information about food consumption of patients ≥60 years of age showed that a decrease in consumption of foods considered to pose a risk for listeriosis occurred in 2006–2007 compared with 2001–2005.

For persons <60 years of age, a 32% increase was observed only in patients with an underlying condition that increased their risk for listeriosis (Figure 2), particularly patients with leukemia (Figure 3). The incidence of maternal/neonatal cases of listeriosis has decreased continuously from 1999 to 2006, with the same trend in the first half of 2007. In 2006, maternal/neonatal cases represented 12% of cases.

Strains Analysis

In 2006, NRC received 280 *L. monocytogenes* strains, which accounted for 96.5% of cases reported to InVS. The distribution of strains by serovar/PCR group did not change from 2001 through 2006 (Table 3). The most common serovar was 4b, which accounted for half of all strains. Analysis of PCR group for strains received during the first 6 months in 2007 shows the same results in terms of distribution. As in previous years (*3*), serovar 4b was predominant among maternal/neonatal cases and central nervous system infection cases and more frequent than among bacteremia cases (Table 4).

Cluster Detection and Investigations

In 2006, 102 pulsovars were identified among the 280 isolates, with 1–30 isolates/pulsovar (Table 5). Pulsed-field gel electrophoresis analysis identified 11 clusters: 9 involving strains of PCR group IVb and 2 of PCR group IIa (Table 5). Results of intensive epidemiologic investigation did not show any cluster suggestive of common-source outbreaks. In 1 cluster involving 14 cases, an *L. monocytogenes* strain of the case-associated pulsovar was identified in a sheep raw milk cheese that had been consumed by 3 patients but not by the other patients in the cluster. The proportion of strains related to a cluster in 2006 (34%) was similar to that in 2003–2005 (35%) (Table 2).

Data from Europe

Complete annual incidence data from 2000 through 2006 were available for 5 European countries (Table 6). In 2000, the median incidence was 4.7 cases/million persons (range 1.9–7.5 cases/million persons); in 2006, it was 6.3 cases/million persons (range 3.5–10.3 cases/million persons) in these countries. Increases were observed for Belgium, Denmark, England, Wales, and Finland. In Sweden, the incidence decreased during 2000–2006. However, the incidence in Sweden was already high in 2000–2001 (5.9–7.5 cases/million persons). Incidence data for at least 5 years during this period were also available for Germany, the Netherlands, and Switzerland. They all observed increases over this period.

In England and Wales, Gillespie et al. compared 2 periods: 1990–2000 and 2001–2004 (*8*). They showed that the increase resulted from a rise in sporadic cases, predominantly in patients ≥60 years of age. The increase was independent of sex, ethnicity, or economic status and occurred in most regions of England and Wales. The proportion of serovar 4b and 1/2 isolates and the proportion of persons with underlying illness did not change during this period. The proportion of bacteremic patients ≥60 years of age increased significantly during 2001–2004 versus 1990–2000

Table 2. Clusters of listeriosis, cluster-associated cases, and sporadic cases, France, 2000–2006

Characteristic	2000	2001	2002	2003	2004	2005	2006
No. clusters detected	9	4	10	11	13	11	11
No. cases belonging to a cluster	53	21	70	78	88	65	98
No. sporadic cases	210	167	150	131	148	156	192

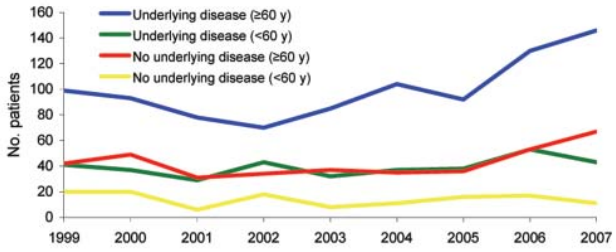

Figure 2. Trends of non–maternal/neonatal listeriosis by presence of underlying disease and age of patients, France, January 1, 1999–June 30, 2007. (Data for 2007 are estimated.)

(85% vs. 76%); after 2000, the risk among persons ≥70 years of age was higher than that for persons 60–69 years of age. During the periods compared by Gillespie et al., the incidence of maternal/neonatal cases did not change.

In Germany, surveillance data showed a continuous increase of cases since 2001, when the national reporting system was introduced. A particularly steep increase was observed in 2005, when the number of cases increased by 72%. Analyzing this increase, Koch and Stark reported that temporal and spatial distribution of cases did not show any clusters suggestive of local outbreaks (*9*). From 2001 through 2005, the number of cases in those ≥60 years of age increased by a factor of 2.6. Among persons ≥80 years of age, almost 4 times as many cases were reported in 2005 as in 2001. In the same period, the annual number of maternal/neonatal cases did not change.

In the Netherlands, until 2005, information was based on data from 15 regional public health laboratories that cover an estimated 44% of the population and from the Netherlands Reference Laboratory for Bacterial Meningitis, which receives isolates from patients with meningitis or septicemia. According to these data, the annual incidence of listeriosis had been stable until 2002 at ≈2 cases/million persons and has increased since 2003 to ≈3 cases/million persons (*10*). Since 2005, an active surveillance that involved all laboratories has been implemented. Cases reported by Doorduyn et al. in 2005 (*10*) corresponded to an incidence of 5.6/million persons (Table 6). Although this increase may have resulted from the strengthening of listeriosis surveillance, the authors do not rule out a genuine recent increase in the number of cases.

In Switzerland, an increase has been observed since 2004. In 2005, an outbreak involving 12 cases (16% of cases reported in 2005) was linked to consumption of a cheese, accounting only for a small part of the upsurge (*11*). In 2006, the incidence remained high (9.1/million persons), although no common-source outbreak was identified.

In Denmark, an increase since 2004 was caused by various subtypes of *L. monocytogenes;* this increase was likely not the consequence of a single outbreak (*12*). This increase involved septicemia cases but not meningitis cases. A further increase was observed in 2006, leading to an incidence of 10.3 cases/million persons.

In Finland, an increase has been observed since 2003. Clusters are detected by routine serogenotyping of strains. In 2003–2004, 2 clusters with 7 cases each were investigated. In 1 cluster, food histories implicated cold fish products (*13*). Clinical and demographic characteristics of cases occurring in 2003–2004 and those occurring in 1999–2000 did not differ.

In Belgium, the incidence has increased since 2003 and reached a peak of 8.6 cases/million persons in 2004 (*14*). The large increase in 2004 was mainly caused by increasing cases in the Flemish community, and the proportion of isolates of serovar 1/2 was unusually high (68%). Therefore, the occurrence of a common-source outbreak in 2004 cannot be ruled out.

Discussion

Surveillance data in France show an upsurge in human listeriosis cases in 2006; this increase was confirmed in January–June 2007 such that the annual incidence is now at its highest level since 1998, when mandatory reporting was introduced. This upsurge is due to an increase among persons ≥60 years of age who were not pregnant and among persons <60 years of age who had a predisposing medical condition.

The methods and conditions of listeriosis surveillance in France have remained unchanged since 1998, and the sensitivity of the mandatory reporting system is high (*15*). In the 1990s, large outbreaks of listeriosis increased awareness among physicians and microbiologists. Because no

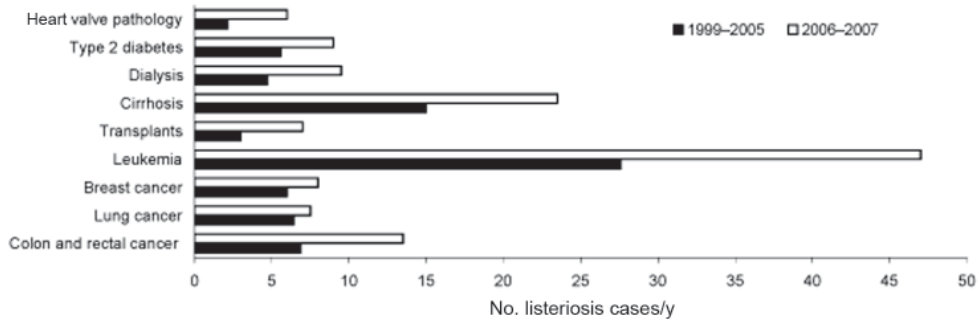

Figure 3. Trends (no. cases of listeriosis/y) by underlying medical condition, France, January 1, 1999–June 30, 2007.

Table 3. Distribution of strains of *Listeria monocytogenes* isolated from human case-patients by serovar, by year

Characteristic	1999	2000	2001	2002	2003	2004	2005	2006	2007*
No. of strains	240	222	186	202	197	233	212	280	151
Serovar 1/2a, %	27	33	33	22	26	30	24	29	27
Serovar 1/2b, %	20	16	22	18	22	11	17	17	15
Serovar 1/2c, %	5	3	3	4	5	4	3	4	4
Serovar 4b, %	48	48	42	55	47	55	56	50	54
Other serovar, %	<1	0	0	<1	0	0	0	<1	<1

*Through Jun 30.

large outbreaks occurred since 2000, this upsurge is most likely not the result of better reporting or raised diagnostic awareness. The increase of bacteremic cases could be an artifact caused by higher hospitalization rates among the elderly, increased frequency of performing blood cultures, or increased sensitivity of the blood-culturing systems. However, from 2005 through 2006, for persons ≥60 years of age, the incidence of listeriosis jumped 39%, the hospitalization rates increased <1%, and data from the national insurance scheme showed a 20% reduction in blood cultures. Because instrumented blood culture systems have been used in hospitals for several years with no demonstrated improvement in the sensitivity of detecting *L. monocytogenes*, the increase of bacteremic infections does not appear to be due to diagnostic practices.

In 2006, the proportion of cases related to clusters remained stable; the clusters did not account for the upsurge in incidence. Also, multiple *L. monocytogenes* strains were responsible for the increase in cases. Because of the above reasons, the increase in incidence in France is unlikely to be due to a common-source outbreak.

In several European countries, similar trends of increasing listeriosis case numbers have been observed. For countries with a long history of listeriosis surveillance, such as England, Wales, Switzerland, and Denmark, the observed upsurge is likely genuine. But even for countries with recently introduced or strengthened listeriosis surveillance systems, such as the Netherlands and Germany, the observed upsurge is not attributed to a surveillance artifact. The upsurge is due to an increase in cases in the same patient groups.

In France, Germany, England, and Wales, the increased incidence occurred predominantly in patients ≥60 years of age. The number of maternal/neonatal cases is declining in all countries. Clusters suspected or confirmed to represent common-source outbreaks contributed to the increased incidence in some countries, such as in Switzerland in 2005, in northeast England in 2003, in Finland in 2003–2004, and possibly the Netherlands and Belgium in 2004. However, in none of these countries did these clusters account for the upsurge in incidence.

Many of these same epidemiologic features may also be occurring in the United States, where a decline in the incidence of listeriosis from 1996 to 2003 was reported on FoodNet websites (*16*). The incidence of listeriosis declined from 4.1 to 2.3/million persons from 1996 to 2003; the percentage of maternal/neonatal cases dropped from 15% to 11% during this period. After dropping to a record low incidence of 2.7 cases/million persons in 2004, the incidence of listeriosis cases reported on FoodNet websites increased to 3.0 cases/million persons in 2005 and 3.1 cases/million in 2006 (*17*).

The reasons for the increased incidence remain unclear. The incidence of listeriosis in France decreased substantially from 1987 through 1997 because of control measures implemented by the food industry in response to several large outbreaks (*15*). After mandatory reporting was implemented, incidence further declined from 4.5 cases/million persons during 1999–2000 to ≈3.5 cases/million persons during 2001–2003. As this reduction concerned all population groups (regardless of whether they were target groups for food recommendations), this further decline was essentially attributed to a reduction in exposure to contaminated food products (*15*). We are not aware of any changes in the control measures used by the food industry that could have increased exposure to contaminated foods in 2006 and 2007. However, in spite of these control measures, we cannot rule out that common food stuffs have been more frequently or more heavily contaminated with *L. monocytogenes* in the past 2 years (2006–2007).

Concerned by the large amount of disease attributable to hypertension-related conditions, in 2002, the French Food

Table 4. Distribution of *Listeria monocytogenes* strains isolated in 2006 from human case-patients, by serovar and clinical forms

Characteristic	No. (%) 1/2a	No. (%) 1/2b	No. (%) 1/2c	No. (%) 4b	No. (%) other	Total no.
Not maternal/neonatal	76 (31)	43 (18)	11 (4)	115 (47)	1 (<1)	246
Central nervous system infection	13 (24)	9 (16)	2 (4)	30 (54)	1 (2)	55
Bacteremia	54 (31)	31 (18)	9 (5)	81 (46)	0 (0)	175
Focal infections	9 (56)	3 (19)	0 (0)	4 (25)	0 (0)	16
Maternal/neonatal	4 (12)	6 (18)	0 (0)	24 (70)	0 (0)	34
Total	80 (29)	49 (17)	11 (4)	139 (50)	1 (<1)	280

Table 5. Description of the diversity, according to serovar, of *Listeria monocytogenes* strains isolated in 2006 for strains belonging to serovars 1/2a, 1/2b, 1/2c, and 4b

Characteristic	1/2a	1/2b	1/2c	4b	Total
No. *L. monocytogenes* strains	80	49	11	139	279
No. pulsovar	42	26	6	28	102
No. pulsovar found once	32	18	4	13	67
Range of frequency for each pulsovar, min–max	1–17	1–10	1–5	1–30	1–30
No. clusters	2	0	0	9	11
Index of diversity	0.931	0.923	0.727	0.893	0.968

Safety Agency recommended a 20% reduction in average salt intake spread over 5 years (*18*). Consequently, the food industry reduced the salt content of selected products, such as ready-to-eat meat products. Evidence from routine food safety investigations indicates that a substantial proportion of ready-to-eat products, such as meat and fish products, may be contaminated by *L. monocytogenes* (*15*). The recently reduced salt content in some of these products, if contaminated, may have contributed to the growth of the organism and increased the likelihood of infection when the products were consumed by susceptible persons. To verify this hypothesis, surveys to determine not only the frequency but also the level of contamination by *L. monocytogenes* of these ready-to-eat food stuffs were initiated in 2008.

In France, we also observed an absolute and relative increase in patients with hematologic malignancies. Improved treatment has likely resulted in an increased number of patients surviving longer with these malignancies. Nevertheless, we are not aware of a sudden and recent increase in the number of these patients that could explain the upsurge. Further investigations are needed to assess whether changes in treatments for these patients may have contributed to an increased susceptibility for illness.

Conclusion

The epidemiology of listeriosis in Europe is changing; the incidence is increasing, and the distribution of cases is shifting, primarily affecting elderly persons and those with predisposing medical conditions. The absence of large outbreaks suggests that there may be increasing exposure to foods that have sporadic or low-level *Listeria* contamination and that have some ability to support growth of *Listeria* organisms. The relative contributions of host and environmental factors need further study.

Acknowledgments

We are indebted to clinicians, biologists, and persons from local health departments, Institut Pasteur, Institut de Veille Sanitaire, Ministry of Health, Ministry of Agriculture, and Ministry of Finance, who contribute to human listeriosis surveillance in France. We thank Edith Laurent, Véronique Vaillant, and Alexandre Leclercq for their help. We thank Bob Adak for fruitful discussions.

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References

1. de Valk H, Jacquet C, Goulet V, Vaillant V, Perra A, Simon F, et al. Surveillance of *Listeria* infections in Europe. Euro Surveill. 2005;10:251–5.

Table 6. Annual incidence of listeriosis in 8 European countries

Country	Source	2000	2001	2002	2003	2004	2005	2006
Belgium	Scientific Institute of Public Health*	4.7	5.6	4.3	7.3	8.6	5.9	6.4†
Denmark	Statens Serum Institut‡	7.5	7.1	5.2	5.4	7.6	8.5	10.3†
England-Wales	Health Protection Agency§	1.9	2.8	2.6	4.5	4.0	3.5	3.5
Finland	National Public Health Institute¶	3.5	5.4	3.8	7.9	6.7	6.8	8.5
Germany	Robert Koch Institute#		2.6	2.9	3.1†	3.6†	6.2	6.2†
Netherlands	National Institute of Public Health**			2	3	3	5.6††	3.9†
Sweden	Swedish Institute for Infectious Disease Control‡‡	5.9	7.5	4.4	5.3	4.7	4.4	4.6
Switzerland	Office Fédéral de la Santé Publique§§		5.1	3.8	6.1	7.8	9.8	9.1

*www.iph.fgov.be/bacterio/iframes/rapports/2005/Listeria_2005_FR_web.pdf.
†www.efsa.europa.eu/EFSA/efsa_locale-178620753812_1178671312912.htm.
‡www.ssi.dk/sw44830.asp.
§www.hpa.org.uk/infections/topics_az/listeria/data_ew.htm.
¶www3.ktl.fi/stat.
#www.eurosurveillance.org/em/v11n06/1106-124.asp.
**www.rivm.nl/infectieziektenbulletin/bul1712/art_listeria.html.
††Introduction of active surveillance.
‡‡www.smittskyddsinstitutet.se/in-english/statistics/listeriosis.
§§www.bag.admin.ch/k_m_meldesystem/00733/00804.

2. Hedberg C. Listeria in Europe: the need for a European surveillance network is growing. Euro Surveill. 2006;11:75–6.
3. Goulet V, Jacquet C, Martin P, Vaillant V, Laurent E, de Valk H. Surveillance of human listeriosis in France, 2001–2003. Euro Surveill. 2006;11:79–81.
4. Goulet V, Jacquet C, Laurent E, Rocourt J, Vaillant V, de Valk H. Surveillance of human listeriosis in France in 1999 [in French]. Bul Epid Heb. 2001;34:161–5.
5. Seeliger HPR, Höhne K. Serotyping of *Listeria monocytogenes* and related species. In: Bergan T, Norris J, editors. Methods in microbiology. New York: Academic Press; 1979. p. 31–48.
6. Doumith M, Buchrieser C, Glaser P, Jacquet C, Martin P. Differentiation of the major *Listeria monocytogenes* serovars by multiplex PCR. J Clin Microbiol. 2004;42:3819–22.
7. Graves LM, Swaminathan B. PulseNet standardized protocol for subtyping *Listeria monocytogenes* by macrorestriction and pulsed-field gel electrophoresis. Int J Food Microbiol. 2001;65:55–62.
8. Gillespie IA, McLauchlin J, Grant KA, Little CL, Mithani V, Penman C, et al. Changing pattern of human listeriosis, England and Wales, 2001–2004. Emerg Infect Dis. 2006;12:1361–6.
9. Koch J, Stark K. Significant increase of listeriosis in Germany–epidemiological patterns 2001–2005. Euro Surveill. 2006;11:85–8.
10 Doorduyn Y, de Jager CM, van der Zwaluw WK, Wannet WJ, van der Ende A, Spanjaard L, et al. First results of the active surveillance of *Listeria monocytogenes* infections in the Netherlands reveal higher than expected incidence. Euro Surveill. 2006;11:E060420.4.
11. Bille J, Blanc DS, Schmid H, Boubaker K, Baumgartner A, Siegrist HH, et al. Outbreak of human listeriosis associated with tomme cheese in northwest Switzerland, 2005. Euro Surveill. 2006;11:91–3.
12 Listeriosis 1998–2005. EPI-NEWS. Copenhagen, Denmark: Statens Serum Institut; 2006. p. 42–3.
13. Lyytikainen O, Nakari UM, Lukinmaa S, Kela E, Nguyen Tran Minh N, Siitonon A. Surveillance of listeriosis in Finland during 1995–2004. Euro Surveill. 2006;11:82–5.
14. Yde M. National Reference Center for Listeria. Activity report for 2005 [in French]. Bruxelles: Institut Scientifique de Santé Publique; 2006. p. 16.
15. Goulet V, de Valk H, Pierre O, Stainer F, Rocourt J, Vaillant V, et al. Effect of prevention measures on incidence of human listeriosis, France, 1987–1997. Emerg Infect Dis. 2001;7:983–9.
16. Voetsch AC, Angulo FJ, Jones TF, Moore MR, Nadon C, McCarthy P, et al. Reduction of incidence of invasive listeriosis in foodborne diseases active surveillance Network sites. 1996–2003. Clin Infect Dis. 2007;44:513–20.
17. Centers for Disease Control and Prevention. Preliminary FoodNet data in the incidence of infection with pathogens transmitted commonly through food–10 states, 2006. MMWR Morb Mortal Wkly Rep. 2007;56:336–9.
18. Agence française de sécurité sanitaire des aliments (AFFSA). Report on salt: evaluation and recommendation. Maison-Alfort (France): AFFSA; 2002 [cited 2008 Mar 3]. Available from http://www.afssa.fr/Documents/NUT-Ra-Sel.pdf

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Transmission of Avian Influenza Virus (H3N2) to Dogs

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In South Korea, where avian influenza virus subtypes H3N2, H5N1, H6N1, and H9N2 circulate or have been detected, 3 genetically similar canine influenza virus (H3N2) strains of avian origin (A/canine/Korea/01/2007, A/canine/Korea/02/2007, and A/canine/Korea/03/2007) were isolated from dogs exhibiting severe respiratory disease. To determine whether the novel canine influenza virus of avian origin was transmitted among dogs, we experimentally infected beagles with this influenza virus (H3N2) isolate. The beagles shed virus through nasal excretion, seroconverted, and became ill with severe necrotizing tracheobronchitis and bronchioalveolitis with accompanying clinical signs (e.g., high fever). Consistent with histologic observation of lung lesions, large amounts of avian influenza virus binding receptor (SAα 2,3-gal) were identified in canine tracheal, bronchial, and bronchiolar epithelial cells, which suggests potential for direct transmission of avian influenza virus (H3N2) from poultry to dogs. Our data provide evidence that dogs may play a role in interspecies transmission and spread of influenza virus.

Influenza A virus, a member of the genus *Orthomyxovirus,* is an economically important virus that causes disease in humans, pigs, horses, and fowl (*1*). A crucial feature in the ecology and epidemiology of influenza virus is interspecies transmission (*2*). The emergence of new virus subtypes and their interspecies transmission is of great concern; measures to counteract their spread are vital for preventing influenza epidemics and pandemics. One of the basic mechanisms of interspecies transmission of influenza virus is direct transfer of an essentially unaltered virus from 1 species to another (*3*); however, some factors restrict this transfer. In particular, the presence or absence of host species–specific influenza virus binding receptors in the upper and lower respiratory tracts serves to prevent such cross-species or zoonotic transmission. Human influenza viruses bind to glycolipids or glycans that contain terminal sialyl-galactosyl residues with α 2,6-gal linkages (SAα 2,6-gal), whereas avian influenza viruses bind to residues with SAα 2,3-gal linkages (*4*). Examples of interspecies transmission of influenza viruses include recent human infections with the H5N1 subtype of avian influenza virus, and in canine infections with the H3N8 subtype of equine influenza virus (*3,5*). In addition, influenza infections were recently reported in species (canine, feline) that historically do not carry influenza virus (*6*). However, most directly transmitted infections of entire influenza viruses from a natural host species to a new host species do not result in sustained transmission in the new host species (*3*). Therefore, establishing new, long-lived influenza virus lineage is uncommon and difficult (*7*).

We report interspecies transmission of a complete avian influenza virus (H3N2) to dogs and the emergence of a new canine influenza virus associated with acute respiratory disease in South Korea, where avian influenza viruses (H3N2, H5N1, H6N1, and H9N2) currently circulate or have been previously detected (*8*). We investigated pathogenicity of the isolated virus in experimental dogs and evaluated localization of SAα 2,6-gal and SAα 2,3-gal linkages in upper and lower canine respiratory tracts.

Materials and Methods

Outbreak Histories

From May through September 2007, cases of severe respiratory disease occurred in animals at 3 veterinary clinics located 10–30 km apart in Kyunggi Province and 1 kennel located in Jeolla Province (southern part of South

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Korea). The first case, which occurred in May, was identified in a 5-year-old miniature schnauzer that had nasal discharge for 3 days and sneezing for 2 days, after which the signs subsided and the dog recovered. In August, another case was identified in a 3-year-old cocker spaniel that had fever, cough, nasal discharge, and anorexia and died after the onset of clinical signs. In September, severe respiratory disease was identified in 2 Jindo dogs (a Korean breed of hunting dog that originated on Jindo Island) and a 3-year-old Yorkshire terrier. These animals had severe cough, fever, and nasal discharge and died 2 days after visiting the same animal hospital. Finally, an outbreak of canine influenza occurred in an animal clinic in which all 13 dogs housed in a shelter facility were found to be infected with the same virus; their clinical signs were nasal discharge, cough, and high fever. Of the dogs in the affected kennel in Jeolla Province, paired serum samples showed that 47 (90%) of 52 were seropositive for canine influenza virus (H3N2) at the first sampling and that 100% were seropositive by the second sampling.

Nasal swabs from the miniature schnauzer, cocker spaniel, and Yorkshire terrier were submitted to Animal Genetics, Inc. (Suwon, South Korea) for reverse transcription–PCR (RT-PCR) and testing with a commercial rapid influenza virus antigen detection kit (Animal Genetics, Inc.). Hemagglutinin inhibition (HI) tests were performed according to the World Organization for Animal Health recommendations; commercial nucleocapsid protein (NP)–based ELISA (Animal Genetics, Inc.) was used for serologic testing.

RT-PCR and Sequencing

Nasal swabs from the above-mentioned 3 dogs were also used to isolate the influenza A virus by inoculation into 11-day-old chicken eggs. After 3–4 days of incubation, allantoic fluids were clarified by low speed centrifugation, and these fluids were shown to agglutinate chicken erythrocytes. Virus RNA was extracted from allantoic fluids by using Trizol LS (Molecular Research Center, Inc., Cincinnati, OH, USA) according to the manufacturer's instructions. RT-PCR was performed under standard conditions with random hexamer primers. Isolated influenza virus was subtyped by RT-PCR analysis by using primers specific for canine, swine, and avian hemagglutinin 3 (H3) genes. Primers for the detection of viral genes H3, neuraminidase 2 (N2), polymerase basic protein (PB) 1, PB2, polymerase acidic protein (PA), NP, matrix protein (M), and nonstructural protein (NS) were designed by using the Primer 3 program with modifications (Whitehead Institute, Massachusetts Institute for Technology Center for Genome Research, Boston, MA, USA).

For PCR, pairs of primers were used to detect target genes. cDNA (2 μL) was mixed with a reaction mixture containing 2.5 μL of 10× Taq DNA polymerase buffer, 1.5 mmol/L $MgCl_2$, 2.0 μL of dNTPs (2.5 mmol/L/μL), 1 μL of each specific primer (10 pmol/L each), and 1 μL of Taq DNA polymerase (Promega, Madison, WI, USA). Distilled water was added to make a final volume of 25 μL. PCR was performed by reaction initiation at 94°C for 10 min, amplification for 32 cycles at 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s, and by final extension at 72°C for 10 min. The reaction was held at 4°C until further use. PCR products were analyzed by electrophoresis in 1.5% agarose gel containing ethidium bromide. Sequences of the isolated virus genes were edited and analyzed by using Bioedit software (www.mbio.ncsu.edu/BioEdit/bioedit.html). Phylogenetic trees were generated by using the MEGALIGN program (DNASTAR, Madison, WI, USA) with the ClustalX alignment algorithm (www.megasoftware.net).

Experimental Infection with Isolated Virus

We experimentally reproduced viral infection in 10-week-old conventional beagle puppies that had been divided into inoculated (I) and noninoculated (NI) groups. Group I puppies (n = 9) were inoculated intranasally with 2 mL of virus isolate with a titer of $10^{6.9}$ 50% egg infectious dose (EID_{50})/0.1 mL; group NI puppies (n = 6) were inoculated intranasally with 2 mL of sterile phosphate buffered saline. Before they were inoculated, the animals were sedated by intramuscular injection of 0.1 mg/kg acepromazine malate (Bayer, Seoul, South Korea). Clinical signs of infection were monitored for 7 days after inoculation, and feces and nasal discharge were examined for virus shedding by RT-PCR for 10 days after inoculation. To detect antibodies against nucleoprotein and HI for hemagglutinin, we analyzed convalescent-phase serum samples from 3 puppies in each group for virus-specific antibodies by ELISA (Animal Genetics, Inc.). HI tests were performed according to World Organization for Animal Health–recommended procedures (*9*). At 3, 6, and 9 days postinoculation (dpi), 3 group I puppies and 2 group NI puppies were humanely euthanized for gross and histopathologic examination. All necropsy procedures were performed by veterinary pathologists. All organs from dogs and pigs (positive control) were rapidly immersed in 10% neutral formalin buffer to prevent autolysis and stored overnight. To detect influenza A virus antigens in group I or group NI tissues, we performed immunohistochemical examination by using goat anti–influenza A virus antibody (1:100; Chemicon, Temecula, CA, USA). To determine the presence or absence of SA α2,3-gal linkages comprising avian influenza virus receptors and SA α2,6-gal linkages comprising human influenza receptors in the respiratory tracts of noninfected puppies, lectin-based staining was performed as previously reported (*10*). Porcine tissue served as a positive control. All experimental procedures were approved by an independent animal care

and use committee, and the guidelines of National Veterinary Research and Quarantine Service for the reproduction of pathogenesis in dogs were respected.

Results

Isolation of Virus

Nasal swabs from the miniature schnauzer, cocker spaniel, and Yorkshire terrier were positive for influenza virus and negative for other pathogens, including canine distemper virus, canine parainfluenza-2 virus, and *Bordetella bronchiseptica*. The isolated viruses were designated A/canine/Korea/01/2007 (H3N2), A/canine/Korea/02/2007 (H3N2), and A/canine/Korea/03/2007 (H3N2).

Nucleotide Sequences

Eight gene segments (H3, N2, PB1, PB2, PA, NP, M and NS) of each isolated canine influenza virus were sequenced (EU127500, H gene; EU127501, N gene), and homologous sequences were sought in GenBank (Table). Sequences from avian influenza viruses that displayed homologies from 95.5% to 98.9% were identified for all 8 gene segments from 1 of the 3 subtype H3N2 canine isolates (A/canine/Korea/01/2007). The HA and NA genes of this isolate showed greatest identity with those of Korean avian influenza virus isolate S11, and the NS gene showed greatest identity to that of avian influenza virus (A/chicken/Nanchang/7-010/2000 [H3N6]) isolated from Chinese chickens. All the other genes, including PB1, PB2, PA, NP and M, were closely related to those of avian influenza virus isolated from ducks in Hong Kong, Japan, and China.

Phylogenetic Relationships

Phylogenetic analysis indicated that the canine influenza virus isolates from South Korea belonged to a different cluster than those of equine and canine influenza subtype H3N8 viruses. The HA and NA genes of the canine isolate (A/canine/Korea/01/2007 [H3N2]) were closely related to those of avian influenza virus (H3N2) from South Korea (Figure 1).

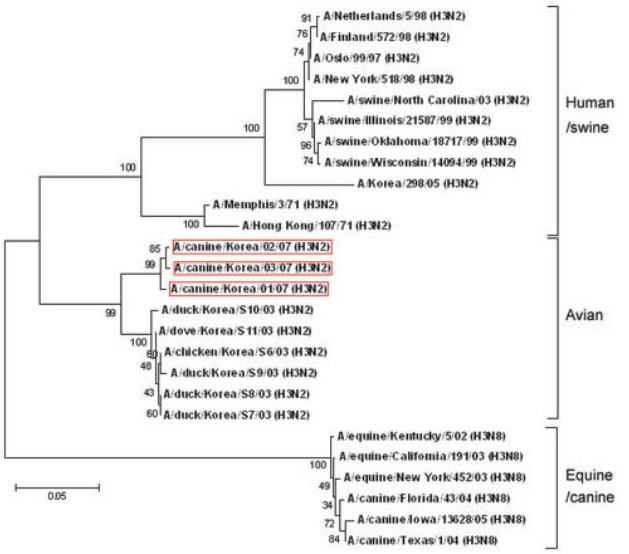

Figure 1. Phylogenetic relationship among hemagglutinin genes of canine influenza virus isolates. Tree of hemagglutinin genes from representative isolates from dog, human, bird, pig, and horse. Scale bar represents a difference of 5%.

Serologic Responses to Inoculation

All group I puppies had negative serologic assay results before inoculation. Group NI control puppies remained negative throughout the experiment.

In nucleoprotein-specific ELISA, the percent inhibition values for group I at 6 dpi were substantially higher than those for group NI (Figure 2); and the HI antibody titers of group I (HI titer 80) were induced at 8 dpi.

Clinical Responses to Challenge

Clinical signs, including sneezing and nasal discharge in group I, were observed at 2–7 dpi. The rectal temperatures of group NI animals remained below 39°C throughout the experiment. At 24 h after inoculation, fever developed in group I puppies (mean rectal temperature 40.14°C) (Figure 2) and lasted through 6 dpi.

Table. Homology of the genes of A/canine/Korea/01/2007 influenza virus (H3N2) isolated in South Korea with related sequences in GenBank*

Gene†	Virus with the highest identity	Source	Identity, %	Accession no.
HA	A/chicken/Korea/S6/2003 (H3N2)	Avian	96.6	AY862607
NA	A/dove/Korea/S11/2003 (H3N2)	Avian	97.4	AY862644
PB1	A/duck/Yangzhou/02/2005 (H8N4)	Avian	98.9	EF061124
PB2	A/duck/Zhejiang/11/2000 (H5N1)	Avian	97.6	AY585523
PA	A/duck/Hokkaido/120/2001 (H6N2)	Avian	95.9	AB286878
NP	A/duck/Hong Kong/Y439/97 (H9N2)	Avian	95.5	AF156406
M	A/duck/Jiang Xi/1850/2005 (H5N2)	Avian	97.5	EF597295
NS	A/chicken/Nanchang/7-010/2000 (H3N6)	Avian	97.5	AY180648

*Influenza virus lineage of all RNA segments is avian.
†HA, hemagglutinin, NA, neuraminidase; PB, polymerase basic protein; PA, polymerase acidic protein; NP, nucleocapsid protein; M, matrix protein, NS, nonstructural protein.

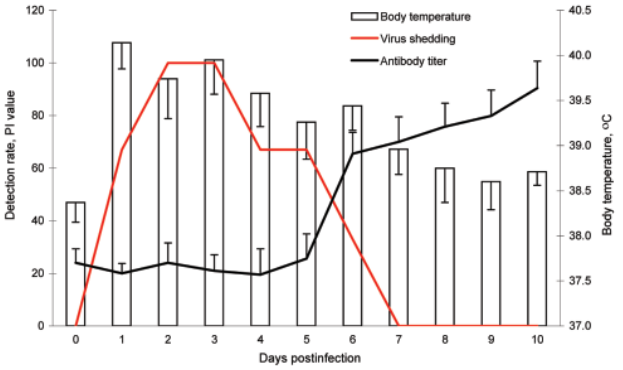

Figure 2. Body temperature, virus shedding, and antibody seroconversion after challenge with canine influenza virus. Body temperature was increased from 1 day postinoculation (dpi) and slowly decreased to normal temperature by 7 dpi. Virus shedding was detected from 1 dpi to 6 dpi by reverse transcription–PCR. However, the ELISA antibody titers increased after 6 dpi. Antibody titers were regarded as positive if percent inhibition (PI) was >50.

Virus Shedding

Influenza virus was not detected in feces. However, for group I puppies, virus shedding in nasal discharge began at 1 dpi and continued to 6 dpi; the highest titers, $10^{6.1}(EID_{50}/0.1$ mL), were reached by 4 dpi. RT-PCR products generated from shed viruses were sequenced and identified as identical to the inoculated virus.

Histopathologic Findings

Gross lesions were limited to the lungs and were characterized by multifocal to coalescing reddish consolidation. In tissues collected on 3, 6 and 9 dpi, histopathologic lesions were observed in the trachea and lungs, and extrapulmonary lesions were absent in puppies infected with the isolate (A/canine/Korea/01/2007 [H3N2]). Severe virus-induced necrosis and inflammation of the upper (trachea and bronchi) and lower (bronchiole and alveoli) respiratory tracts were noted on histologic examination. Although minor differences in the severity of the histologic findings were observed among the 9 infected dogs, all infected dogs shared the following histopathologic features regardless how long after inoculation tissues were collected: 1) moderate to severe multilobular or diffuse necrotizing tracheobronchitis with suppurative inflammation in the lumina and squamous metaplasia of the tracheobronchial epithelium (Figure 3, panel B); 2) moderate to severe multilobular or diffuse necrotizing bronchiolitis and alveolitis (i.e., bronchioalveolitis, occasionally accompanied by chronic peribronchiolar and perivascular inflammation) (Figure 3, panels D and E); and 3) mild to moderate multilobular or diffuse thickening of alveolar septa by infiltrates of inflammatory cells, such as interstitial pulmonary macrophages. At 3, 6, and 9 dpi, large amounts of influenza A virus antigen were found in bronchial and bronchiolar epithelium and lumens (Figure 3, panel F).

Receptor Binding Assay

Consistent with the histologic lung lesions, large amounts of SAα 2,3-gal were found on the surface of bronchial and bronchiolar epithelial cells of group NI puppies and were rarely found on tracheal epithelial cells (Figure 4). In contrast, SAα 2,6-gal was not detected in tracheal, bronchial, or bronchiolar epithelial cells, which suggests that canine species may have a lesser role as intermediate hosts for transmission of human influenza viruses to dogs than for avian influenza viruses.

Discussion

Because all genes of the canine isolates were of avian influenza virus origin, we concluded that the entire genome of the avian influenza virus had been transmitted to the dogs. Transmission of avian influenza A virus to a new mammalian species is of great concern, because it potentially allows the virus to adapt to a new mammalian host, cross new species barriers, and acquire pandemic potential.

Transmission of an entire avian influenza virus to an unrelated mammalian species is a rare event. Several outbreaks of avian influenza infection have occurred in mammals. Influenza virus (H7N7) of avian origin was isolated from the lungs and brains of dead seals. In addition, it was replicated to high titers in ferrets, cats, and pigs and caused conjunctivitis in humans (*11,12*). Avian origin influenza virus (H4N5) was reported as the cause of infection and death in harbor seals along the New England coastline (*13*), and avian origin influenza (H5N1) infection was identified in a dog after ingestion of a duck infected with subtype H5N1 during an outbreak in Thailand in 2004 (*14*).

Previously, outbreaks of hemorrhagic pneumonia caused by equine influenza virus (H3N8) were noted in racing dogs, and a human influenza virus (H3N2) was isolated from dogs (*15,16*). However, these reports provide limited serologic and virologic evidence for influenza virus infection in dogs. We report the emergence of a new canine influenza virus strain that causes acute respiratory disease in dogs and that differs from previous outbreaks of equine influenza virus (H3N8) infections.

Concerning the possible mechanism of avian influenza virus transmission to dogs, we posit that this transmission results from feeding dogs untreated minced meats of ducks or chickens. In South Korea, untreated duck and chicken meats, including internal organs and heads, have been widely used to feed dogs for fattening in local canine farms or kennels. In a previous study, avian influenza virus (H3N2) was isolated from ducks and chickens sold at live-bird markets in South Korea. Live-bird markets are thought to constitute "a missing link in the epidemiology of avian

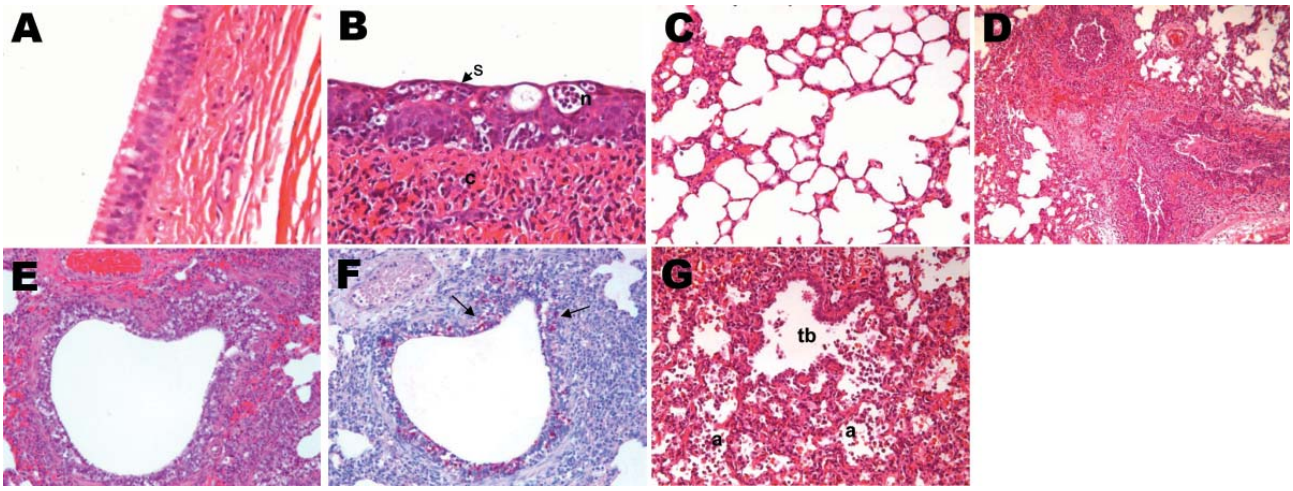

Figure 3. Histopathologic lesions in the trachea and lungs of control (A and C) or experimentally infected (B, D–F) dogs (A/canine/Korea/01/2007 [H3N2]) at different days postinoculation (dpi). A) Control dog at 9 dpi, showing normal pseudostratified columnar epithelium lining of the trachea; original magnification ×400. Hematoxylin and eosin (HE) stain. B) Influenza-infected dog at 9 dpi, showing necrotizing tracheitis characterized by necrosis (n), squamous metaplasia (s), and hyperplasia of the epithelium and nonsuppurative inflammation (c) in the connective tissue; original magnification ×400. HE stain. C) Control dog at 3 dpi, showing normal alveoli; original magnification ×200. HE stain. D) Influenza-infected dog at 3 dpi, showing severe diffuse necrotizing bronchitis and bronchiolitis with suppurative inflammation in the lumina; original magnification ×100. HE stain. E) Influenza-infected dog at 6 dpi, showing severe necrotizing bronchiolitis; original magnification ×200. HE stain. F) Influenza-infected dog at 6 dpi (serial section of E), showing large amounts of influenza A virus antigens (red stain; arrows) in the bronchiolar epithelium and lumen. Immunohistochemistry; Red Substrate (Dako, Carpinteria, CA, USA); Mayer's hematoxylin counterstain. G) Influenza-infected dog at 9 dpi, showing severe necrotizing alveolitis with accumulation of necrotic cells in terminal bronchioles (tb) and alveoli (a); original magnification ×200. HE stain.

influenza viruses" because they bring together numerous hosts, such as chickens, ducks, turkeys, geese, and doves, in a high-density setting, which represents an ideal environment for virus interspecies transmission (*17,18*). The S11 strain, whose HA and NA genes showed the greatest identity to those of the A/canine/Korea/01/2007 (H3N2) isolates from dogs, was isolated from a tracheal swab of a healthy chicken and is nonpathogenic in poultry (*8*). These observations support the hypothesis that avian influenza virus (H3N2) strains could be transmitted by feeding infected poultry by-products to dogs (*2*).

It is also possible that cross-species transmission of influenza virus occurs directly by aerosol transmission from infected birds to susceptible dogs as a consequence of close contact between the 2 species. Lectin-staining results showed that canine upper (trachea and bronchi) and lower

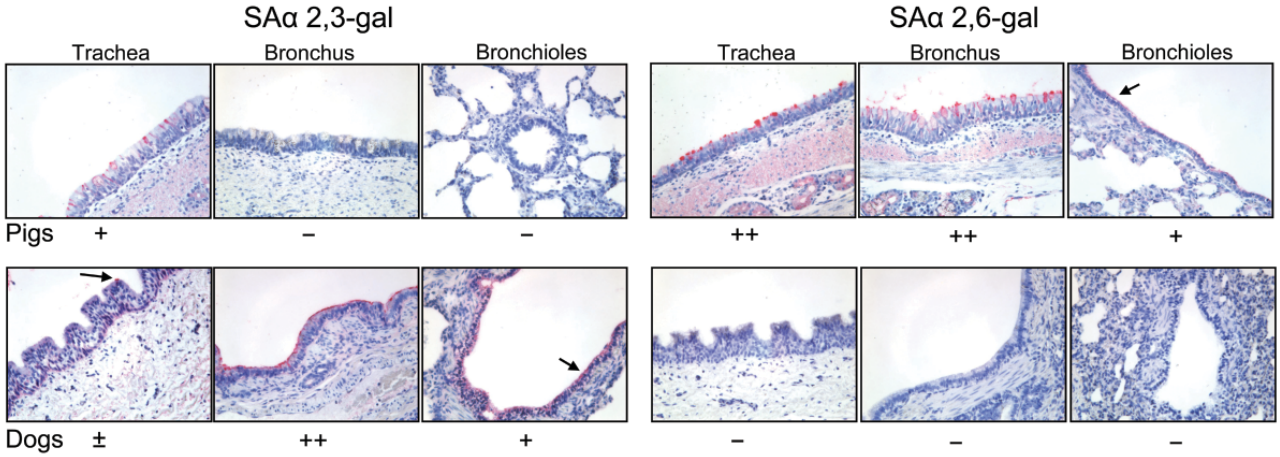

Figure 4. Lectin staining (red stain) for SAα 2,3-gal (avian influenza virus receptors) and SAα 2,6-gal (human influenza virus receptors) in canine trachea, bronchus, and bronchioles, together with porcine tissues as a positive control. Original magnification all x300. −, no staining; ±, rare or few positive cells; +, moderate numbers of positive cells; and ++, many positive cells.

(bronchiole) respiratory tract epithelium cells display SAα 2,3-gal, to which avian influenza viruses bind, making possible a direct transmission of avian influenza viruses from poultry to dogs. Additionally, according to the animal hospital veterinarian, this outbreak was traced to a Jindo dog purchased at a live-animal market in Kyunggi Province that sold chicken, duck, pheasant, rabbit, cats, pet dogs, and other dogs. The Jindo dog was hospitalized at the local animal hospital and may have infected the other pet dogs at the hospital. This epidemiologic result also suggests that the novel canine influenza virus of avian origin was transmitted within canine species.

Antigenic and phylogenetic analyses showed that the HA and NA genes of the A/canine/Korea/01/2007 (H3N2) isolate are closely related to isolates identified in 2003 from chickens and doves in South Korea. Furthermore, HA genes of canine influenza isolates were different from recent isolates from swine in South Korea (*19*). The other genes of the canine influenza isolate are more closely related to those of the subtype H9N2 isolate found in ducks from Hong Kong, the subtype H6N2 isolate from ducks in Japan, and several other avian influenza strains from southeastern China in 2000 through 2005. This finding suggests that multiple variants of subtype H3 influenza viruses may be circulating in these regions and causing disease in pet dogs.

Our experimental reproduction of the disease caused by this isolate induced severe pathologic changes and showed that infected dogs excreted influenza virus (H3N2) in nasal discharge but not in feces. This finding suggests that dog-to-dog transmission of subtype H3N2 could occur through the nasal route and that dog-to-dog transmission of the virus could play an important role in the epizootiology of the disease.

In our study, virologic, serologic, pathologic, and phylogenetic analyses showed cross-species infection of an entire avian influenza A virus (H3N2) to another mammalian species, dogs. Evidence of avian influenza virus infection in pet dogs raises the concern that dogs may be become a new source of transmission of novel influenza viruses, especially where avian influenza viruses are circulating or have been detected.

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References

1. Wright PF, Webster RG. In: Orthomyxoviruses. Philadelphia: Lippincott Williams & Wilkins; 2001. p.1533–9
2. Webster RG. Influenza: an emerging disease. Emerg Infect Dis. 1998;4:436–41.
3. Crawford PC, Dubovi EJ, Castleman WL, Stephenson I, Gibbs EPJ, Chen L, et al. Transmission of equine influenza virus to dogs. Science. 2005;310:482–5.
4. Suzuki Y. Sialobiology of influenza: molecular mechanism of host range variation of influenza viruses. Biol Pharm Bull. 2005;28:399–408.
5. Guan Y, Poon LL, Cheung CY, Ellis TM, Lim W, Lipatov AS, et al. H5N1 influenza: a protean pandemic threat. Proc Natl Acad Sci U S A. 2004;101:8156–61.
6. Keawcharoen J, Oraveerakul K, Kuiken T, Fouchier RA, Amonsin A, Payungporn S, et al. Avian influenza H5N1 in tigers and leopards. Emerg Infect Dis. 2004;10:2189–91.
7. Webster RG, Bean WJ, Gorman OT, Chambers TM, Kawakowa Y. Evolution and ecology of influenza viruses. Microbiol Rev. 1992;56:152–79.
8. Choi YK, Seo SH, Kim JA, Webby RJ, Webster RG. Avian influenza viruses in Korean live poultry markets and their pathogenic potential. Virology. 2005;332:529–37.
9. World Organization of Animal Health. Manual of diagnostic tests and vaccines for terrestrial animals. 5th ed. Paris: The Organization; 2005.
10. Wan H, Perez DR. Quail carry sialic acid receptors compatible with binding of avian and human influenza viruses. Virology. 2006;346:278–86.
11. Webster RG, Geraci J, Petursson G, Skirnisson K. Conjunctivitis in human beings caused by influenza A virus of seals. N Engl J Med. 1981;304:911.
12. Webster RG, Hinshaw VS, Bean WJ, Van Wyke KL, Geraci JR, St Aubin DJ, et al. Characterization of an influenza A virus from seals. Virology. 1981;113:712–24.
13. Hinshaw VS, Bean WJ, Webster RG, Rehg JE, Fiorelli P, Early G, et al. Are seals frequently infected with avian influenza viruses? J Virol. 1984;51:863–5.
14. Songserm T, Amonsin A, Jam-on R, Sae-Heng N, Pariyothorn N, Payungporn S, et al. Fatal avian influenza A H5N1 in a dog. Emerg Infect Dis. 2006;12:1744–7.
15. Chang CP, New AE, Taylor JF, Chang HS. Influenza virus isolations from dogs during a human epidemic in Taiwan. Int J Zoon. 1976;3:61–4.
16. Houser RE, Heuschele WP. Evidence of prior infection with influenza A/Texas/77 (H3N2) virus in dogs with clinical parainfluenza. Can J Comp Med. 1980;44:396–402.
17. Liu H, Liu X, Cheng J, Peng D, Jia L, Huang Y. Phylogenetic analysis of the hemagglutinin genes of twenty-six avian influenza viruses of subtype H9N2 isolated from chickens in China during 1996–2001. Avian Dis. 2003;47:116–27.
18. Liu M, He S, Walker D, Zhou N, Perez DR, Mo B, et al. The influenza virus gene pool in a poultry market in south central China. Virology. 2003;305:267–75.
19. Song DS, Lee JY, Oh JS, Lyoo KS, Yoon KJ, Park YH, et al. Isolation of H3N2 swine influenza virus in South Korea. J Vet Diagn Invest. 2003;15:30–4.

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Efficacy of Aerial Spraying of Mosquito Adulticide in Reducing Incidence of West Nile Virus, California, 2005

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Epidemic transmission of West Nile virus (WNV) in Sacramento County, California, in 2005 prompted aerial application of pyrethrin, a mosquito adulticide, over a large urban area. Statistical analyses of geographic information system datasets indicated that adulticiding reduced the number of human WNV cases within 2 treated areas compared with the untreated area of the county. When we adjusted for maximum incubation period of the virus from infection to onset of symptoms, no new cases were reported in either of the treated areas after adulticiding; 18 new cases were reported in the untreated area of Sacramento County during this time. Results indicated that the odds of infection after spraying were ≈6× higher in the untreated area than in treated areas, and that the treatments successfully disrupted the WNV transmission cycle. Our results provide direct evidence that aerial mosquito adulticiding is effective in reducing human illness and potential death from WNV infection.

West Nile virus (WNV; genus *Flavivirus*, family *Flaviviridae*) is transmitted to humans through the bite of an infected female mosquito and can cause clinical manifestations such as acute febrile illness, encephalitis, flaccid paralysis, and death (*1*). In California, WNV was first identified in 2003, during which time the virus was detected in 6 southern counties and 3 infected persons were identified (*2*). The following year, WNV spread northward from southern California to all 58 counties in the state, resulting in 779 human WNV cases and 28 deaths (*3,4*). In 2005, 880 human WNV cases and 19 related deaths were identified in California; 3,000 cases were reported nationwide (*5,6*). In contrast to 2004, when most of the WNV activity was concentrated in southern California, activity in 2005 occurred primarily in the northern part of the Central Valley of California, where Sacramento County, the epicenter of WNV activity in the United States that year, had more human cases (163) than any other county in the nation (*7*).

In northern California, the principal urban and rural vectors of WNV are *Culex pipiens* and *Cx. tarsalis*, respectively (*8–10*). To reduce WNV transmission and human exposure to mosquitoes in 2005, the Sacramento-Yolo Mosquito and Vector Control District (SYMVCD) implemented a battery of control practices from their Integrated Pest Management plan (*11*), an ecosystem-based strategy focused on long-term control of mosquito populations (D. Brown, SYMVCD, pers. comm.). Despite the district's intensified efforts (which began in March 2005) to control larval mosquitoes and to spot-treat for adult mosquitoes by using truck-mounted equipment, by August 2005 the county had reached the epidemic response level designated by the California Mosquito-Borne Virus Surveillance and Response Plan (*12,13*). Per the response plan, SYMVCD determined the appropriate response and control measures through the analysis of 8 surveillance factors, which provided a semiquantitative measure of transmission risk (D. Brown, pers. comm.). Rapidly escalating risk for WNV transmission to humans in Sacramento County was indicated by high mosquito abundance and infection prevalence; high numbers of sentinel chicken seroconversions;

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and record numbers of dead bird reports, equine cases, and human cases, including ≈24 confirmed human infections by early August (*8,10,14*). Following state guidelines, and in consultation with local public health officials, SYMVCD initiated aerial adulticiding in Sacramento County in August 2005 to rapidly reduce the abundance of infected mosquitoes and decrease the risk for WNV transmission to humans (D. Brown, pers. comm.). Despite a 60-year history of the aerial application of mosquito control products in California (*15*), this was the first instance within the state of aerial adulticiding over a large urban area.

Although published studies on aerial application of adulticides have documented reductions in mosquito abundance and infection prevalence along with concurrent or subsequent decreases in human cases (*16–19*), no published study to date has directly assessed the efficacy of such control efforts in reducing incidence of human disease by comparing distribution of clinical cases within treated and untreated areas. The objective of our study was to evaluate the efficacy of adulticide applications for reducing human cases of WNV; we compared the proportion and incidence of cases in the treated and untreated areas of Sacramento County in 2005 before and after aerial treatments. The proportion and incidence of these cases were also compared with those of the rest of California.

Methods

Data Collection

Human WNV case data were reported to the California Department of Public Health from the Sacramento County Department of Health and Human Services and other local health departments throughout the state by using a standardized case history form. A total of 177 human infections were reported within Sacramento County in 2005, with onsets of illness ranging from June through October. Of 177 infections, 163 were clinical cases and 14 were asymptomatic infections; the former was confirmed by immunoglobulin (Ig) G and IgM antibody assays of serum or cerebrospinal fluid samples. Of 163 case records, 7 had no date-of-onset information and 4 others had no residential address. Consequently, the Sacramento County human dataset used in this study comprised 152 records that contained spatial and temporal attributes.

Residential addresses were imported into ArcMap 9.1 geographic information systems software (Environmental Systems Research Institute, Inc., Redlands, CA, USA) and geocoded by using the software's 2005 StreetMap USA Plus AltNames street dataset. All remaining unmatched addresses were geocoded by using Tele Atlas 2006 (Tele Atlas, Lebanon, NH, USA), NAVTEQ 2006 (NAVTEQ, Chicago, IL, USA.), GDT 2005 (Geographic Data Technology, Inc., Lebanon, NH, USA), and TIGER 2006 (US Census Bureau, Washington, DC, USA) datasets. Population size estimates for the study areas defined below were calculated in ArcMap by selecting census blocks that had their center (centroid) in each defined region (Table 1) (*20*). All data were mapped by using the NAD83 USA Contiguous Albers Equal Area Conic coordinate system.

Adulticide Application

Aerial adulticide applications were intended to create aerosolized clouds of insecticide that would contact, and consequently kill, airborne adult *Culex* spp. mosquitoes. SYMVCD targeted areas for treatment on the basis of levels of mosquito infection prevalence that had been previously associated with epidemic transmission within an urban setting (minimum infection rate per 1,000 female *Culex* spp. tested >5.0) (*12*). The district contracted with ADAPCO Vector Control Services (ADAPCO, Inc., Sanford, FL, USA) to apply adulticide by using 2 Piper Aztec aircraft (Piper Aircraft, Inc., Vero Beach, FL, USA) over an area of 222 km^2 in northern Sacramento County on the nights of August 8–10, 2005 (northern treated area) and an area to the south of 255 km^2

Table 1. Number of human cases of infection with West Nile virus by location and temporal classification, California, 2005*

Area†	Total	Pretreatment‡	Posttreatment§	Postincubation¶	Population#
Treated, northern	34	28	6	0	221,828
Treated, southern	21	20	1	0	338,579
Buffer, northern	13	9	4	3	94,399
Buffer, southern	8	5	3	1	50,127
Untreated	76	41	35	18	518,566
Sacramento County	152	103	49	22	1,223,499
California	670	357	313	197	32,648,149

*Only cases with known date of onset of illness and location information (i.e., Sacramento County at the address level and California at the county level) are included in the analysis.
†California excluding Sacramento County.
‡Refers to cases with onset of illness up to and including the last date that aerial adulticiding was conducted (ending 22 Aug for the southern treated area and southern buffer zone and 10 Aug for all other areas).
§Refers to cases with onset of illness after the last date that aerial adulticiding was conducted (beginning 23 Aug for the southern treated area and southern buffer zone and 11 Aug for all other areas).
¶Refers to cases with onset of illness >14 days after the first date that aerial adulticiding was conducted (beginning 4 Sep for the southern treated area and southern buffer zone and 23 Aug for all other areas).
#Population data source: UA Census 2000 TIGER/Line data made available in shapefile format through Environmental Systems Research Institute, Inc. (Redlands, CA, USA) (*20*).

on the nights of August 20–22, 2005 (southern treated area) (D. Brown, unpub. data) (Figure 1). Coverage was similar each night; repeated applications were intended to increase efficacy (D. Brown, pers. comm.).

The applied compound was Evergreen EC 60–6 insecticide (MGK, Minneapolis, MN, USA), a product composed of 6% pyrethrin/60% piperonyl butoxide (*8*). It was applied at the maximum rate according to the label, 0.0025 pounds of pyrethrins per acre (ultra-low volume dispersal), by 2 Micronair AU4000 atomizer nozzles (Micron Sprayers, Ltd, Bromyard, Herefordshire, UK) on each aircraft, with a swath width of 1,300 feet and expected droplet spectrum volume mean diameters of 32.1 and 36.3 microns for the 2 planes (D. Brown and G. Goodman, unpub. data). Conditions during each night of spraying included wind speeds of 4–10 knots/h and temperatures/dew points of 27°C/14°C (northern treatment) and 33°C/12°C (southern treatment) (D. Brown, unpub. data). Planes began flying at ≈8:00 PM each night and flew for 3–6 h at 130 knots/h (D. Brown, unpub. data). The aircraft flew at altitudes of 61.0 m in the northern treated area and 91.4 m (because of obstacles such as tall towers and buildings) in the southern treated area (R. Laffey, SYMVCD, unpub. data, D. Markowski, pers. comm.). The Wingman GX aerial guidance and recording system (ADAPCO, Inc.), coupled with the Aircraft Integrated Meteorological Management System (AIMMS-20; Aventech Research, Inc., Barrie, Ontario, Canada), modeled the effective drift of released compounds on the basis of real-time meteorologic conditions (D. Brown, pers. comm.). Flight and treatment data were imported into ArcMap for mapping and analysis.

Figure 1. Map of northern and southern aerial adulticiding treatment areas in Sacramento County, California, 2005, showing the 2 urban areas treated by the Sacramento-Yolo Mosquito and Vector Control District (SYMVCD). Horizontal bars represent swaths of spray clouds created by individual passes of the aircraft, as defined by the spray drift modeling systems. Gaps within spray clouds were caused by factors such as towers and buildings that altered the flight of the aircraft (G. Goodman, SYMVCD, pers. comm.). These gaps were assumed to have negligible effect in this study; no human cases occurred within any gaps. Gray region surrounding much of the spray zones represents the urbanized area of Sacramento; urbanized area is defined by the US Census Bureau as a densely settled territory that contains ≥50,000 persons (*21*). For display purposes, we used the NAD83 HARN California II State Plane coordinate system (Lambert Conformal Conic projection). Inset shows location of treatment areas in California.

Case Classification and Analysis

Despite the spray drift modeling systems' high degree of accuracy, variable and incomplete spray application was expected at the edges of the modeled spray cloud (D. Markowski, pers. comm.). Factors contributing to this phenomenon include the intrinsic margin of error of the aircrafts' spray drift modeling systems, the extrinsic margin of error caused by factors not detectable or taken into account by the modeling system (i.e., wind gusts, minor changes in aircraft altitude or speed, and other operational variables), and nonoverlapping spray clouds during different nights of application (D. Markowski, pers. comm.). Through consultation with ADAPCO, Inc., this variable and incomplete application at the perimeter was taken into account by delineating a 0.8-km (0.5-mile) buffer within the outermost range of the modeled spray clouds for each treated area (D. Markowski, pers. comm.). Nonbuffered areas of the spray regions (henceforth referred to as treated areas) were considered the most accurate representation of the actual spray application for this analysis, and any WNV cases that occurred within buffer zones were considered separately from those within treated areas. All human cases from Sacramento County that did not occur within treated areas or buffer zones were assigned to the untreated subset of cases, which served as the comparison (control) group for this study.

Cases were further classified by date of onset of illness into pretreatment and posttreatment groups; temporal classification for the untreated area and the rest of California followed that of the northern treated area (Table 1). Because of the relatively lengthy and variable human WNV incubation period, persons who became infected just before the spray events could have become symptomatic up to 14 days later (*22,23*). To exclude from analysis any infections that may have been acquired just before the spray events, posttreatment cases that had an onset of illness >14 days after spraying (counting from the first night of application) were also included in a postincubation subset.

The null hypothesis, that the proportion of cases in treated and untreated areas was equal to that of the respective population size estimates, was tested for pretreatment and posttreatment groups with the exact binomial test for goodness of fit by using VassarStats (http://faculty.vassar.edu/lowry/VassarStats.html). Second, significance of proportions of human cases before and after spraying within treated and untreated areas was evaluated with the Fisher exact test of independence by using SAS version 9.1.3 (SAS Institute Inc., Cary, NC, USA). The null hypothesis of this test was that there was no significant association between occurrence of adulticiding and temporal classification of cases (i.e., pretreatment or posttreatment). Third, relative risk (RR) and odds ratio (OR) of infection in the untreated area compared with those in treated areas were calculated by using cumulative incidence of WNV in each region before and after spraying (*24*). To evaluate whether buffer zones had any effect on results, all calculations were repeated by using cases from buffer zones and treated areas combined, as well as cases from buffer zones alone.

Assumptions

As is standard practice in most epidemiologic studies, residential addresses of patients were assumed to be locations of disease transmission; this is also consistent with other WNV studies (*25–31*). The assumption that WNV was transmitted to persons at their place of residence is supported by the fact that WNV mosquito vectors feed primarily from dusk to dawn, and also by findings that persons who spent >2 h outdoors during this time without wearing insect repellant had the highest WNV seroprevalence (*31*).

Because of the random sampling requirement for tests of statistical significance, we must assume that various human populations had an equal likelihood of becoming clinically ill before aerial treatment and that no preexisting factors contributed to a differential in disease experience. Although construction of a multilevel, spatial correlation model is beyond the scope of this study, several important properties of the populations sufficiently support our assumption of homogeneity. Despite the geographic size of the untreated area being ≈6× that of the treated areas combined (2,101 vs. 361 km^2, Figure 2), population size estimates of both areas were comparable (518,566 vs. 560,407, Table 1) (*20*). Furthermore, the preponderance of cases in the treated (100%, 55/55), buffer (95%, 20/21), and untreated (87%, 66/76) areas was located within the urbanized area of Sacramento, which constitutes 27% (686 of 2,578 km^2) of the total area of the county (Figure 1) (*20*). Additionally, most cases in the untreated area were located either between the northern and southern treated areas or immediately north of the northern treated area, and >94% (143/152) of all cases were located within 4.8 km (3 miles) of treated areas. This staggered configuration of treated and untreated areas, along with the general proximity of cases within 1 urban region, supported the assumption of homogeneity of populations at risk and created a natural experiment for comparative analyses between treated and untreated areas.

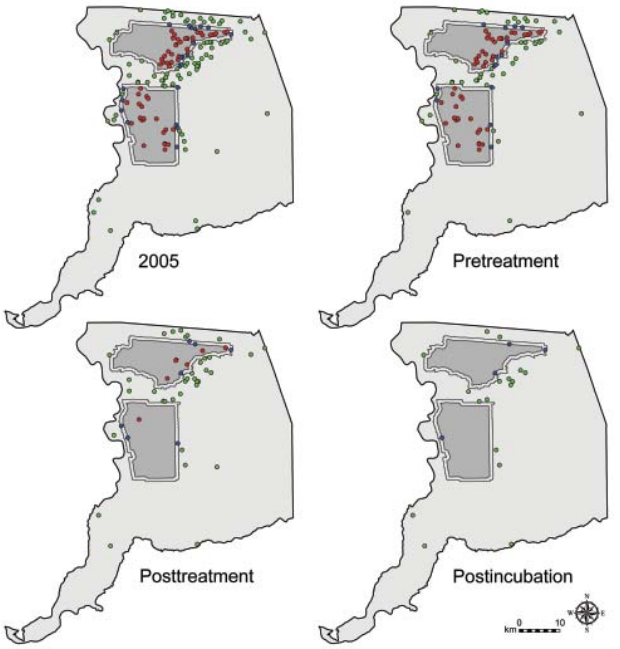

Figure 2. Locations of treated areas and human cases of West Nile virus by temporal classification, Sacramento County, California, 2005. Shown are treated areas (dark gray), surrounding 0.8-km buffers (thin regions around dark gray areas), untreated areas (light gray), and location of human cases within each of these regions (red, blue, and green circles, respectively). For display purposes, we used the NAD83 HARN California II State Plane coordinate system (Lambert Conformal Conic projection).

Results

The observed proportion of pretreatment cases in treated areas to those in the untreated area was not significantly different from the expected proportion on the basis of population size estimates ($p = 0.7508$, Table 2). Similarly, none of the proportions of pretreatment cases in any combination of treated areas and buffer zones were different from those of the untreated area. However, after adulticiding, all proportions of cases in treated areas were lower than that in the untreated area. Proportions of posttreatment cases in buffer zones were not different from those in the untreated area.

There was a significantly lower proportion of posttreatment cases within combined treated areas compared with that in the untreated area ($p<0.0001$, Table 2). Proportions of posttreatment to pretreatment cases within each of the individual treated areas were also significantly lower than that for the untreated area (northern treated area $p = 0.0053$; southern treated area $p = 0.0003$). After com-

Table 2. Statistical test results for West Nile virus cases, Sacramento County, California, 2005*

	Goodness of fit†		Independence‡
Area	Pretreatment	Posttreatment	Posttreatment vs. pretreatment
Treated, both	0.7508	**<0.0001**	**<0.0001**
Treated, northern	0.0650	**0.0391**	**0.0053**
Treated, southern	0.2983	**<0.0001**	**0.0003**
Treated plus buffer, both	0.6195	**<0.0001**	**0.0005**
Treated plus buffer, northern	0.1015	**0.0314**	**0.0069**
Treated plus buffer, southern	0.4568	**<0.0001**	**0.0029**
Buffer, both	0.5140	0.5744	0.3309
Buffer, northern	0.5592	0.5065	0.3745
Buffer, southern	0.5990	1.0000	0.7237

*Numbers of cases were combined for multiple areas; geographically corresponding buffer zones were added where noted. Numbers are 2-tailed p values. Statistically significant associations (p<0.05) are in **boldface**.
†Exact binomial goodness-of-fit test for observed proportion of cases in listed area(s) to cases in untreated area compared with the expected proportion based on population size estimates.
‡Fisher exact test of independence for 2 × 2 contingency tables containing numbers of pretreatment and posttreatment cases for listed area(s) and the untreated area.

bining cases from treated areas and buffer zones, proportions of posttreatment versus pretreatment cases were again significantly lower (both treated areas plus buffers p = 0.0005; northern treated area plus buffer p = 0.0069; southern treated area plus buffer p = 0.0029). However, none of the proportions of posttreatment versus pretreatment cases in buffer zones alone compared with those in the untreated area were significantly different (both buffer zones p = 0.3309; northern buffer zone p = 0.3745; southern buffer zone p = 0.7237).

The last human case that occurred in treated areas had an onset of illness 12 days after inception of spraying, within the 14-day maximum range of the human WNV incubation period. Thus, when the incubation period was taken into account, there were no new human WNV cases reported in either treated area after adulticiding (postincubation cases, Table 1, Figure 3). In contrast, 18 new cases were reported from the untreated area during this time; the last case occurred 59 days after inception of spraying. The frequency of these postincubation cases relative to the overall number of cases in the untreated area (24%) was consistent with that for the rest of the state (29%) but inconsistent with that for treated areas (0%).

Normalizing number of cases in each region by respective population size estimate showed the increase in incidence levels throughout the year (Figure 4). Statewide (excluding Sacramento County and cases without onset data), cumulative incidence in 2005 was 2.1/100,000 population, and the temporal pattern of incidence throughout the year was similar to that of the untreated area. On the basis of cumulative incidence within each region before aerial treatment, RR for the untreated area compared with that for treated areas was 0.9231 (95% confidence interval [CI] 0.6085–1.400), which did not differ from unity. After treatment, RR was 5.403 (95% CI 2.400–12.16), with an OR of 5.853 (5.403/0.9231, 95% CI 2.351–14.58) in favor of infection in the untreated area than in treated areas; RR and OR differed from unity. Similarly, RRs for the untreated area compared with those for treated areas and buffer zones combined were 0.8990 (95% CI 0.6059–1.334) and 3.398 (95% CI 1.829–6.316) before and after adulticiding, respectively, with an OR of 3.780 (3.398/0.8990, 95% CI 1.813–7.882). Conversely, RRs for the untreated area versus the buffer zones alone were 0.8162 (95% CI 0.4450–1.497) and 1.393 (95% CI 0.6190–3.137) before and after adulticiding, respectively, with an OR of 1.707 (1.393/0.8162, 95% CI 0.6198–4.703); the RRs and OR did not differ from unity.

Discussion

Evaluation of efficacy is essential for assessing appropriateness of insecticide applications. However, such studies assessing the ability of adulticides to directly affect human incidence of WNV have been nonexistent. Our findings, coupled with corroborating evidence of a reduction in the abundance of *Cx. pipiens* (*8*), indicate that aerial application of pyrethrin in 2005 successfully disrupted the WNV transmission cycle, and that this treatment was responsible for an abrupt decrease in the number of human cases within treated areas compared with that in the untreated area. These results provide direct evidence that aerial spraying to control adult mosquitoes effectively reduced human illness and potential deaths from WNV infection.

With respect to population size estimates, proportions of pretreatment cases in all treated areas and buffer zones were not different from that in the untreated area, which validates comparability of the baseline populations. Similarly, none of the pretreatment RRs deviated from unity, which supports the assumption that treated and untreated areas had an equal likelihood, on the basis of population size, of containing a clinical case before the adulticiding, and that no preexisting factors contributed to differing disease incidence rates during that time. These conditions are important for verifying that the untreated area was a valid

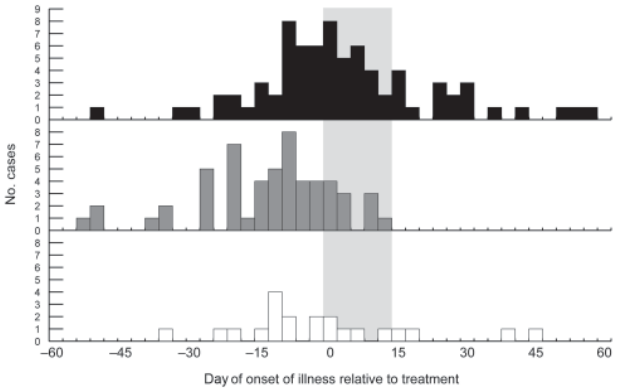

Figure 3. Human cases of West Nile virus (WNV), Sacramento County, California, 2005, by region and date of onset of illness. Black bars show cases within untreated area; gray bars show cases within northern and southern treated areas combined; and white bars show cases within northern and southern buffer zones combined. Values along the x-axis (days) are grouped into sets of 3 and labeled with the date farthest from 0. Each of the 3 days of adulticiding within the treated areas and buffer zones was considered to be 0; for the untreated area, the dates of the northern adulticiding (August 8–10) were considered to be 0. The wide gray vertical band represents time from the first day of treatment to the maximum range of the human WNV incubation period 14 days later.

comparison group for use in statistical analyses.

Comparisons of buffer zones with the untreated area indicated no differences between posttreatment RR or the proportions of posttreatment cases within the 2 areas, which supports the assumption of reduced spray efficacy at the perimeter of the modeled spray cloud. This finding may have implications for future aerial applications and efficacy studies. Additionally, posttreatment infiltration of *Cx. tarsalis* mosquitoes from bordering untreated areas has been a previously documented phenomenon in California and Texas (*19,32–34*). On the basis of mean dispersal distances of *Cx. tarsalis* (0.88 km) and *Cx. pipiens quinquefasciatus* (1.10 km) in California (*35*), use of the 0.8-km buffer in this study also reduced the probability of including in the treatment groups any human infections contracted through posttreatment mosquito infiltration. However, results of all statistical tests remained unchanged after combining the number of cases from buffer zones and treated areas, and these posttreatment reductions of cases still differed from that in the untreated area (Table 2).

Because posttreatment proportions of cases were lower than in the untreated area, we rejected the null hypothesis of goodness-of-fit comparisons. Our results also indicate that there were associations between adulticiding and temporal classification of cases. Therefore, we also rejected the null hypothesis of tests of independence. Furthermore, odds of infection after spraying were ≈6× higher in the untreated area than in treated areas. Without applications of aerial adulticide, more Sacramento residents would have been infected with WNV. This finding supports federal and California WNV response recommendations, which state that "mosquito adulticiding may be the only practical control technique available in situations where surveillance data indicate that it is necessary to reduce the density of adult mosquito populations quickly to lower the risk of WNV transmission to humans" (*36*).

Although there was a negative correlation between aerial treatments and incidence of human cases, causation is predicated upon spraying having a direct effect on mosquito populations. Recent work showed that adulticiding immediately reduced abundance and infection rates of *Culex* spp. mosquitoes compared with rates in an untreated area (*8*). Using factorial 2-way analysis of variance, these researchers compared mean abundances of *Cx. pipiens* and *Cx. tarsalis* from CO_2-baited traps (46 trap nights) in the northern treated area with mean abundances from traps (55 trap nights) in similar urban-suburban habitats within the untreated area of Sacramento County and adjacent Yolo County, 1 week before and 1 week after the August 8 spraying. Abundance of *Cx. pipiens* decreased by 75.0%, and there was a significant interaction between adulticiding and temporal classification (F 4.965, df 1,47, p = 0.031).

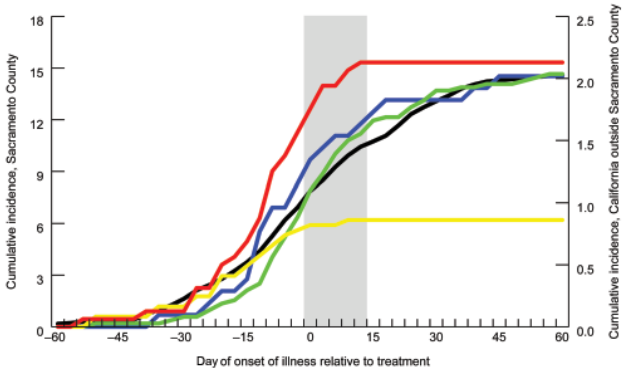

Figure 4. Cumulative incidence of human cases of West Nile virus (WNV) in Sacramento County and California, 2005. Only cases with known date of onset of illness and location information (i.e., Sacramento County at the address level and California at the county level) are included in the analysis. Cumulative incidence is the total no. WNV cases/100,000 population. Green line shows incidence within untreated area; red line shows incidence within northern treated area; yellow line shows incidence within southern treated area; blue line shows incidence within northern and southern buffer zones combined; black line shows incidence within California, excluding Sacramento County. Values along the x-axis (days) are grouped into sets of 3 and labeled with the date farthest from 0. Each of the 3 days of adulticiding within the treated areas and buffer zones was considered to be 0; for the untreated area and the rest of California, the dates of the northern adulticiding (August 8–10) were considered to be 0. The wide gray vertical band represents time from the first day of treatment to the maximum range of the human WNV incubation period 14 days later.

Abundance of *Cx. tarsalis* decreased by 48.7% but the interaction was not statistically significant (F 0.754, df 1,47, p = 0.390). As stated by these researchers, this disparity may have been caused by the presence of "an increasing population of *Cx. pipiens* and an already declining population of *Cx. tarsalis*" at the time of the spraying, and because *Cx. tarsalis* breeds principally in rural areas. Regardless, we reason that *Cx. pipiens* was the primary vector in the Sacramento County epidemic because this species is the principal urban vector in this region (*8–10*), was the most abundant species collected in Sacramento County in 2005 (D.-E.A. Elnaiem, unpub. data), and comprised the highest percentage of WNV-infected mosquito pools (68.3% versus 28.8% for *Cx. tarsalis*) in Sacramento County that same year (*10*).

Additionally, these researchers combined mosquitoes of both species (into pools of ≤50 females) taken from aforementioned traps and others in the northern treated area and untreated area 2 weeks before and 2 weeks after the August 8 adulticiding. Pools of mosquitoes were tested for WNV by using a reverse transcription–polymerase chain reaction, and infection rates were calculated by using a bias-corrected maximum likelihood estimation (www.cdc.gov/ncidod/dvbid/westnile/software.htm). After spraying, infection rates decreased from 8.2 (95% CI 3.1–18.0) to 4.3 (95% CI 0.3–20.3) per 1,000 females in the spray area and increased from 2.0 (95% CI 0.1–9.7) to 8.7 (95% CI 3.3–18.9) per 1,000 females in the untreated area. Furthermore, no additional positive pools were detected in the northern treatment area during the remainder of the year, whereas positive pools were detected in the untreated area until the end of September (D.-E.A. Elnaiem, unpub. data). These independent lines of evidence corroborate our conclusion that actions taken by SYMVCD were effective in disrupting the WNV transmission cycle and reducing human illness and potential deaths associated with WNV.

Historically, human WNV cases in the United States peak in August (*37,38*). This pattern was observed in Sacramento County and the rest of California in 2005, in which 61% (93/152) and 47% (314/670), respectively, of human cases had onset of illness in August. The next highest month was July, during which 27% (41/152) and 29% (195/670) of human cases had onset of illness in the county and the rest of the state, respectively. These findings are consistent with others from Sacramento County in 2005, which indicated that mosquito infection rates peaked in July and August (*10*). Considering early summer amplification within vector populations and length of the human incubation period, WNV remediation efforts would be more effective in limiting illness and death associated with human infection if conducted at the onset of enzootic amplification rather than after occurrence of human cases.

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References

1. Hayes EB, Sejvar JJ, Saki SR, Lanciotti RS, Bode AV, Campbell GL. Virology, pathology, and clinical manifestations of West Nile virus disease. Emerg Infect Dis. 2005;11:1174–9.
2. California Department of Health Services, Vector-Borne Disease Section. Vector-borne diseases in California, 2003: annual report. Sacramento (CA): The Department. 2004 Aug [cited 2007 Jul 25]. Available from http://www.dhs.ca.gov/PS/dcdc/disb/pdf/Vector-Borne%20Diseases%20in%20California,%20Annual%20Report%202003.pdf
3. California Department of Health Services, Vector-Borne Disease Section. Vector-borne diseases in California, 2004: annual report. Sacramento (CA): The Department. 2005 Aug [cited 2007 Jul 25]. Available from http://www.dhs.ca.gov/dcdc/disb/pdf/2004%20VBDS%20Annual%20Report%20final.pdf
4. California Department of Health Services. California's human West Nile virus (WNV) cases, 2004. Updated 2007 Feb 9. Sacramento (CA): The Department; 2005 [cited 2007 Mar 19]. Available from http://westnile.ca.gov/website/maps_data/2004_maps_data/humsum.pdf
5. California Department of Health Services. Human West Nile virus (WNV) cases in California, 2005. Updated 2006 Mar 30. Sacramento (CA): The Department; 2006 [cited 2007 Mar 21]. Available from http://westnile.ca.gov/website/maps_data/2005_maps_data/2005%20Human%20WNV%20Case%20Linelist.pdf
6. Centers for Disease Control and Prevention. West Nile virus activity in the United States. Updated 2007 Feb 23. Atlanta: The Centers; 2006 [cited 2007 Apr 11]. Available from http://www.cdc.gov/ncidod/dvbid/westnile/surv&controlCaseCount05_detailed.htm
7. US Geological Survey. West Nile virus, human, 2005. Updated 2007 May 1. Reston (VA): The Survey; 2006 [cited 2007 Mar 2]. Available from http://diseasemaps.usgs.gov/2005/wnv/wnv_us_human.html
8. Elnaiem DA, Kelley K, Wright S, Laffey R, Yoshimura G, Reed M, et al. Impact of aerial spraying of pyrethrin insecticide on *Culex pipiens* and *Culex tarsalis* (Diptera: Culicidae) abundance and West Nile virus infection rates in an urban/suburban area of Sacramento County, California. J Med Entomol. In press.

9. Nielsen CF, Armijos MV, Wheeler S, Carpenter TE, Boyce WM, Kelley K, et al. Risk factors associated with human infection during the 2006 West Nile virus outbreak in Davis, a residential community in northern California. Am J Trop Med Hyg. 2008;78:53–62.
10. Elnaiem DA, Kelley K, Wright S, Laffey R, Yoshimura G, Armijos V, et al. Epidemic amplification of West Nile virus in Sacramento and Yolo counties, June–September 2005. Proceedings of the California Mosquito Vector Control Association. 2006;74:18–20.
11. Boyce K. Mosquito and mosquito-borne disease management plan. Elk Grove (CA): Sacramento-Yolo Mosquito and Vector Control District. 2005 [cited 2007 Jul 25]. Available from http://www.fightthebite.net/download/Mosquito_Management_Plan.pdf
12. California Department of Health Services. Mosquito and Vector Control Association of California; University of California. California Mosquito-borne Virus Surveillance and Response Plan. Sacramento (CA): The Department. Vector-Borne Disease Section. 2005 Jun [cited 2007 Mar 21]. Available from http://westnile.ca.gov/website/publications/2005_ca_mosq_response_plan.pdf
13. Barker CM, Reisen WK, Kramer VL. California State Mosquito-borne Virus Surveillance and Response Plan: a retrospective evaluation using conditional simulations. Am J Trop Med Hyg. 2003;68:508–18.
14. Weston DP, Amweg EL, Mekebri A, Ogle RS, Lydy MJ. Aquatic effects of aerial spraying for mosquito control over an urban area. Environ Sci Technol. 2006;40:5817–22.
15. Herms WB. Medical entomology, with special reference to the health and well-being of man and animals. 4th ed. New York: Macmillan; 1950.
16. Hopkins CC, Hollinger FB, Johnson RF, Dewlett HJ, Newhouse VF, Chamberlain RW. The epidemiology of St. Louis encephalitis in Dallas, Texas, 1966. Am J Epidemiol. 1975;102:1–15.
17. Palmisano CT, Taylor V, Caillouet K, Byrd B, Wesson DM. Impact of West Nile virus outbreak upon St. Tammany Parish mosquito abatement district. J Am Mosq Control Assoc. 2005;21:33–8.
18. Williams KH, Hollinger FB, Metzger WR, Hopkins CC, Chamberlain RW. The epidemiology of St. Louis encephalitis in Corpus Christi, Texas, 1966. Am J Epidemiol. 1975;102:16–24.
19. Hayes RO, Holden P, Mitchell CJ. Effects of ultra-low volume applications of malathion in Hale County, Texas. IV. Arbovirus studies. J Med Entomol. 1971;8:183–8.
20. US Census Bureau. UA Census 2000 TIGER/Line Files. Redlands (CA): Environmental Systems Research Institute, Inc.; 2007 [cited 2007 Aug 9]. Available from http://arcdata.esri.com/data/tiger2000/tiger_download.cfm
21. Miller CL. Census 2000 TIGER/line files technical documentation. Washington: US Census Bureau; 2002 [cited 2007 Aug 9]. Available from http://www.census.gov/geo/www/tiger/tigerua/ua2ktgr.pdf
22. Petersen LR, Marfin AA. West Nile virus: a primer for the clinician. Ann Intern Med. 2002;137:173–9.
23. Centers for Disease Control and Prevention. West Nile virus: clinical description. 2004 Sep 29 [cited 2007 Mar 16]. Available from http://www.cdc.gov/ncidod/dvbid/westnile/clinicians/pdf/wnv-clinicaldescription.pdf
24. Szklo M, Nieto FJ. Epidemiology: beyond the basics. 2nd ed. Sudbury (MA): Jones and Bartlett; 2007.
25. Ruiz MO, Tedesco C, McTighe TJ, Austin C, Kitron U. Environmental and social determinants of human risk during a West Nile virus outbreak in the greater Chicago area, 2002. Int J Health Geogr. 2004;3:8.
26. Gaulin C, Couillard M, Pilon PA, Tremblay M, Lambert L, Fradet MD, et al. Assessment of surveillance of human West Nile virus infection in Quebec, 2003. Can Commun Dis Rep. 2004;30:97–104.
27. Theophilides CN, Ahearn SC, Grady S, Merlino M. Identifying West Nile virus risk areas: the dynamic continuous-area space-time system. Am J Epidemiol. 2003;157:843–54.
28. Theophilides CN, Ahearn SC, Binkowski ES, Paul WS, Gibs K. First evidence of West Nile virus amplification and relationship to human infections. International Journal of Geographical Information Science. 2006;20:103–15.
29. Warner RD, Kimbrough RC, Alexander JL, Rush Pierce J Jr, Ward T, Martinelli LP. Human West Nile virus neuroinvasive disease in Texas, 2003 epidemic: regional differences. Ann Epidemiol. 2006;16:749–55.
30. Watson JT, Jones RC, Gibbs K, Paul W. Dead crow reports and location of human West Nile virus cases, Chicago, 2002. Emerg Infect Dis. 2004;10:938–40.
31. Mostashari F, Bunning ML, Kitsutani PT, Singer DA, Nash D, Cooper MJ, et al. Epidemic West Nile encephalitis, New York, 1999: results of a household-based seroepidemiological survey. Lancet. 2001;358:261–4.
32. Dow RP, Reeves WC, Bellamy RE. Dispersal of female *Culex tarsalis* into a larvicided area. Am J Trop Med Hyg. 1965;14:656–70.
33. Mitchell CJ, Kilpatrick JW, Hayes RO, Curry HW. Effects of ultra-low volume applications of malathion in Hale County, Texas. II. Mosquito populations in treated and untreated areas. J Med Entomol. 1970;7:85–91.
34. Mitchell CJ, Hayes RO, Holden P, Hill HR, Hughes TB Jr. Effects of ultra-low volume applications of malathion in Hale County, Texas. I. Western encephalitis virus activity in treated and untreated towns. J Med Entomol. 1969;6:155–62.
35. Reisen WK, Milby MM, Meyer RP. Population dynamics of adult *Culex* mosquitoes (Diptera: Culicidae) along the Kern River, Kern County, California, in 1990. J Med Entomol. 1992;29:531–43.
36. Centers for Disease Control and Prevention. Epidemic/epizootic West Nile virus in the United States: guidelines for surveillance, prevention, and control. Fort Collins (CO): The Centers; 2003 [cited 2007 Mar 19]. Available from http://www.cdc.gov/ncidod/dvbid/westnile/resources/wnvguidelines2003.pdf
37. Marfin AA, Petersen LR, Eidson M, Miller J, Hadler J, Farello C, et al. Widespread West Nile virus activity, eastern United States, 2000. Emerg Infect Dis. 2001;7:730–5.
38. Centers for Disease Control and Prevention. West Nile virus activity—United States, January 1–December 1, 2005. MMWR Morb Mortal Wkly Rep. 2005;54:1253–6.

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Cryptococcus neoformans Strains and Infection in Apparently Immunocompetent Patients, China

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To determine the population structure of the cryptococcosis agents in China, we analyzed the genotype of 120 *Cryptococcus neoformans* and 9 *Cryptococcus gattii* strains isolated from 1980 through 2006 from cryptococcosis patients residing in 16 provinces of mainland China. A total of 71% (91/129) of the clinical strains isolated from 1985 through 2006 were from patients without any apparent risk factors. Only 8.5% (11/129) were from AIDS patients; the remaining 20.5% (27/129) were from patients with underlying diseases other than HIV infection. One hundred twenty of the 129 isolates were *C. neoformans* serotype A, mating type *MAT*α strains that exhibited an identical M13-based VNI subtype, which was distinguishable from the reference VNI molecular type. The 9 remaining isolates were serotype B, *MAT*α strains of *C. gattii* and portrayed a typical VGI molecular type. Data analyzed from multilocus sequences showed no variation and that these Chinese *C. neoformans* isolates belong to a cluster that has phylogenetically diverged from the VNI reference strain. Our finding that most cryptococcosis patients in China had no apparent risk factor is in stark contrast with reports from other countries.

Cryptococcosis is caused by 2 species in the genus *Cryptococcus*, *C. neoformans* and *C. gattii* (*1*). *C. neoformans* (serotypes A, D, and AD) is found worldwide and causes cryptococcosis most frequently in AIDS patients (*2*,*3*). *C. gattii* (serotypes B and C) is geographically restricted and is infrequently diagnosed in AIDS patients except in some areas of Africa (*4*). The most widely used approaches for cryptococcal strain typing include the following: PCR fingerprint analysis based on microsatellite DNA using M13 primers (*5*,*6*) and $(GACA)_4$ repeats (*7*), amplified fragment length polymorphisms (*8*), sequence analysis of the rDNA intergenic spacer regions I and II (IGSI and IGSII) (*9*), and multilocus gene sequence typing (MLST) (*10*). These molecular approaches showed genetically distinguishable subgroups for each serotype. For example, phylogenetic analysis of the IGS1-5.8S rDNA-IGSII sequence showed 3 genotypes, 1a, 1b, and 1c, among the strains of serotype A collected worldwide (*9*). Likewise, PCR fingerprint patterns based on M13 microsatellite DNA identified 2 major genotypes among the strains of serotype A. In this group, the VNI strain type was the most common genotype found worldwide (*5*). Furthermore, MLST showed 3 genetic subpopulations among strains of serotype A as well as subpopulations unique to certain geographic areas as was the case with strains from Botswana (VNB) in Africa (*10*). A *C. neoformans* genotype unique to Botswana showed epidemiologic importance of strain type. Rare genotypes of *C. gattii* have been reported from the recent outbreak of cryptococcosis on Vancouver Island in Canada (*11*). Finding *C. gattii* strains of a rare genotype in geographic areas outside of subtropical and tropical zones (*12*) further underscores the epidemiologic importance of this rare genotype.

In spite of extensive global strain typing, isolates from the People's Republic of China have rarely been included (*5*,*8*,*9*). Recently, data released by the Chinese Department of Health indicated that by October 2006, the total number of HIV patients and those identified as AIDS patients was 183,733 and 40,667, respectively (www.chinacdc.net.cn/n272442/n272530/n294176/n340510/15099.html).

From 1999 through 2006, a total of 224 articles on cryptococcosis have been published in Chinese journals; 2,850 case-patients were included. We cited 6 representative reports from these papers (*13*–*18*). In these cases, only 69 strains (0.2 %) were associated with AIDS. The popu-

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lation structure of *C. neoformans* in China, however, has not been fully explored. In this study, we have used various molecular typing approaches to analyze the population structure of the cryptococcosis agent in China.

Materials and Methods

Cryptococcal Isolates

A total of 129 strains isolated from patients included 120 *C. neoformans* and 9 *C. gattii* (online Appendix Table, available from www.cdc.gov/EID/content/14/5/755-appT.htm). The identities of all strains were confirmed according to routine diagnostic tests (*2*). L-canavanine-glycine-bromothymol blue medium was used to differentiate the 2 species (*19*). We used a set of strains representing the known molecular types within the 2 species as a reference: WM 148 (serotype A, VNI), WM 626 (serotype A, VNII), Bt 63 (serotype A, Botswana), WM 628 (serotype D, VNIII), WM629 (serotype AD, VNIV), WM 179 (serotype B, VGI), WM 178 (serotype B, VGII), WM 161 (serotype B, VGIII) and WM 779 (serotype C, VGIV)(6). The strain H99 (serotype A) was also included (*20*). The isolates were stored in 25% glycerol at –80°C until use and were maintained on yeast peptone dextrose (1% yeast extract, 2% peptone, 2% glucose) agar at 25°C during this study.

Serotype and Mating Type

The CryptoCheck kit (Iatron, Tokyo, Japan) was used for serotyping. Mating type was determined by crossing all the *C. neoformans* strains with B-3501 (*MATα*) and JEC20 (*MATa*) and *C. gattii* strains with NIH112 (*MATα*) and NIH198 (*MATa*) on V-8 agar (*11*,*21*). A few strains of *C. neoformans* that showed ambiguous mating results were analyzed by PCR using serotype A *MATα* and *MATa* allele specific primers of either the *STE12* or *STE20* gene (*22*).Yeast cultures were grown overnight in yeast extract glucose broth (0.5% yeast extract, 2% glucose) at 30°C. Genomic DNA was extracted from all strains as previously described (*23*).

Genotyping

Genotyping approaches included PCR and DNA sequencing. PCR fingerprint analysis was based on M13 primers for microsatellite DNA as well as primers containing sequence repeats of $(GACA)_4$ and *URA5*–restriction fragment length polymorphism (RFLP). Sequencing was performed for partial IGSI-5.8S-IGSII region of rDNA cluster, and MLST involving 8 genes located on 7 different chromosomes (*10*). PCR fingerprints using either the microsatellite-specific primer M13 (5′-GAGGGTGGCGGTTCT-3′) (*5*) or the $(GACA)_4$ sequence repeats (*7*) were generated as described. The *URA5*-RFLP profiles were generated after digestion of the PCR fragment containing the *URA5* gene sequence with *Sau*961 and *Hha*1 (*6*). IGSI sequence analysis of the LrDNA gene was derived from PCR amplicon products (≈1.7 kb) generated by the primer pair combination LR11 (5′-TTACCACAGGGATAACTGGC-3′) and 5SR (5′-GGATCGGACGGGGCAGGGTGC-3′) (*9*). The amplification reactions were carried out in microtubes at a final volume of 50 μL. The PCR mix contained 50–100 ng of genomic DNA, 0.5 mmol/L of the forward and reverse primer pairs, 1.0 U DyNAzyme II polymerase, 1.5 mmol/L $MgCl_2$, and 200 mmol/L of each dNTP. The reaction was performed in an MJ Research PTC-100 thermocycler (GMI, Inc., Ramsey, MN, USA) and consisted of a denaturation step at 94°C for 1 min, followed by 40 cycles: 2 min of denaturation at 95°C, 1 min of annealing at 57°C, and 3 min of extension at 72°C. A final elongation step was conducted at 72°C for 7 min. The amplicon product was purified with QIAquick PCR Purification Kit (QIAGEN, Valencia, CA, USA). Amplicon synthesis was confirmed by agarose gel electrophoresis. Sequencing reactions used the forward primer IGS1F (5′-CAG ACGACTTGAATGGGAACG-3′), located at positions 3613–3633 of the LrRNA region and the reverse primer IG2R (5′-ATG CAT AGA AAG CTG TTG G-3′), located at position 791 of the IGS1 region. Sequencing reactions were carried out with an ABI 3730 capillary sequencer (Applied Biosystems, Foster City, CA, USA) and followed the protocol described by Diaz et al. (*9*). Sequencing alignments of the IGS region were executed with MegAlign (DNASTAR, Inc., Madison, WI, USA) and visually corrected. Phylogenetic tree construction used PAUP* version 4.0 (*24*) with parsimony analysis (heuristic search, stepwise addition, random addition sequence, nearest neighbor interchange, 100 maximum tree). Reliability of the character was checked by using bootstrap analysis with 500 replications.

Construction of Dendrogram Based on M13 RFLP

To construct a dendrogram based on M13-generated DNA fingerprints, 12 strains representing the different provinces of China were chosen, along with 6 reference strains. The PCR product of the reference strain VNI was used twice in the panel (adjacent to the size marker and in the last lane). A total of 15 major bands that ranged in size from 3,054 bp to 506 bp were identified across the lanes. Data were coded as 1 and 0; 1 represented the presence of a band and 0, its absence. This data matrix was coded into NEXUS format for input into the program PAUP* (*24*). PAUP* was used to generate a maximum parsimony phylogeny using a heuristic search algorithm. For the heuristic search, 10 random-addition replicates were performed. The starting tree for each replicate was obtained by stepwise addition. Seven replicates returned the same most parsimonious tree, which had a tree score of 18 steps. To quantify clade robustness, 500 nonparametric bootstrap replicates

were performed. Bootstrap percentages >50% are indicated above the branches of the maximum parsimony phylogenetic tree. The tree figure for the maximum parsimony analysis was created using FigTree version 1.0 (http://tree.bio.ed.ac.uk/software/figtree).

MLST

For MLST analysis, DNA fragments of 8 unlinked genes that included *CAP10, GPD, IGS1, LAC1, PLB1, SOD1, TEF1,* and *URE1,* were amplified by PCR (*10*) from 6 randomly chosen *C. neoformans* strains from China. DNA sequencing was carried out by using the dideoxy method, and sequences were compared with previously published sequences from the global collection of serotype A strains (*10*). To visualize the genetic relationships among different MLST genotypes, sequences were automatically aligned by using Sequencher 4.1 (Gene Codes Corp., Ann Arbor, MI, USA); the alignment was imported into MacClade 4.05 and edited manually. Because of the observed incongruence in the genealogies of several genes, combined sequence data for all 88 worldwide isolates (*10*) were analyzed with the neighbor-joining method using uncorrected ("p") genetic distances. Statistical support for the phylogenetic groups was assessed by bootstrap analysis using 1,000 replicate data sets.

Virulence Study

Three Chinese strains of *C. neoformans*, CHC114, CHC186, and CHC193, were compared for their virulence in mice with 3 serotype A reference strains, H99, WM148 (VNI), and WM626 (VNII). Ten 9-week old female BALB/c mice were infected intranasally with 5×10^7 cells of each strain (*23*) and were monitored for survival.

Results

The conditions of most patients (81.3%) were diagnosed at Shanghai Changzheng Hospital; the remaining patients (18.6%) were diagnosed at other hospitals. Patients without any recognizable predisposing factor for cryptococcosis such as HIV infection, malignancies, cirrhosis, organ transplantation, end-stage renal failure, autoimmune disorder, diabetes mellitus, idiopathic CD4 T-cell lymphopenia, sarcoidosis, chronic usage of corticosteroids or other immunosuppressive therapies, and any abnormal symptoms were regarded as patients "without apparent risk factors" (*2*). Since 2002, the period when isolates were obtained from 68% of the patients without apparent risk factors, HIV serologic testing and a battery of immunologic tests were performed on all cryptococcosis patients. Before 2002, each patient's cellular and humoral immune status was routinely determined. Any abnormality in these tests led to further HIV serology. The patients who had otherwise normal test results were also subjected to HIV serologic testing, the results of which were negative.

Of the 120 *C. neoformans* strains obtained from 16 provinces located in the middle to the eastern regions of mainland China (Figure 1), 84 (70 %) strains were isolated from apparently healthy patients and 27 (22.5 %) strains were isolated from patients with risk factors other than HIV infection (online Appendix Table). Only 9 (7.5 %) of the *C. neoformans* strains were isolated from AIDS patients. All 120 isolates were analyzed by PCR fingerprints using M13 primers, by *URA5* RFLP patterns, and by $(GACA)_4$ sequence primers. Notably, all 120 strains of *C. neoformans* yielded an identical M13-based fingerprint pattern that could be distinguished from the reference types. In Figure 2, the M13 fingerprint patterns of 12 of the 120 strains are shown as examples along with the 6 reference strains. The major bands pattern for the serotype A isolates from China was more similar to the VNI than the other reference types (Figure 2, panel A). The *URA5* RFLP (Figure 2, panel B) and $(GACA)_4$ patterns of 120 isolates, however, were identical to that of the VNI type (online Appendix Figure 1, available from www.cdc.gov/EID/content/14/5/755-appG1.htm).

The phylogenetic tree for maximum parsimony analysis showed the strains to be closely related to the reference strain VNI and the H99 strain (Figure 3). The H99 strain had the same M13 fingerprint pattern as that of the VNI strain. Genotyping by sequence analysis of the IGSI-rDNA region indicated that the *C. neoformans* strains from China belonged to the *C. neoformans* genotype 1a (online Appendix Figure 2, available from www.cdc.gov/EID/content/14/5/755-appG2.htm). Genotype 1a is the major genotype found among the serotype A strains of *C. neoformans* collected worldwide and follows a clonal pattern (*9*).

MLST, performed using 8 unlinked genes from 6 randomly chosen strains, showed identical sequences for the *CAP10, GPD, LAC1, PLB1, SOD1, IGSI, TEF1*, and *URE1* genes. These results corroborate the homogeneity observed with various PCR fingerprint patterns. Notably, the Chinese strains of *C. neoformans* formed a cluster with 7 strains previously reported by Litvintseva et al.

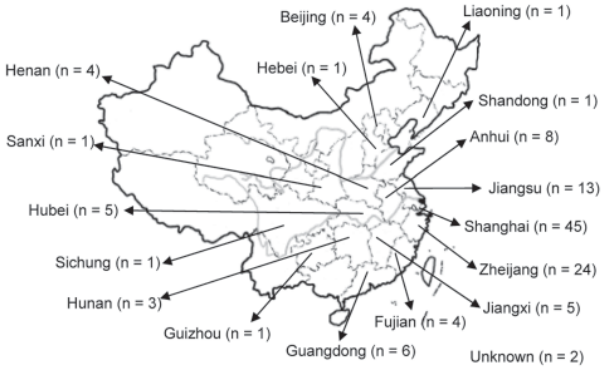

Figure 1. Mainland China. The numbers in parentheses represent strains used in this study that were isolated during 1980–2006 from each region.

Table. Information on 7 *Cryptococcus neoformans* strains previously determined as M5 of MLST*

Strain	Geographic origin	Source	MAT	Year	MLST type	VN type	Reference
Jt743	Italy	Unknown	α	Unknown	M5	VNI	S. Maesaki
Jp1086	Japan	Human lung	α	1999	M5	VNI	S. Maesaki
Jp1088	Japan	Human lung	α	1999	M5	VNI	(*10*)
mal 212 i	Malawi	CSF	α	1999	M5	VNI	(*25*)
C48	USA	Bronchial lavage	α	2001	M5	VNI	(*10*)
C8	USA	CSF	α	2001	M5	VNI	(*10*)
A5 36–17	USA	Pigeon excreta	α	2002	M5	VNI	(*10*)

*MLST, multilocus sequence typing; MAT, mating type; CSF, cerebrospinal fluid.

(Table) (*10*) and formed a distinct cluster, M5, that appeared to have diverged from the M1 genotype to which the VNI reference strain belongs (Figure 4). Two strains in the M5 that clustered with the Chinese strains, jp1086 and jp1088, were isolated in Japan and the M-13 fingerprint pattern of all 7 strains (Table) tested was identical to that of the Chinese strains (online Appendix Figure 3, available from www.cdc.gov/EID/content/14/5/755-appG3.htm). Our genotyping data indicates that M13-based PCR fingerprinting together with the MLST are powerful tools that enable discrimination of different strain types. The 3 Chinese *C. neoformans* strains randomly chosen to assess virulence were considerably less virulent than the H99 strain and moderately to significantly more virulent than VNI and VNII reference strains (Figure 5).

The H99 strain, the type strain of *C. neoformans* var. *grubii* (*26*), clustered with VNI strain based on the MLST data. The previous MLST tree, which did not place the H99 strain in the same cluster, A1 + A3 (= M1), as VNI was determined to be due to an error introduced during the sequencing process (*10*). The sequences from 8 genes that we sequenced matched 100% with the nucleotide sequence data posted in the H99 genomic database.

Nine isolates of *C. gattii* were all obtained from the eastern regions of China. Except for 1 strain from Shangdong, and 1 from Shanghai, all strains were recovered from provinces located south of Shanghai with a warm climate. Five (55%) of the 9 strains of *C. gattii* were from the Zhejiang province where *Eucalyptus* trees are commonly found. Of the 9 *C. gattii* strains, 2 were isolated from AIDS patients and 7 were isolated from otherwise normal patients. All of the *C. gattii* strains were serotype B, of *MATα* mating type with typical PCR patterns of the VGI type (Figure 6). IGS sequences from 4 randomly chosen strains belonged to the genotype 4b (*9*).

Discussion

From 1985 to 2007, a total of 7,372 cases of cryptococcosis were reported in China. However, documentation on these patients is mostly unavailable, and fragmented documentation is available for only 1,999 cases. Among them, 323 (16.2%) cases seem to have occurred in patients with no underlying disease and who were considered otherwise healthy; 215 (10.8%) cases occurred in AIDS patients (Z. Yao et al., unpub. data).

This study provides a large scale population analysis of *C. neoformans* strains isolated from 129 patients with well-documented clinical cases of cryptococcosis that were treated at the Shanhai Changzheng Hospital. All patients were assessed for the common predisposing factors for cryptococcosis. Patients without any of these predisposing factors were regarded as "with no apparent risk factor." HIV serology was performed for all cases that have occurred since 2002. A total of 68% (56/82) of the patients had no underlying disease. Patient outcomes were monitored for 2 years following treatment at which time patients were determined to be cured of cryptococcosis.

Consistent with previous reports, all 120 clinical strains of *C. neoformans* isolated from China were serotype A and *MATα* (*2*). In contrast to the genotypic diversity of clinical *C. neoformans* serotype A strains found in other countries, such as Brazil, Australia, and the United States (*5*,*27*), the

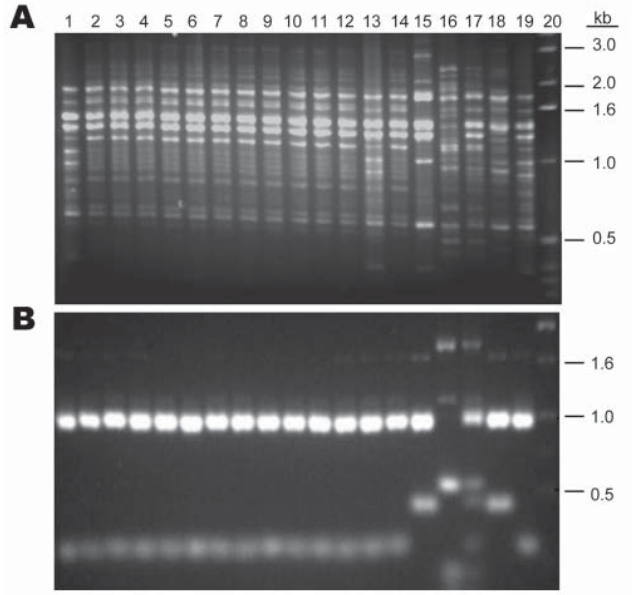

Figure 2. DNA fingerprint patterns of 12 *Cryptococcus neoformans* strains from China and the molecular type reference strains. A) M13-based PCR pattern. B) URA5 restriction fragment-length polymorphism. Lanes: 1, VNI; 2–12, 11 Chinese strains; 13, H99; 14, Chinese strain B-4587; 15, VNBt63; 16, VNI; 17, VNIII; 18, VNII; 19, VNI; 20, marker.

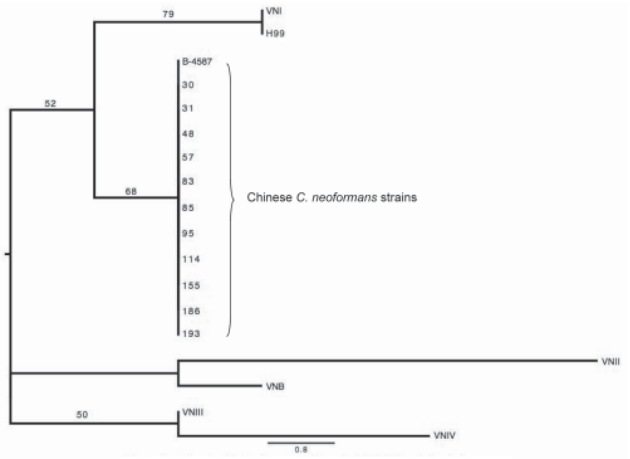

Figure 3. The phylogenetic tree for maximum parsimony analysis composed on the basis of the M13-based PCR pattern of 12 Chinese *Cryptococcus neoformans* strains. Numbers above the branches represent bootstrap support percentages based on 500 replicates. The scale bar represents the inferred number of steps along a branch of the tree.

Chinese *C. neoformans* strains showed remarkable genetic homogeneity. This was evident not only in the patterns based on various PCR fingerprints but also by the lack of diversity in MLST results. A report from India also showed relatively low genetic diversity among 57 clinical *C. neoformans* isolates. However, a serotype D strain was found, indicating that the population structure of *C. neoformans* in India is less homogeneous than that in China (*28*). Remarkably, a majority of the Chinese strains caused cryptococcosis in persons without any recognizable immune defect or underlying disease. Although a considerable number of AIDS patients have been identified, 8.5% of the 129 strains isolated across mainland China were recovered from AIDS patients; 71% were isolated from otherwise healthy persons. This is in stark contrast to the >80% AIDS-associated cryptococcosis cases reported in Europe and the United States and the 69% reported in Africa (*29–31*). Although markedly lower than the frequency in China, a relatively high number of cases of non-AIDS associated cryptococcosis due to *C. neoformans* were documented in Australia, New Zealand (*32*), and India (*28*). Most patients in Australia and New Zealand, however, were immunocompromised (*32*). The report from India showed that 41% of the cryptococcosis patients also had HIV infections; the remaining patients were determined to have no known immune defect (*28*). Information on the underlying diseases in these HIV-free Indian patients, however, was not recorded.

We recognize the possibility that 71% of the Chinese cryptococcosis patients without apparent underlying disease may have had subtle defects in immunity that may have predisposed them to cryptococcosis. Alternatively, genetic factors may play an important role in the unusually high non–AIDS-associated cryptococcosis in China. A relationship between common functional genetic polymorphisms of the low-affinity Fc gamma receptor genes, FCGR2A, -3A, and -3B, and the risk of cryptococcosis in HIV-uninfected patients was recently reported (*33*). It would be of interest to investigate the relationship between the genetic polymorphisms of the 3 genes in the immunocompetent Chinese cryptococcocis patients.

The M13 based PCR fingerprints of the Chinese *C. neoformans* strains were identical to each other and similar but distinguishable from VNI. For convenience throughout the discussion, we will hereafter refer to the M13 pattern of the Chinese strains as VNIc. The MLST-based phylogenetic tree also showed that the VNIc diverged from WM148 and formed a separate cluster with 7 previously analyzed strains (*10*). The 7 strains that cluster with the VNIc strains had originated from 3 different continents, which suggests that the VNIc type is not unique to China and apparently follows a widespread cosmopolitan distribution. We found that the 7 strains that cluster with Chinese strains have exactly the same M13 PCR pattern as the VNIc type. Whether most of these 8 strains were also from immunocompetent patients is not known. Since analysis of the IGSI region of

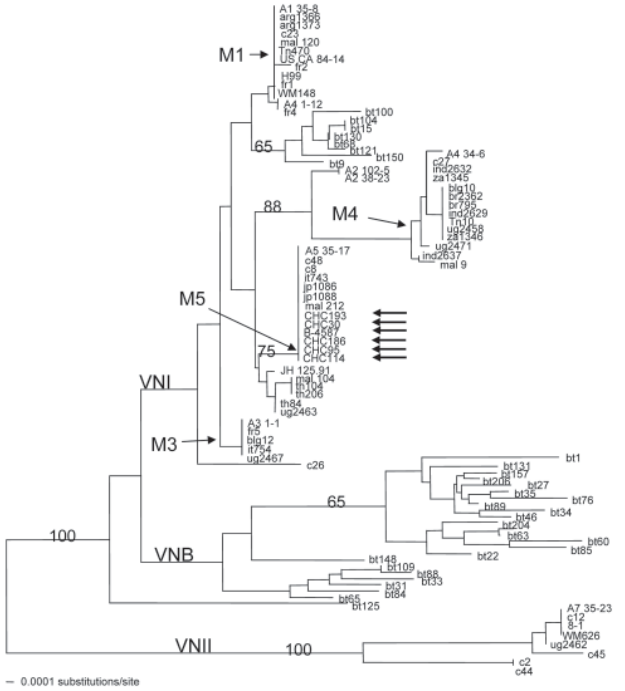

Figure 4. Genetic relationship of multilocus sequence typing (MLST) genotypes among 94 isolates of *Cryptococcus neoformans* serotype A (88 strains from Litvintseva et al. [*10*]) and 6 representative Chinese strains) visualized by the neighbor-joining dendrogram. Numbers on each branch indicate the bootstrap values >50%, based on 500 replicates. Vertical lines represent strains with identical genotypes. Arrows indicate MLST results for Chinese strains.

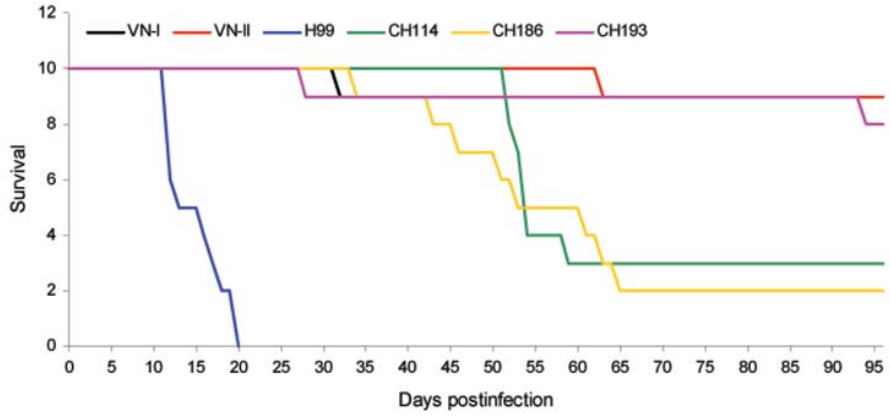

Figure 5. Virulence in mice. Mice were intranasally infected with 5×10^7 yeast cells from the 3 *Cryptococcus neoformans* Chinese strains, CHC114, CHC193, and CHC 186, and compared with H99 and the reference strains VNI and VNII for survival.

the VNIc strains showed it to be identical with WM148, the M13 fingerprint analysis appears to have greater discriminatory power in distinguishing the VNIc strains from the typical VNI type than IGSI analysis. The *URA5* RFLP analysis or the $(GACA)_4$-based PCR fingerprints also had lower resolution power than the M13 pattern analysis because the 2 methods could not distinguish VNIc from the WM148 strain.

We do not know if VNIc is the most common type in China because the present study only analyzed clinical strains. Environmental strains should also be isolated and undergo molecular typing. Such a study would offer insight into the importance of the VNIc strain type in relation to infection in otherwise healthy persons in China. In general, the occurrence of cryptococcosis in patients without any apparent immune defect or underlying disease is relatively rare (*2*,*34*). In the United States, the incidence of cryptococcosis in immunocompetent patients has been estimated at 0.2/1,000,000 population per year in California (*35*); 0.9/100,000 in Atlanta, Georgia; and 0.93/100,000 in Alabama (*36*).

The presence of the VGI type in China is not surprising because it is the most common *C. gattii* strain type identified in Southeast Asia and most of the *C. gattii* strains obtained were from the southeastern part of China. Although the total number of the *C. gattii* strains studied is small, the percentage of AIDS-associated *C. gattii* infections in China is higher than expected. Other than from certain geographic areas in Africa (*4*), *C. gattii* strains are rarely isolated from AIDS patients (*2*,*32*). In Australia where *C. gattii* is prevalent, for example, only 1 of 47 clinical strains of *C. gattii* was isolated from a patient with AIDS (*32*).

In our previous study, the urease-negative strain B-4578, which had been isolated from a cryptococcosis patient in China, was as virulent in mice as was the highly virulent H99 strain (*23*). Because the strain B-4578 was also of the VNIc molecular type, it was tempting to assume that strains isolated from immunocompetent patients in China would similarly be highly virulent in experimental animals. Indeed, the 3 strains of the VNIc type isolated from different provinces tested in mice were more virulent than the VNI reference strain. However, they were considerably less virulent than the H99 strain, which had been isolated from an immunocompromised patient (*20*). This suggests that a wide variation in virulence exists among the strains of the VNIc molecular type. Whether the degree of cryptococcal strain virulence manifested in mice is comparable to the human host remains unknown. Our experience with strain NIH12 has shown that virulence in mice does not necessarily correlate with that of the human host. The strain NIH12 is one of the most virulent serotype D strains tested in BALB/c mice (*37*); it only caused a chronic localized infection without dissemination in the human host. The patient infected by the NIH12 strain had sarcoidosis and a chronic, localized, osteomyelitis lesion later developed in the hip, which was cured by amphotericin B treatment without any dissemination or recurrence.

Because all 129 of the cryptococcal strains were isolated from Chinese, primarily immunocompetent, patients, one can ask whether any susceptibility difference to cryptococcosis is related to ethnicity. Australian studies have indicated a higher frequency of cryptococcosis in Aborigines (*38*) than in whites, and in Los Angeles, disease incidence was reported to be twice as frequent in Hispanics than in whites (*39*). However, data regarding ethnic differences in susceptibilities are scant and unconvincing. Since most of the 129 strains were isolated from non-AIDS patients in China, possible differences may exist in the reporting systems for cryptococcosis cases among AIDS patients and among non-AIDS patients. While some strains were isolated from patients in Henen, Yunan, and Xinjian provinces where HIV/AIDS was more prevalent, most strains were from patients in regions where HIV/AIDS was not prevalent. Although the HIV status of some otherwise apparently healthy patients was unknown, they did fully recover after antifungal treatment. The outcome of the AIDS patients with cryptococcosis, however, is unknown because they were transferred to a quarantined fa-

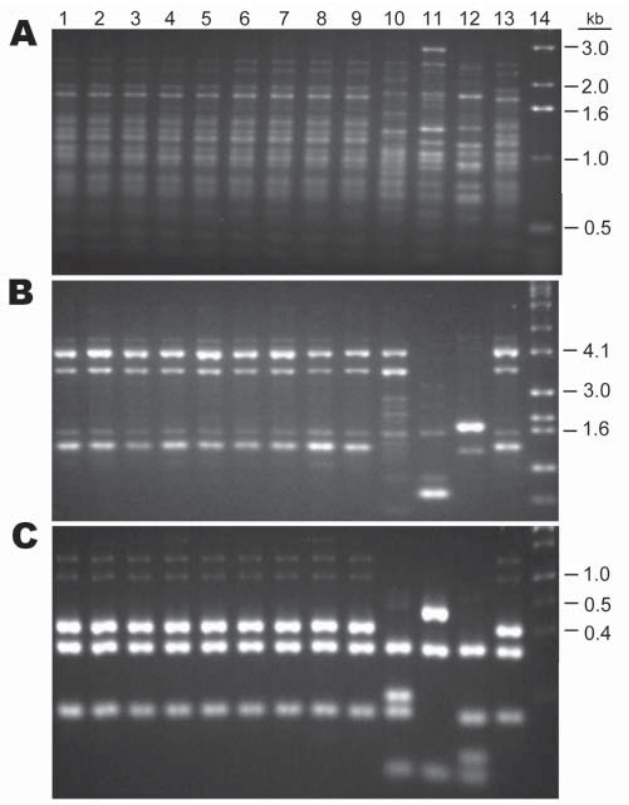

Figure 6. Comparison of the PCR patterns of 9 Chinese *Cryptococcus gattii* isolates with reference *C. gattii* strains. A) M13, B) $(GACA)_4$, C) URA5. Lanes: 1–9, Chinese strains; 10, VGIV; 11, VGIII; 12, VGII; 13, VGI; 14, marker.

cility soon after they were diagnosed to be HIV positive. To determine whether Chinese AIDS patients are more resistant to cryptococcosis than AIDS patients in other countries, differences in how AIDS patients are handled in China should be investigated.

In conclusion, this study reveals a strikingly homogeneous cryptococcal population belonging to a subtype of VNI in China. The high incidence of cryptococcosis in immunocompetent patients in China contrasts with reports from other countries.

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References

1. Kwon-Chung KJ, Varma A. Do major species concepts support one, two or more species within *Cryptococcus neoformans?* FEMS Yeast Res. 2006;6:574–84.
2. Kwon-Chung KJ, Bennett JE. Medical mycology. Philadelphia: Lea & Febiger; 1992.
3. Mitchell TG, Perfect JR. Cryptococcosis in the era of AIDS—100 years after the discovery of *Cryptococcus neoformans.* Clin Microbiol Rev. 1995;8:515–48.
4. Litvintseva AP, Thakur R, Reller LB, Mitchell TG. Prevalence of clinical isolates of *Cryptococcus gattii* serotype C among patients with AIDS in sub-Saharan Africa. Eukaryot Cell. 2005;192:888–92.
5. Meyer W, Marszewska K, Amirmostofian M, Igreja RP, Hardtke C, Methling K, et al. Molecular typing of global isolates of *Cryptococcus neoformans* var. *neoformans* by polymerase chain reaction fingerprinting and randomly amplified polymorphic DNA — a pilot study to standardize techniques on which to base a detailed epidemiological survey. Electrophoresis. 1999;20:1790–9.
6. Meyer W, Castaneda, A, Jackson, S, Huynh M, Castaneda E, IberoAmerican Cryptococcal Study Group. Molecular typing of IberoAmerican *Cryptococcus neoformans* isolates. Emerg Infect Dis. 2003;9:189–95.
7. Cogliati M, Allaria M, Tortorano AM, Viviani MA. Genotyping *Cryptococcus neoformans* with specific primers designed from PCR-fingerprinting bands sequenced using a modified PCR-based strategy. Med Mycol. 2000;38:97–103.
8. Boekhout T, Theelen B, Diaz M, Fell JW, Hop WC, Abeln EC, et al. Hybrid genotypes in the pathogenic yeast *Cryptococcus neoformans*. Microbiology. 2001;147:891–907.
9. Diaz MR, Boekhaut T, Kiesling T, Fell J. Comparative analysis of the intergenic spacer regions and population structure of the species complex of the pathogenic yeast *Cryptococcus neoformans*. FEMS Yeast Res. 2005;5:1129–40.
10. Litvintseva AP, Thakur R, Vilgalys R, Mitchell TG. Multilocus sequence typing reveals three genetic subpopulations of *Cryptococcus neoformans* var. *grubii* (serotype A), including a unique population in Botswana. Genetics. 2006;172:2223-38.
11. Kidd SE, Hagen F, Tscharke RL, Huynh M, Bartlett KH, Fyfe M, et al. A rare genotype of *Cryptococcus gattii* caused the cryptococcosis outbreak on Vancouver Island (British Columbia, Canada). Proc Natl Acad Sci U S A. 2004;101:17258–63.
12. Kwon-Chung KJ, Bennett JE. Epidemiologic differences between the two varieties of *Cryptococcus neoformans*. Am J Epidemiol. 1984;120:123–30.
13. Huang LB, Zhang ZJ, Xu GY, Murong SX. Clinical analysis of 32 cryptococcal meningitis patients [in Chinese]. J Clin Neurol. 2002;15:371–2.
14. Wu CH, Wang HY, Yang BS, Lu X, Wang JM, Lin JS. Analysis of the outcome of 46 patients with cryptococcal meningitis [in Chinese]. Zhonghua Shen Jing Ge Za Zhi. 2002;35:102.
15. Zu LP, Peng XZ, Shi YZ, Weng XH. One case of AIDS associated disseminated cryptococcosis [in Chinese]. Chin J Infect Dis. 2004;22:301.
16. Gua AH, Hu XQ. Clinical features of 101 cryptococcal meningitis [in Chinese]. Chin J Infect Dis . 2005;38:445–7.
17. Gu JL, Wen H, Chen JH, Chen YC, Li JC. Clinical analysis of 39 cryptococcal meningitis [in Chinese]. J Clin Dermatol. 2005;34:801–3.
18. Chen JH, Wen H, Chen SX, Wu JH, Zhu YJ, Xu H. The adverse events of amphotericin B and its management during the treatment of cryptococcal meningitis [in Chinese]. Acad J Second Military Med Univ. 2005;26:456–7.
19. Kwon-Chung KJ, Polacheck I, Bennett JE. Improved diagnostic medium for separation of *Cryptococcus neoformans* var. *neoformans* (serotypes A and D) and *Cryptococcus neoformans* var. *gattii* (serotypes B and C). J Clin Microbiol. 1982;15:535–7.

20. Perfect JR, Lang SD, Durack DT. Chronic cryptococcal meningitis: a new experimental model in rabbits. Am J Pathol. 1980;101:177–94.
21. Kwon-Chung KJ, Bennett JE, Rhodes JC. Taxonomic studies on *Filobasidiella* species and their anamorphs. Antonie Van Leeuwenhoek. 1982;48:25–38.
22. Lengeler KB, Cox GM, Heitman J. Serotype AD strains of *Cryptococcus neoformans* are diploid or aneuploid and are heterozygous at the mating-type locus. Infect Immun. 2001;69:115–22.
23. Varma A, Wu S, Guo N, Liao W, Lu G, Li A, et al. Identification of a novel gene *URE2* that functionally complements a urease negative clinical strain of *Cryptococcus* neoformans. Microbiology. 2006;152:3723–31.
24. Swofford DL. PAUP*: Phylogenetic analysis using parsimony (*and other methods). Version 4. Sunderland (MA): Sinauer Associates, Inc.; 2002.
25. Bell M, Archibald LK, Nwanyanwu O, Dobbie H, Tokars J, Kazembe P. et al. Seasonal variation in the etiology of bloodstream infections in a febrile inpatient population in a developing country. Int J Infect Dis. 2001;5:63–9.
26. Franzot SP, Salkin IF, Casadevall A. *Cryptococcus neoformans* var. *grubii*: separate varietal status of *Crytococcus neoformans* serotype A isolates. J Clin Microbiol. 1999;37:838–40.
27. Barreto de Oliveira MT, Boekhaut T, Theelen B, Hagen F, Baroni FA, Lazera MS, et al. *Cryptococcus neoformans* shows a remarkable genotypic diversity in Brazil. J Clin Microbiol. 2004;42:1356–9.
28. Jain N, Wickes BL, Keller SM, Fu J, Casadevall A, Jain P, et al. Molecular epidemiology of clinical *Cryptococcus neoformans*. J Clin Microbiol. 2005;43:5733–42.
29. Dromer F, Mathoulin S, Dupont B, Laporte A. Epidemiology of cryptococcosis in France: a 9 year survey (1985–1993). French Cryptococcosis Study Group. Clin Infect Dis. 1996;23:82–90.
30. Hajjeh RA, Conn LA, Stephen DS, Baughman W, Hamill R, Graviss E, et al. Cryptococcosis: population-based multistate active surveillance and risk factors in human immunodeficiency virus-infected persons. Cryptococcal Active Surveillance Group. J Infect Dis. 1999;179:449–54.
31. Moosa MYS, Coovadia YM. Cryptococcal meningitis in Durban, South Africa: a comparison of clinical features, laboratory findings, and outcome for human immunodeficiency virus (HIV)-positive and HIV-negative patients. Clin Infect Dis. 1997;24:131–4.
32. Chen S, Sorrell T, Nimmo G, Speed B, Currrie B, Ellis D, et al. Epidemiology and host-and variety-dependent characteristics of infection due to *Cryptococcus neoformans* in Australia and New Zealand. Clin Infect Dis. 2000;31:499–08.
33. Meletiadis J, Walsh TJ, Choi EH, Pappas PG, Ennis D, Douglas J, et al. Study of common functional genetic polymorphisms of FCGR2A, 3A and 3B genes and the risk for cryptococcosis in HIV-uninfected patients. Med Mycol. 2007;45:513–8.
34. Casadevall A, Perfect JR. *Cryptococcus neoformans*. Washington: ASM Press; 1998.
35. Friedman GD. The rarity of cryptococcosis in northern California: the 10-year experience of a large defined population. Am J Epidemiol. 1983;117:230–4.
36. Hajjeh RA, Conn LA, Stephens DS, Baughman W, Hamill R, Graviss E, et al. Population-based multistate active surveillance and risk factors in human immunodeficiency virus-Infected persons. J Infect Dis. 1999;179:449–54.
37. Kwon-Chung KJ, Hill WB. Sexuality and pathogenicity of *Filobasidiella neoformans* (*Cryptococcus neoformans*). In: Vanbreuseghem R, DeVroey C, editors. Sexuality and pathogenicity of fungi. New York: Masson; 1981. p. 243–50.
38. Lo D. Cryptococcosis in the Northern Territory. Med J Aust. 1976;2:825,828.
39. Sorvillo F, Beall G, Turner PA, Beer VL, Kovacs AA, Kerndt PR. Incidence and factors associated with extrapulmonary cryptococcosis among persons with HIV infection in Los Angeles County. AIDS. 1997;11:673–9.

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etymologia

Cryptococcus neoformans

[krip´´ to-kok´əs ne´´o for-mənz], from the Greek—*krypto* (hidden), *kokkos* (berry), *neos* (new); and Latin—*forma* (form)

C. neoformans is an encapsulated yeastlike fungus of the family *Cryptococcaceae*. It was first described in 1894 by German pathologist Otto Busse, who observed the cells in a tumor from the tibia of a woman with sarcoma. Found worldwide in nests and droppings of pigeons, it is the most common species that causes cryptococcosis in humans. The effects range from asymptomatic infection to meningitis, pneumonia, or disseminated disease. The crucial factor is the immune status of the host. With the global emergence of AIDS, the incidence of cryptococcosis is increasing and now represents a major life-threatening infection in these patients.

Sources: Dorland's Illustrated Medical Dictionary, 31st edition. Philadelphia: Saunders Elsevier; 2007; http://www.emedicine.com/med/TOPIC482.HTM

Risk Factors for Sporadic Shiga Toxin–producing *Escherichia coli* Infections in Children, Argentina[1]

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We evaluated risk factors for sporadic Shiga toxin–producing *Escherichia coli* (STEC) infection among children in Argentina. We conducted a prospective case–control study in 2 sites and enrolled 150 case-patients and 299 controls. The median age of case-patients was 1.8 years; 58% were girls. Serotype O157:H7 was the most commonly isolated STEC. Exposures associated with infection included eating undercooked beef, living in or visiting a place with farm animals, and contact with a child <5 years of age with diarrhea. Protective factors included the respondent reporting that he or she always washed hands after handling raw beef and the child eating more than the median number of fruits and vegetables. Many STEC infections in children could be prevented by avoiding consumption of undercooked beef, limiting exposure to farm animals and their environment, not being exposed to children with diarrhea, and washing hands after handling raw beef.

Shiga toxin–producing *Escherichia coli* (STEC) infections cause nonbloody diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome (HUS) (*1*). HUS is characterized by hemolytic anemia, thrombocytopenia, and renal failure (*2*,*3*). No specific treatment exists for HUS, and the mortality rate among children with the syndrome is 5% (*3*–*5*). *E. coli* O157:H7, which was first identified as a human pathogen in 1982 (*6*), is the STEC serotype most frequently isolated from persons with diarrhea (*7*). Other STEC serotypes can cause a similar illness. STEC has been isolated from the feces of many farm animals, including ruminants (e.g., cattle, sheep, and water buffalo) and nonruminants (e.g., horses, dogs, rabbits, and pigs) (*8*–*10*). STEC infections are transmitted to humans through contaminated food (*11*), water (*12*,*13*), and contact with infected persons (*14*) or animals (*15*,*16*).

Argentina had the highest rates of HUS globally, 10.4 and 12.2 cases/100,000 children <5 years of age in 2001 and 2002, respectively (*17*). In Argentina, HUS is the leading cause of acute renal failure among children; in 1 study, after at least 10 years of follow-up, ≈20% of Argentine children had low creatinine clearances (*5*). HUS is responsible for 20% of kidney transplants among children and adolescents in Argentina (*18*). In studies in the 1990s, evidence of STEC infection was found in 59% of Argentinean HUS case-patients, and *E. coli* O157 was the predominant serogroup (*19*,*20*). In 2000, HUS became reportable in Argentina, and sentinel sites began screening for STEC on all routine stool cultures. Given the high rate of HUS, the lack of definitive treatment, and the high morbidity, primary prevention of STEC infections is needed to lower the incidence of childhood kidney disease. However, controlled epidemiologic studies to identify risk factors associated with STEC infection have not been conducted in Argentina. To evaluate risk factors for sporadic STEC infection, we conducted a case-control study in 2 sites, Mendoza and Buenos Aires cities and their surroundings.

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Methods

Case Ascertainment

Patients were enrolled from January 2001 through December 2002 from the public tertiary-care pediatric hospitals, Hospital "Dr. Humberto Notti" in Mendoza, which serves an urban and semirural area, and Hospital "Dr. Juan P. Garrahan" in Buenos Aires, which serves an urban area. Study personnel detected STEC cases through daily review of the hospitals' laboratory records and detected diarrhea-associated HUS cases through biweekly discussions with nephrologists.

Definitions

A case of STEC infection was defined as illness in a previously healthy child <16 years old who was evaluated at a participating institution and who had either culture-confirmed O157 STEC diarrhea, culture-confirmed non-O157 STEC diarrhea, or definite diarrhea-associated HUS. For convenience, we included only children permanently residing within 15 km of each institution. Local investigators stated that the characteristics of the population within each of these areas were similar to that within 50 or 100 km of the respective hospitals. We chose these 2 areas so that the population serviced would be similar to the Argentine population living in areas with a high incidence of HUS.

Definite HUS was defined as acute microangiopathic hemolytic anemia, thrombocytopenia, and renal impairment as determined by 1) hematocrit <30% with microangiopathic changes on peripheral blood smear (e.g., schistocytes, burr cells, helmet cells, or red cell fragments); 2) platelet count <150,000/mm^3; and 3) serum creatinine concentration >2 standard deviations above the upper limit of normal for age and sex (*21*); or abnormal urinary sediment by dipstick, i.e., hematuria (≥2+) or proteinuria (≥2+). HUS was considered diarrhea-associated when a diarrheal illness preceded HUS by ≤3 weeks. We excluded children with a family history of HUS, secondary HUS (e.g., drug-associated), or HUS associated with pneumococcal infection. Probable HUS was defined as an illness that met only 2 of the laboratory criteria in a patient with culture-confirmed STEC infection.

Case–Control Study

For each patient enrolled, 2 age- and neighborhood-matched control children without gastrointestinal illness in the 2 weeks before the matched case's illness onset were identified. We neighborhood-matched control children to control for socioeconomic status, which was not a factor of interest, was a possible confounder, and was quite variable in the study areas. In urban areas, a trained interviewer sought controls by walking, starting at the case-patient's home, going to the third house from the nearest corner, and continuing to every house on the block, then to the block facing the case-patient's residence, then to other blocks in a clockwise fashion, until 2 eligible and consenting controls were interviewed. In nonurban areas, the interviewer randomly chose a cardinal direction and then sought controls beginning from the third residence from the case patient's house in that and the opposite direction until controls were found. Informed consent was obtained from the adult primary caregiver, who was interviewed with a standardized questionnaire, administered in person. Most eligible controls were enrolled; information on potential controls who were excluded or chose not to participate was not kept.

The questionnaire (available upon request from the corresponding author) had 89 major questions and was divided into 3 major sections: characteristics of and treatment for the illness (19 questions), exposures (55 questions), and demographics (15 questions). Exposure questions were divided into sections that dealt with human contacts, care and feeding of young children, water sources and treatment, beef, other meats, fruits and vegetables, meat handling at home, animal contact, swimming, and travel. Many exposure questions had several parts (e.g., if beef consumed, was it pink; if chicken consumed, indicate if at home, restaurant, or other location). Almost all responses were measured discretely (i.e., categorically). Responses to each question were treated independently, except for those about fruits and vegetables, which were combined and dichotomized at the median; this analysis method was checked against other choices and found robust. All exposure questions for case-patients and controls were about the 7 days before the onset of illness. Interviews of case-patients were conducted a median of 12 days (range 3–41) after diarrhea onset; control interviews were conducted a median of 15 days (range 1–41) after diarrhea onset in the matched case. Controls were age-matched to case-patients by using the following groups: <12 months; 1–5 years ± 1 year; 6–9 years ± 2 years; 10–15 years ± 3 years.

Laboratory Methods

Fecal samples were plated onto sorbitol-MacConkey agar directly, and after enrichment at 37°C for 4 h in trypticase soy broth were supplemented with cefixime (50 ng/mL) and potassium tellurite (25 mg/mL). The confluent growth zone and colonies were screened for *stx*1, *stx*2, and *rfb*O157 genes by a multiplex PCR (*22*,*23*). Isolates with *stx*1 or *stx*2 genes were identified by standard biochemical methods. *Stx*-positive colonies were serotyped (*24*) and characterized at the Argentina National Reference Laboratory (*11*,*25*).

Statistical Analysis

Data analysis used 3 steps: an initial univariate analysis, a second univariate analysis adjusted for highly significant factors of prior or secondary interest, and a final multivariable model-building analysis. Four factors were ultimately chosen in the second univariate analysis as a fixed set from which to explore further model-building, based on epidemiologic sensibility, strong association, and stability of subsequent adjusted associations. Single- and multiple-variable conditional logistic regression models were used to evaluate associations between the outcome and exposure variables. At each step, risk factors that were statistically significant ($p \leq 0.05$) and had biologic plausibility were selected for further modeling. In multivariable model-building, we pursued forward, backward, and the best subset selection strategies, as well as manual strategies. Standard methods were used to assess model fit, including residual analyses. Maximum likelihood parameter estimates from these models were used to calculate point estimates and confidence intervals for odds ratios, referred to henceforth as matched odds ratios. Exact analysis was used where small sample size would make asymptotic analysis suspect, and Mantel-Haenszel odds ratios were computed when maximum likelihood estimates did not exist.

Exploratory and sensitivity analyses were performed on subsets of the data defined by location and subcategory of disease status. Because the subsets by site (Buenos Aires and Mendoza) and by serogroup (O157, non-O157) were small and most factors examined had already been demonstrated as risky or protective in the larger dataset, we used a p value of 0.10 to assess significance by site and serogroup. For similar reasons, we did not perform multivariable analyses on these subsets. Three patients with mixed STEC infection and 2 *stx*-positive HUS patients without STEC isolated were excluded from the analysis by serogroup. Data were analyzed with Epi Info 2000 (Centers for Disease Control and Prevention, Atlanta, GA, USA) and SAS 9.0 (SAS Institute, Inc., Cary, NC, USA) software. The study was approved by the hospitals' ethics committees as well as the institutional review boards of the Ministry of Health of Argentina and CDC.

Results

Case and Control Characteristics

Among 157 eligible case-patients, 150 (96%) were enrolled; the parents of 1 child refused, and interviewers could not contact the parents of 6. The hospital "Dr. Juan P. Garrahan" in Buenos Aires enrolled 54% of the cases, and the hospital "Dr. Humberto Notti" in Mendoza enrolled 46%. Among the 150 enrolled cases, 17 met both entry criteria of culture-confirmed STEC infection and definite HUS, 82 met only the criterion of culture-confirmed STEC infection, and 51 met only the criterion of definite HUS. In addition to the patients with definite HUS, 10 patients with culture-confirmed STEC infection had probable HUS.

The median age of case-patients was 1.8 years (range 4 months–14 years). The median age of the 299 controls was similar (2.0 years; range 1 month–17 years); 58% of case-patients versus 43% of controls were female ($p = 0.01$); 134 (89%) case-patients were urban residents.

Clinical Findings

Among the 150 case-patients, clinical findings included bloody diarrhea (84%) and vomiting (71%). Ninety-four (63%) were hospitalized for a median of 4.5 days (range 1–14 days). Among the 78 case-patients with definite HUS (dHUS n = 68) or probable HUS (pHUS n = 10), 37 (47%) had peritoneal dialysis (34 dHUS 50%, 3 pHUS 30%), 2 (3%) had hemodialysis (all dHUS), and 69 (88%) received erythrocyte transfusions (62 dHUS, 7 pHUS).

Stool Cultures and Characterization of Isolates

Among the 99 case-patients with culture-confirmed STEC infection, 96 had a single STEC isolated: 58 (60%) were O157:H7, and 38 (40%) non-O157. Among the 99 with STEC isolated, the proportion who had O157 was 82% (14 of 17) among children with definite HUS, 90% (9 of 10) among children with probable HUS, and 53% (38 of 72) among children without HUS. Among the 38 non-O157 isolates, the serotype frequency was 29% O145:NM, 11% O26:H11, 11% O113:H21, 8% O174:H21, 5% O8:H19, 5% O145:H25, 5% ONT:NM, and 3% (1 each) O2:H11, O15:H27, O25:NM, O58:H40, O91:H7, O103:H2, O103:H25, O111:NM, O121:H19, and O171:H2.

Risk Factors Overall

Analysis of single variable associations, when the fixed adjustment factors were controlled for, identified dietary habits and animal exposures linked to illness (Table 1). General dietary habits linked to STEC illness included eating at a social gathering, eating any meal prepared at home, and drinking from a baby bottle left at room temperature for ≥2 hours. Many beef-related exposures were significantly associated with STEC infection (Tables 1, 2). Eating beef outside the home and eating undercooked beef (described as uncooked, red and juicy, or pink) anyplace was associated with illness. Eleven percent of case-patients but only 5% of controls consumed *jugo de carne* (liquid squeezed from a tender, usually lightly cooked piece of beef, and spoon-fed); case-patients with this exposure ranged from 7 months to 9 years old. Living in or visiting a place with farm animals, contact with farm animals (including horses, pigs, poultry, and cattle), and contact with cattle manure were associated with illness. Risky exposures that suggest

person-to-person transmission from young children included contact with a child <5 years of age, attending daycare or kindergarten, and contact with a child <5 years of age with diarrhea. Wearing diapers was also linked to illness. No significant differences between case-patients and controls were found in the distribution of most variables that

Table 1. Univariate analysis of risk factors for Shiga toxin–producing *Escherichia coli* infections, unadjusted and adjusted, Buenos Aires and Mendoza, Argentina, 2001–2002

Risk factors	% Case-patients† (N = 150)	% Controls† (N = 299)	Unadjusted univariate analysis			Adjusted univariate analysis*			Sites‡
			mOR	95% C‡	p value	mOR	95% CI	p value	
General dietary habits									
Eating at a social gathering	18	8	2.79	1.4–5.3	0.002	3.77	1.8–8.1	0.0007	B, M
Eating any meal prepared at home	93	88	2.26	1.0–5.0	0.047	3.22	1.3–7.7	0.009	B
Drinking from baby bottle left at room temperature for ≥2 h	71	63	1.70	1.0–2.8	0.043	1.89	1.1–3.4	0.029	B
Beef-related dietary habits									
Eating beef outside home	22	15	1.70	1.0–2.9	0.06	2.18	1.2–4.1	0.014	B
Eating meatballs	3	0	0.46	0.3–0.8	0.004	15.00§	1.7–136.2	0.005	M
Eating breaded beef (*milanesa*)	3	0.3	10.00	1.2–85.6	0.036	13.45	1.4–125.0	0.022	B
Eating undercooked beef any place	29	14	2.69	1.6–4.5	0.0002	2.65	1.5–4.8	0.001	B
Eating undercooked piece of beef	19	9	2.46	1.4–4.4	0.003	2.38	1.2–4.6	0.010	B
Eating undercooked ground beef	11	5	2.41	1.1–5.1	0.021	2.70	1.1–6.5	0.026	B
Eating undercooked beef outside home	5	0.3	14.00	1.7–113.8	0.014	25.04	2.6–242.4	0.005	B
Eating undercooked beef at home	26	14	2.33	1.4–3.9	0.001	2.23	1.2–4.0	0.008	B
Teething on undercooked beef	11	2	4.83	1.9–12.4	0.001	4.00	1.4–11.4	0.010	B
Consuming *jugo de carne*¶	11	5	2.19	1.2–4.0	0.009	3.23	1.3–7.8	0.009	B, M
Eating undercooked piece of beef	18	9	2.21	1.2–4.0	0.009	2.05	1.1–4.0	0.033	B
Eating undercooked steak	13	6	2.34	1.2–4.6	0.015	2.03	0.0–4.3	0.060	B
Eating salami at home	19	11	2.19	1.2–4.0	0.009	2.22	1.1–4.5	0.027	B
Exposure to animals or their environment									
Living in or visiting a place with farm animals	13	5	3.49	1.5–7.9	0.003	4.86	1.9–12.8	0.001	B, M
Contact with farm animals	11	5	2.25	1.0–4.8	0.036	4.45	1.7–11.6	0.002	B, M
Contact with cattle manure	3	1	4.33	0.8–22.8	0.084	9.03	1.0–86.1	0.050	M
Contact with horses	10	4	2.76	1.2–6.4	0.02	5.02	1.7–14.5	0.003	M
Contact with pigs	5	2	2.13	0.7–6.2	0.20	3.80	1.0–13.4	0.041	M
Contact with poultry	6	4	1.68	0.7–4.2	0.26	2.90	1.0–8.2	0.050	M
Contact with cattle	4	2	1.92	0.6–6.5	0.29	3.51	0.8–14.7	0.085	M
Person-to-person transmission									
Contact with a child <5 y	80	67	2.08	1.3–3.4	0.003	2.05	1.2–3.5	0.009	B, M
Attending daycare or kindergarten	17	9	2.87	1.4–5.9	0.004	2.34	1.1–5.1	0.034	B, M
Contact with a child <5 y with diarrhea	15	6	3.61	1.6–8.4	0.003	2.54	1.0–6.6	0.050	M
Other variables									
Wearing diapers	72	62	2.63	1.4–5.0	0.003	2.12	1.0–4.3	0.036	–
Nonparental household income	56	40	1.89	1.3–2.8	0.002	1.98	1.2–3.2	0.005	B

*Adjusted by the fixed adjustment factors shown in Table 2. mOR, matched odds ratio; CI, confidence interval.
†The denominator (number of respondents) for case-patients varied from 146 to 150, except for contact with a child <5 y with diarrhea in which the number was 119. The denominator for controls varied from 292 to 299, except for contact with a child <5 y with diarrhea in which the number was 263.
‡Denotes adjusted univariate analysis significant in Buenos Aires (B), Mendoza (M), or neither site (–).
§Cochran-Mantel-Haenszel odds ratio.
¶Liquid squeezed from a tender, usually lightly cooked piece of beef, and spoon-fed.

relate to socioeconomic status (e.g., number of bedrooms, water supply, garbage disposal, educational level of parents). However, case households were more likely to have nonparental income.

Four protective factors were identified, all related to beef (Table 2). These were the child eating meatballs at home; the child eating *empanadas* (fried or baked pastries with ground beef filling) at home; the child eating meat pie at home; and the respondent always washing hands with soap and water after handling raw beef. The factors controlled for in the adjusted univariate analysis were eating more than the median number of fruits and vegetables, male sex, having a nonparent respondent, and the respondent always washing hands after handling raw beef; all were protective (Tables 1, 2).

On multivariable logistic regression analysis, significant risk factors for STEC infection that remained were eating undercooked beef outside the home (odds ratio [OR] 17.63, 95% confidence interval [CI] 1.6–197.4, p = 0.02), living in or visiting a place with farm animals (OR 6.61, 95% CI 1.5–28.8, p = 0.01), contact with a child <5 years of age with diarrhea (OR 3.29, 95% CI 1.0–10.4, p = 0.04), and having nonparental household income (OR 2.21, 95% CI 1.2–4.0, p = 0.01). Eating ground beef at home (meatballs, *empanadas*, or meat pie) remained protective (OR 0.36, 95% CI 0.1–0.9, p = 0.03). With this model, the fixed adjustment factors had significant estimated protective associations as follows: eating more than the median number of fruits and vegetables (OR 0.31, 95% CI 0.1–0.6, p = 0.0007), male sex (OR 0.34, 95% CI 0.2–0.7, p = 0.001), having a nonparent respondent (OR 0.34, 95% CI 0.2–0.7, p = 0.001), and the respondent always washing hands after handling raw beef (OR 0.23, 95% CI 0.1–0.6, p = 0.001).

Risk Factors by Site and Etiology

We performed a univariate adjusted analysis for all variables by site (Tables 1, 2). For every variable that was significantly risky or protective in the combined analysis, the OR went in the same direction in the site-specific analysis (Buenos Aires 81 cases, Mendoza 69 cases), although the association was not always statistically significant. Many dietary habits, most of which were beef associated, were significantly associated with illness in Buenos Aires, whereas fewer reached statistical significance in Mendoza. Consuming *jugo de carne* was significantly associated with illness in both sites; however, 19.1% of case-patients from Mendoza consumed this item compared with only 4.9% from Buenos Aires.

Risk and protective factors were also analyzed separately for patients with culture-confirmed O157 or non-O157 STEC infection (Table 3). These 2 groups were similar in age, sex, and site distribution. The risk and protective factors among these 2 groups were similar to those of all study participants. Among patients with O157 STEC infection, illness was significantly associated with eating at a social gathering, with many meat-related dietary habits, exposures related to farm animals and their environment, wearing diapers, and having a nonparental household income. Protective factors included several related to eating beef at home and buying beef less than once a week. Among the smaller group of patients with non-O157 STEC infection, the only risk factors significantly linked to illness were drinking from a bottle left at room temperature, drinking formula (a factor not identified in the full group), eating a piece of beef outside the home, teething on undercooked beef at home, contact with a child <5 years of age with diarrhea, wearing diapers, and

Table 2. Univariate analysis of protective factors for Shiga toxin–producing *Escherichia coli* infections, unadjusted and adjusted, and adjustment factors, Buenos Aires and Mendoza, Argentina, 2001–2002

Characteristic	% Case-patients† (N = 150)	% Controls† (N = 299)	Unadjusted univariate analysis			Adjusted univariate analysis*			Sites‡
			mOR	95% CI	p value	mOR	95% CI	p value	
Protective factors									
Eating meatballs at home	17	29	0.46	0.3–0.8	0.004	0.44	0.2–0.8	0.010	M
Eating meat pie at home	11	23	0.40	0.2–0.8	0.004	0.47	0.2–0.9	0.025	–
Eating *empanadas* at home	20	34	0.43	0.3–0.7	0.001	0.49	0.3–0.9	0.016	M
Respondent always washing hands with soap and water after handling raw beef	50	64	0.53	0.3–0.8	0.004	0.57	0.3–0.9	0.019	B, M
Fixed adjustment factors									
Eating more than the median number of fruits and vegetables	33	51	0.42	0.3–0.7	0.0002	–	–	–	B, M
Male sex	43	57	0.57	0.4–0.9	0.01	–	–	–	B, M
Having a nonparent respondent	3	1	0.29	0.1–0.8	0.009	–	–	–	M
Respondent always washing hands after handling raw beef	74	90	0.27	0.1–0.5	0.0001	–	–	–	B, M

*Adjusted by the fixed adjustment factors shown. mOR, matched odds ratio; CI, confidence interval.
†The denominator (number of respondents) for case-patients varied from 144 to 150. The denominator for controls varied from 298 to 299.
‡Denotes adjusted univariate analysis significant in Buenos Aires (B), Mendoza (M), or neither site (–).

living in an overcrowded condition. Eating meat pie at home was protective for this group.

Discussion

This first study of risk factors for sporadic STEC infections in Argentina demonstrates a broad range of factors associated with transmission. Undercooked beef in many forms was the most risky food. The presence of *E. coli* O157 in beef purchased in Argentina has also been demonstrated microbiologically (*25*). Beef, especially undercooked ground beef, is well recognized as a vehicle for *E. coli* O157:H7 infections (*26*). Our results also suggest that many STEC infections are acquired in the home as a result of breaches in kitchen hygiene in relation to beef; washing hands after handling raw beef, especially with soap and water, was protective. The protective effect of consuming some beef products at home is further evidence of the important role of the food preparer. Few beef-related factors were significantly risky in Mendoza, suggesting that this population may consume less undercooked beef; however, consumption of *jugo de carne*, a risky food, was much more common in Mendoza.

Table 3. Adjusted univariate analysis of risk and protective factors for Shiga toxin–producing *Escherichia coli* (STEC) O157 and non-O157 STEC, Buenos Aires and Mendoza, Argentina 2001–2002*

	STEC O157				Non-O157 STEC			
Characteristics	% Case-patients† (n = 58)	% Controls† (n = 116)	mOR	p value	% Case-patients† (n = 38)	% Controls† (n = 75)	mOR	p value
Risk factors								
Dietary habits								
Eating at a social gathering	19	8	9.79	<0.01	21	7	2.82	NS
Drinking from a baby bottle left at room temperature for ≥2 h	75	76	1.32	NS	66	53	3.78	<0.05
Drinking formula (milk)‡	3	3	1.91	NS	11	1	12.70	<0.05
Meat-related dietary habits§								
Eating breaded beef (*milanesa*) at restaurant	5	0	15.00¶	<0.05	3	1	2.02	NS
Eating a piece of beef outside home§	12	5	4.68	<0.05	13	1	7.56	<0.10
Eating undercooked beef at any place	29	10	3.69	<0.05	29	17	1.96	NS
Teething on undercooked beef at home	16	3	4.15	<0.10	8	1	12.78	<0.10
Consuming *jugo de carne*¶	16	4	3.24	<0.10	8	7	2.22	NS
Eating undercooked piece of beef	22	8	3.29	<0.10	16	11	1.25	NS
Eating salami at home	24	10	3.73	<0.05	11	8	1.28	NS
Eating ham‡	40	25	2.52	<0.10	16	25	0.36	NS
Eating beef soup‡	36	48	0.44	<0.10	45	40	1.31	NS
Exposure to animals or their environment								
Living in or visiting a place with farm animals	18	5	11.83	<0.01	13	5	2.76	NS
Contact with farm animals at any place	14	5	6.08	<0.05	13	7	3.39	NS
Contact with horses	12	5	4.51	<0.05	13	4	6.78	NS
Person-to-person transmission								
Contact with a child <5 y with diarrhea	20	5	6.29	NS	26	4	6.93	<0.05
Other variables								
Wearing diapers	82	70	2.83	<0.10	82	65	9.34	<0.10
Nonparental household income	58	42	2.06	<0.10	45	47	1.02	NS
Living in overcrowded condition‡	22	15	1.77	NS	31	1	3.06	<0.10
Protective factors								
Eating meat pie at home	17	23	0.77	NS	5	19	0.19	<0.10
Eating *empanadas* at home	16	37	0.17	<0.01	18	33	0.37	NS
Eating ground beef at home‡	69	82	0.21	<0.05	60	73	0.29	NS
Eating breaded beef (*milanesa*) at home	49	67	0.39	<0.05	45	56	0.77	NS
Buying beef <1 time/wk‡	76	93	0.24	<0.05	87	89	0.43	NS

*mOR, matched odds ratio; NS, not significant (p>0.10).
†For STEC O157, the denominator (number of respondents) for case-patients varied from 56 to 58, except for contact with a child <5 y with diarrhea, in which the number was 51. The denominator for their controls varied from 112 to 116, except for this same factor, in which the number was 104. For non-O157 STEC, the denominator (number of respondents) varied from 37 to 38, except for contact with a child <5 y with diarrhea, in which the number was 31. The denominator for their controls varied from 73 to 75, except for this same factor, in which the number was 67.
‡All significant associations except these were also significant associations in the total dataset with 150 cases.
§The term "meat" includes ground beef.
¶Liquid squeezed from a tender, usually lightly cooked piece of beef, and spoon-fed.

Whereas ground beef consumed as hamburgers is frequently implicated in North America, the spectrum of major risky beef items we identified in Argentina is wider. Other beef-based items linked to sporadic STEC infections in this study included ground beef, pieces of beef, pieces of tender "teething" beef, beef *milanesa* (breaded beef), steak, *jugo de carne*, and salami. Salami and other types of beef are uncommon causes of STEC outbreaks (*27*). Among 183 foodborne *E. coli* O157 outbreaks reported in the United States from 1982 to 2002, 41% were linked to ground beef, but only 6% to other beef items (*28*). These findings highlight the importance of conducting studies locally to determine local risk factors and corresponding control measures. The risk we demonstrated from contact with farm animals and their environment supports studies from other areas that this is an important mode of transmission (*9*,*29*–*32*). The variety of animals to which exposure conferred risk, including some which have never been directly implicated as a source of STEC infections, suggests widespread contamination of farm environments. Our data also indicate that person-to-person spread is an important mode of STEC transmission in Argentina, as evidenced by the increased risk for illness from contact with a young child with diarrhea and from attendance at a daycare or kindergarten. The neighborhood-matched study design limited the likelihood of identifying socioeconomic factors, but the finding that case households were more likely than controls to have nonparental household income suggests that they were poorer.

To our knowledge, this is the largest study of risk factors for sporadic non-O157 STEC infection. Most exposures that were risky for the 150 case-patients also had high ORs for the non-O157 STEC case-patients, suggesting that similar exposures are risky; however, few of the risks were statistically significant in the subgroup. This finding may partially reflect the small size (38 cases) and diversity (14 serogroups) of the non-O157 STEC subgroup. Others have also reported outbreaks and sporadic cases of non-O157 STEC infections caused by cattle-related items (*1*,*9*). However, a study from Belgium of both O157 and non-O157 STEC infection found that consumption of fish but not beef was risky (*33*). Our finding that drinking infant formula was risky only in the non-O157 subgroup merits further study. Powdered infant formula is a known source of invasive infections in infants (*34*). With larger studies, strains that are less likely to be pathogens can be excluded and serotype-specific risk factors can be examined.

To our knowledge, others have not reported male sex as a protective factor (or female sex as a risk factor) for STEC infection. However, others have reported female sex as a risk factor for *E. coli* O157–associated hemolytic anemia (*35*). The reason for this sex difference is not known. We do not know why having a nonparent responder to the questionnaire was associated with a lower risk for STEC infection. However, it suggests a setting in which help with childcare is available from family members or paid care providers.

Our finding that eating a wider variety of fruits and vegetables was protective against STEC infection merits further investigation. A varied diet may increase resistance to disease by providing bowel flora that help to protect against colonization with pathogens by providing compounds that block bacterial adhesions, as has been postulated for urinary tract infections (*36*), or by some other mechanism (*37*).

Our study had several limitations. First, we included as STEC cases children with diarrhea-associated HUS who did not have laboratory confirmation of STEC infection. However, other data indicate that almost all diarrhea-associated HUS cases in children are due to STEC infection (*7*). To our knowledge, *Shigella dysenteriae* type 1, the only other known cause of diarrhea-associated HUS (*38*), has not been isolated from ill persons in Argentina in recent decades. Inclusion of these HUS cases provided power needed for the analysis; a subanalysis examining these cases alone indicates that they did not introduce any extraneous associations into the analysis of the full dataset (data not shown). Second, we analyzed multiple exposures, which can lead to finding associations by chance alone. However, the factors we identified are plausible biologically and supported by other evidence. Third, features inherent to the study design may have led to finding risk and protective factors that were not representative of the Argentine population. Although the study population included those with urban, suburban, and rural residences, our inclusion of patients only within 15 km of study hospitals likely resulted in exclusion of some very rural segments of the population. Fourth, our neighborhood matching of controls may have led to overmatching on some fixed environmental features, but decreased the chance of identifying risk factors that were surrogates for differences in socioeconomic factors. We matched for neighborhood to control for socioeconomic status both to decrease the number of significant factors that were difficult to change and because we expected most of the causal pathways to relate to food and animal exposures. Fifth, we studied 2 geographically separated populations. Our subanalysis indicated that some risky factors were more prominent in 1 location. Studies of other Argentine populations may identify risky practices important in those populations.

We considered using the multivariable model as the complete basis for describing our results. However, we were able to create many multivariable models of similar strength that varied in the factors that remained significant. In all multivariable models, some important factors dropped out, but those factors varied. Because no one multivariable

model adequately described the findings, we chose to present both the multivariable model that had the strongest individual predictors and the adjusted univariate analysis. The latter retains some important factors amenable to intervention, such as eating undercooked beef at home.

Measures are needed to decrease the likelihood of persons in Argentina consuming food contaminated with STEC. Effective safety practices at all stages of the food chain must be ensured. In particular, the contamination of beef by STEC O157 should be reduced. Major efforts to educate the Argentine public and the food industry could help to reduce these serious illnesses. Social research is needed to better understand practices involving giving meat for teething and *jugo de carne* to young children. Ensuring that beef is well cooked is a key message. Education is needed to explain the risks related to exposure to farm animals and the ability of people to protect themselves by washing hands.

Evidence indicates that measures instituted by industry, in response to government regulations, recalls, and outbreak investigations, are critical in decreasing STEC infections. After a large outbreak due to ground beef in 1993 in which 4 children died (*39*), the US Department of Agriculture declared *E. coli* O157:H7 an adulterant in ground beef; retail beef from lots known to contain the organism must now be recalled. In 2002, a recall of >18 million pounds of ground beef with *E. coli* O157 contamination (*40*), and a new USDA directive (*41*), galvanized the US beef industry to institute more aggressive pathogen control measures, including testing of all lots of beef trimmings or ground beef for *E. coli* O157 in plants (R. Huffman, American Meat Institute Foundation, pers. comm.). Implementation of prevention measures by industry, government, and consumers could result in a decrease in the incidence of STEC infections in children, and thereby decrease the incidence of childhood kidney disease from HUS in Argentina with its associated human and economic costs.

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References

1. Griffin PM, Tauxe RV. The epidemiology of infections caused by *Escherichia coli* O157:H7, other enterohemorrhagic *E. coli*, and the associated hemolytic uremic syndrome. Epidemiol Rev. 1991;13:60–98.
2. Gianantonio C, Vitacco M, Mendilaharzu F, Rutty A, Mendilaharzu J. The hemolytic uremic syndrome. J Pediatr. 1964;64:478–91.
3. Boyce TG, Swerdlow DL, Griffin PM. *Escherichia coli* O157:H7 and the hemolytic uremic syndrome. N Engl J Med. 1995;333:364–8.
4. Martin DL, MacDonald KL, White KE, Soler JT, Osterholm MT. The epidemiology and clinical aspects of the hemolytic uremic syndrome in Minnesota. N Engl J Med. 1990;323:1161–7.
5. Spizzirri FD, Rahman RC, Bibiloni N, Ruscasso JD, Amoreo OR. Childhood hemolytic uremic syndrome in Argentina: long term follow-up and prognostic features. Pediatr Nephrol. 1997;11:156–60.
6. Riley LW, Remis RS, Helgerson SD, McGee HB, Wells JG, Davis BR, et al. Hemorrhagic colitis associated with a rare *Escherichia coli* O157:H7 serotype. N Engl J Med. 1983;308:681–5.
7. Banatvala N, Griffin PM, Greene KD, Barrett TJ, Bibb WF, Green JH, et al. The United States national prospective hemolytic uremic syndrome study: microbiologic, serologic, clinical, and epidemiologic findings. J Infect Dis. 2001;183:1063–70.
8. Ørskov F, Ørskov I, Villar JA. Cattle as reservoir of verotoxin-producing *Escherichia coli* O157:H7. Lancet. 1987;2:276.
9. Caprioli A, Morabito S, Brugère H, Oswald E. Enterohaemorrhagic *Escherichia coli*: emerging issues on virulence and modes of transmission. Vet Res. 2005;36:289–311.
10. Meichtri L, Miliwebsky E, Gioffre A, Chinen I, Baschkier A, Chillemi G, et al. Shiga toxin–producing *Escherichia coli* in healthy young beef steers from Argentina: prevalence and virulence properties. Int J Food Microbiol. 2004;96:189–98.
11. Rivas M, Caletti MG, Chinen I, Refi SM, Roldan CD, Chillemi G, et al. Home-prepared hamburger and sporadic hemolytic uremic syndrome, Argentina [letter]. Emerg Infect Dis. 2003;9:1184–6.
12. Swerdlow DL, Woodruff BA, Brady RC, Griffin PM, Tippen S, Donnell HD Jr, et al. A waterborne outbreak in Missouri of *Escherichia coli* O157:H7 associated with bloody diarrhea and death. Ann Intern Med. 1992;117:812–9.
13. Friedman MS, Roels T, Koehler JE, Feldman L, Bibb WF, Blake P. *Escherichia coli* O157:H7 outbreak associated with an improperly chlorinated swimming pool. Clin Infect Dis. 1999;29:298–303.
14. Belongia EA, Osterholm MT, Soler JT, Ammend DA, Braun JE, MacDonald KL. Transmission of *Escherichia coli* O157:H7 infection in Minnesota child day-care facilities. JAMA. 1993;269:883–8.
15. Renwick SA, Wilson JB, Clarke RC, Lior H, Borczyk AA, Spika J, et al. Evidence of direct transmission of *Escherichia coli* O157:H7 infection between calves and human. J Infect Dis. 1993;168:792–3.
16. Crump JA, Sulka AC, Langer AJ, Schaben C, Crielly AS, Gage R, et al. An outbreak of *Escherichia coli* O157:H7 infections among visitors to a dairy farm. N Engl J Med. 2002;347:555–60.
17. Rivas M, Miliwebsky ES, Chinen I, Deza N, Leotta GA. Síndrome urémico hemolítico: asociación con la infección por *Escherichia coli* productor de toxina Shiga. Medicina (B Aires). 2006;66 (suppl. III):27–32.
18. Exeni R. Síndrome urémico hemolítico. Archivos Latinoamericanos de Nefrología Pediátrica. 2001;1:35–56.

19. Rivas M, Balbi L, Miliwebsky ES, Garcia B, Tous MI, Leardini NA, et al. Síndrome urémico hemolítico en niños de Mendoza: su asociación con la infección por *Escherichia coli* productor de toxina Shiga. Medicina (B Aires). 1998;58:1–7.
20. Miliwebsky E, Balbi L, Gomez D, Wainsztein R, Cueto Rua M, Roldan C, et al. Síndrome urémico hemolítico en niños de Argentina: su asociación con la infección por *Escherichia coli* productor de toxina Shiga. Bioquímica y Patología Clínica. 1999;63:113–21.
21. Schwartz GJ, Haycock GB, Spitzer A. Plasma creatinine and urea concentration in children: normal values for age and sex. J Pediatr. 1976;88:828–30.
22. Pollard DR, Johnson WM, Lior H, Tyler SD, Rozee KR. Rapid and specific detection of verotoxin genes in *Escherichia coli* by the polymerase chain reaction. J Clin Microbiol. 1990;28:540–5.
23. Paton AW, Paton JC. Detection and characterization of Shiga toxigenic *Escherichia coli* by using multiplex PCR assays for *stx*1, *stx*2, *eae*A, enterohemorrhagic *E. coli hly*A, *rfb*O111, and *rfb*O157. J Clin Microbiol. 1998;36:598–602.
24. Ørskov F, Ørskov I. Serotyping of *Escherichia coli*. In: Bergan T, editor. Methods in microbiology. Vol 14. London: Academic Press; 1984. p. 43–112.
25. Chinen I, Tanaro JD, Miliwebsky E, Lound LH, Chillemi G, Ledri S, et al. Isolation and characterization of *Escherichia coli* O157:H7 from retail meats in Argentina. J Food Prot. 2001;64:1346–51.
26. Voetsch AC, Kennedy MH, Keene WE, Smith KE, Rabatsky-Ehr T, Zansky S, et al. Risk factors for sporadic Shiga toxin-producing *Escherichia coli* O157 infections in FoodNet sites, 1999–2000. Epidemiol Infect. 2007;135:993–1000.
27. Paton AW, Voss E, Manning PA, Paton JC. Molecular microbiological investigation of an outbreak of hemolytic-uremic syndrome caused by dry fermented sausage contaminated with Shiga-like toxin–producing *Escherichia coli.* J Clin Microbiol. 1996;34:1622–7.
28. Rangel JM, Sparling PH, Crowe C, Griffin PM, Swerdlow DL. Epidemiology of *Escherichia coli* O157:H7 outbreaks, United States, 1982–2002. Emerg Infect Dis. 2005;11:603–9.
29. Locking ME, O'Brien SJ, Reilly WJ, Wright EM, Campbell DM, Coia JE, et al. Risk factors for sporadic cases of *Escherichia coli* O157 infection: the importance of contact with animal excreta. Epidemiol Infect. 2001;127:215–20.
30. Wells JG, Shipman LD, Greene KD, Sowers EG, Green JH, Cameron DN, et al. Isolation of *Escherichia coli* serotype O157:H7 and other Shiga-like toxin–producing *E. coli* from dairy cattle. J Clin Microbiol. 1991;29:985–9.
31. Elder RO, Keen JE, Siragusa GR, Barkocy-Gallagher GA, Koohmaraie M, Laegreid WW, et al. Correlation of enterohemorrhagic *Escherichia coli* O157 prevalence in feces, hides, and carcasses of beef cattle during processing. Proc Natl Acad Sci U S A. 2000;97:2999–3003.
32. Kassenborg HD, Hedberg CW, Hoekstra M, Evans MC, Chin AE, Marcus R, et al. Emerging Infections Program FoodNet Working Group. Farm visits and undercooked hamburgers as major risk factors for sporadic *Escherichia coli* O157:H7 infection: data from a case-control study in 5 FoodNet sites. Clin Infect Dis. 2004;38(Suppl 3):S271–8.
33. Pierard D, Crowcroft N, De Bock S, Potters D, Crabbe G, Van Loock F, et al. A case-control study of sporadic infection with O157 and non-O157 verotoxin-producing *Escherichia coli.* Epidemiol Infect. 1999;122:359–65.
34. Centers for Disease Control and Prevention. *Enterobacter sakazakii* infections associated with use of powdered infant formula—Tennessee, 2001. MMWR Morb Mortal Wkly Rep. 2002;51:297–300.
35. Rowe PC, Walop W, Lior H, Mackenzie AM. Haemolytic anaemia after childhood *Escherichia coli* O157:H7 infection: are females at increased risk? Epidemiol Infect. 1991;106:523–30.
36. Kontiokari T, Nuutinen M, Uhari M. Dietary factors affecting susceptibility to urinary tract infection. Pediatr Nephrol. 2004;19:378–83.
37. Taylor CE, Higgs ES. Micronutrients and infectious diseases: thoughts on integration of mechanistic approaches into micronutrient research. J Infect Dis. 2000;182(Suppl 1):S1–4.
38. Bhimma R, Rollins NC, Coovadia HM, Adhikari M. Post-dysenteric hemolytic uremic syndrome in children during an epidemic of *Shigella dysentery* in Kwazulu/Natal. Pediatr Nephrol. 1997;11:560–4.
39. Bell BP, Goldoft M, Griffin PM, Davis MA, Gordon DC, Tarr PI, et al. A multistate outbreak of *Escherichia coli* O157:H7–associated bloody diarrhea and hemolytic uremic syndrome from hamburgers. JAMA. 1994;272:1349–53.
40. Centers for Disease Control and Prevention. Multistate outbreak of *Escherichia coli* O157:H7 infections associated with eating ground beef—United States, June–July, 2002. MMWR Morb Mortal Wkly Rep. 2002;51:637–9.
41. Naugle AL, Holt KG, Levine P, Eckel R. Food Safety and Inspection Service regulatory testing program for *Escherichia coli* O157:H7 in raw ground beef. J Food Prot. 2005;68:462–8.

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Invasive Group A Streptococcal Disease in Nursing Homes, Minnesota, 1995–2006

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Nursing home residents are at high risk for invasive group A streptococcal (GAS) disease, and clusters of cases in nursing homes are common.To characterize the epidemiologic features of invasive GAS disease in nursing homes, we conducted active, statewide, population- and laboratory-based surveillance in Minnesota from April 1995 through 2006. Of 1,858 invasive GAS disease cases, 134 (7%) occurred in nursing home residents; 34 of these cases were identified as part of 13 clusters. Recognizing cases of GAS disease in nursing homes posed challenges. Measures to ensure identification of case-patients as residents of specific nursing homes need to be included in standard guidelines for the prevention and control of invasive GAS disease in this setting.

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Streptococcus pyogenes, or group A *Streptococcus* (GAS), is most commonly associated with noninvasive conditions such as pharyngitis and impetigo but can also cause severe invasive GAS infections such as necrotizing fasciitis and streptococcal toxic shock syndrome (STSS) (*1*–*3*). Risk factors for invasive GAS disease include advanced age, diabetes mellitus, cardiac disease, chronic obstructive pulmonary disease, cancer, immunocompromising conditions, and varicella (*4*,*5*). Most nursing home residents have at least one of these risk factors, which makes this population especially vulnerable to invasive GAS disease. An estimated 8,950 to 11,500 (3.5/100,000 population) invasive cases and 1,050 to 1,850 deaths occur in the United States annually (*6*). The incidence among persons ≥65 years of age of 9.4/100,000 population is almost 3 times that of the general population (*6*).

CME ACTIVITY

Invasive Group A Streptococcal Disease in Nursing Homes, Minnesota, 1995–2006

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Learning Objectives

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

- Identify the risk factors for invasive group A streptococcal (GAS) disease
- Compare the incidence of invasive GAS disease among persons >65 years of age with that of the general population in the United States
- Identify factors most likely to contribute to GAS outbreaks in nursing homes
- Describe the case-fatality ratio of GAS disease among older patients
- Describe the pattern of invasive GAS disease in nursing homes

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Most of the literature about GAS disease in nursing homes has focused on acute outbreaks with little attention paid to sporadic disease in this setting (*7–19*). Factors contributing to these outbreaks include GAS-infected caregivers, inadequate infection control measures, resident-to-resident spread, and the presence of a chronically infected or persistently colonized resident (*7–17*). On the basis of our experience with GAS surveillance in Minnesota, we suspect that a lack of recognition of GAS disease occurrence within nursing homes may also be a contributing factor.

We describe the occurrence of invasive GAS disease among residents of nursing homes in Minnesota over 11 years, the challenges we encountered with surveillance, and our efforts to prevent and control the spread of disease in this setting. Our findings will be useful in the development of guidelines for the prevention and control of GAS disease in nursing homes.

Methods

Surveillance

We began active, statewide, population- and laboratory-based surveillance for invasive GAS disease in April 1995 through Active Bacterial Core surveillance (ABCs), part of the Emerging Infections Program of the Centers for Disease Control and Prevention (CDC) (*20*). The population of Minnesota was 4.9 million in 2000. Invasive GAS disease is defined as GAS isolated from a normally sterile site such as blood or cerebrospinal fluid or from a wound when accompanied by STSS or necrotizing fasciitis (*20*). To ensure complete case capture, laboratories either submit computerized lists of all GAS-positive cultures from normally sterile sites at least monthly or are contacted twice monthly by Minnesota Department of Health (MDH) staff, and audits are completed routinely. Hospital infection control practitioners then complete standard report forms for cases of invasive disease, and all GAS isolates are sent to the MDH Public Health Laboratory. All GAS isolates undergo pulsed-field gel electrophoresis (PFGE) with *Sma*1 by methods described elsewhere, with the exception that an *Enterococcus* isolate was used as the standard (*21*). PFGE patterns are evaluated both visually and with BioNumerics software (Applied Maths, Kortrijk, Belgium) by using the dice coefficient. For patterns to be considered indistinguishable, they must visually appear identical and the DNA patterns must differ by <1.5% with respect to molecular weight. Isolates are also sent to CDC for *emm* typing (*22*).

Beginning in 1995, information about a case-patient's residence was collected on the case report form, including street address, city, state, and ZIP code. In 1998, a question was added about whether the case-patient had been a resident of a nursing home, long-term care facility (LTCF), or other chronic care facility for at least 30 days before the date the culture was collected. Persons living in group homes, prisons, rehabilitation hospitals, or who were going to facilities for daily outpatient therapy were not included. Beginning in 2002, the name of the facility was collected. Addresses for case-patients ≥55 years of age were also checked by a reverse address directory to see whether they corresponded with that of an LTCF. Information about size, location, and classification of LTCFs was collected from a directory of Minnesota licensed, certified, and registered healthcare facilities.

For the purposes of this study, only case-patients who could be confirmed as residents of nursing homes were included because accurate denominator data were only available for this group. In Minnesota, a nursing home is defined as a facility that provides nursing care to ≥5 persons who are not in need of acute care facilities but require nursing supervision on an inpatient basis. Denominators for calculating incidence were derived from the 2000 US Census data as reported by the Minnesota State Demographic Center, which describe the population living in group quarters by age and type of quarters (*23*).

Cluster Identification

We defined a nursing home cluster as ≥2 cases in residents of a nursing home in which isolates were nearly identical as determined by PFGE (PFGE patterns within a 3-band difference [*24*]) during a 12-month period. PFGE patterns were used for cluster identification because they were more discriminating than *emm* types (e.g., several PFGE patterns were typically found to correspond to 1 *emm* type, while PFGE patterns were not found to have multiple *emm* types), and PFGE was readily available in our laboratory. We chose 12 months because we observed that invasive cases with indistinguishable patterns sometimes occurred many months apart within a facility. Beginning in 1995, case reports were reviewed regularly by address, facility name, and PFGE pattern to look for clusters.

EpiInfo version 6.0 (www.cdc.gov/epiinfo/Epi6/EI6-dnjp.htm) was used for statistical analysis. χ^2 test was used to determine statistical significance of differences in proportions for discrete variables, and a *t* test was used to determine whether the difference in means was significant for continuous variables.

Intervention

Since 1997, whenever a cluster was identified through ABCs, the nursing home was contacted by MDH and encouraged to conduct retrospective and enhanced prospective surveillance for invasive and noninvasive GAS infections. This surveillance included reviewing culture logs to identify noninvasive GAS infections and residents with chronic or recurrent infections and reviewing reported staff illnesses to identify a possible source of GAS. Clinical examples of

possible GAS infections were provided so the facility could consider the possibility of earlier undiagnosed GAS disease and recognize new suspect cases. For prospective surveillance, the nursing home was encouraged to obtain cultures for any suspected infection and to ask laboratories to save GAS isolates for PFGE and *emm* typing. A follow-up letter and packet of information about GAS and infection control measures were also sent to the nursing home. We offered assistance of further investigation but most often had no further contact with the facility. In 2004, after noting that we seldom observed another case at a facility after we contacted them regarding a cluster, we began to contact a facility any time we received a report of a single case.

In Minnesota, MDH staff cannot conduct an investigation in a nursing home without an invitation from the facility. Only 2 facilities with clusters requested our assistance. These investigations have been described in detail elsewhere (*25*,*26*), but in both instances cultures were collected from residents and staff (all residents and staff at facility G; all but 1 resident who refused and 3 staff members in the affected unit at facility B). Those with positive GAS cultures were treated with antimicrobial agents (clindamycin at facility G and penicillin and rifampin at facility B). Formal infection control educational sessions were provided for staff on the same day that cultures were collected.

Results

Surveillance

From April 1995 through 2006, 1,858 cases of invasive GAS disease were reported among Minnesota residents; 642 (35%) were in persons ≥65 years of age. One hundred seventy-five case-patients were identified as LTCF residents on their case report forms. Twenty-three of these case-patients resided in non–nursing home settings such as assisted living or group homes, and we were unable to determine the type of setting for 18 of those designated as LTCF residents. One hundred thirty-four (7%) of our case-patients were known to be nursing home residents. The number and percentage of all cases associated with nursing homes fluctuated over time; from 6 to 21 cases were identified among nursing home residents annually, representing 3%–12% of all case-patients each year (Figure 1). Seasonal variation of invasive GAS infections was noted among both the general population and nursing home residents (Figure 2), with peak incidence in the winter and spring and little disease noted in late summer and early fall.

The age of nursing home case-patients ranged from 36 to 100 years of age (median 84 years); 58% were women, 87% had positive blood cultures, 36% had bacteremia without another focus of infection, 32% had cellulitis, and 12% had pneumonia. The case-fatality ratio of all case-patients with invasive GAS was 12%. Among case-patients ≥65 years of age, the case-fatality rate of nursing home resident case-patients (n = 121) was 35% compared with 18% of case-patients who were not nursing home residents (n = 521).

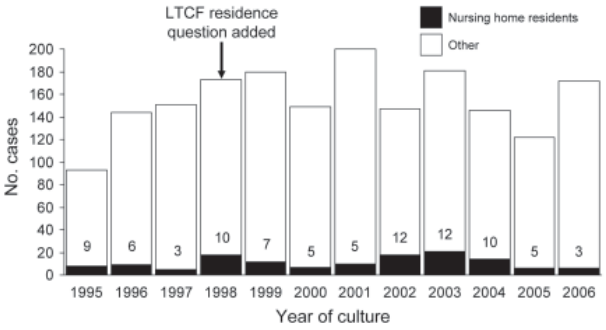

Figure 1. Annual number of cases of invasive group A streptococcal infections and percentage of cases occurring among nursing home residents, April 1995–2006. LTCF, long-term care facility.

In 2000, 37,542 Minnesota residents ≥65 years of age lived in nursing homes, and 556,724 lived in their own homes or other group quarters. During 2000, the incidence of invasive GAS infections among Minnesota nursing home residents ≥65 years was 18.6 cases/100,000 population compared with 6.8 cases/100,000 among those ≥65 years who did not reside in nursing homes. Estimated annual incidence for nursing home residents ≥65 years varied from 13.3 cases/100,000 in 1997 to 50.6 cases/100,000 in 2003, while the estimated incidence for non–nursing home residents ≥65 years was 8.6 and 9.3 cases/100,000 during the same years.

Emm type was available for 117 (87%) of the nursing home case-patient isolates. Of 21 different *emm* types identified, 4 (*emm* 1 [21%], *emm* 89 [15%], *emm* 28 [13%], and *emm* 03 [11%]) accounted for 60% of the isolates. Among 1,416 (82%) non–nursing home case-patients, 5 *emm* types accounted for 62% of the isolates (*emm* 1 [24%], *emm* 28 [13%], *emm* 03 [11%], *emm* 12 [10%], and *emm* 89 [4%]). Although total numbers of cases varied considerably from one year to the next, the proportion of disease caused by the most common *emm* types fluctuated little.

Cluster Identification

Of the 444 licensed nursing homes in Minnesota, 91 (20%) were known to have at least 1 case of invasive GAS disease during the study period. Sixty-seven (74%) of these facilities had a single case; 13 facilities had 2 cases; and 11 facilities had ≥3 cases. Of 24 facilities that had ≥2 cases, 13 (54%) met the definition of a cluster as previously defined (Table). We found that PFGE patterns for isolates from the same facilities were either indistinguishable from each other or distinctly different (>3 bands different).

Four nursing homes that had clusters also had additional cases that did not fit the definition for inclusion in

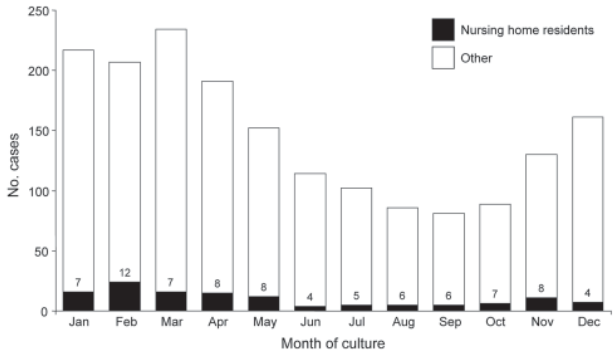

Figure 2. Number of cases of invasive group A streptococcal infections and percentage of cases occurring among nursing home residents by month of culture, Minnesota, 1995–2006.

the cluster, either because a case isolate had a distinctly different PFGE pattern, a case did not occur within 1 year of the other cases, or both. One of these facilities had 4 such cases. In addition, 11 other nursing homes each had 2–3 cases that did not fit the definition of a cluster.

All but 2 clusters were caused by the most common *emm* types. *Emm* 1 was the most common cause of invasive disease (causing 24% of all cases) and also the cause of 5 (38%) clusters.

Eighteen of 21 pairs (86%) of consecutive cases occurring within 12 months of each other in the same facility had matching PFGE patterns, while 13 (93%) of 14 pairs of consecutive cases occurring within 3 months had matching patterns. The occurrence of a third case in a nursing home was not dependent on the first 2 case isolates having the same PFGE patterns; 6 (46%) of 13 facilities in which the first 2 case isolates had different PFGE patterns and 5 (45%) of 11 facilities in which the first 2 case isolates had matching PFGE patterns subsequently had more cases.

No significant difference was found for age, sex, or type of infection between cluster and sporadic cases. Forty-one percent of case-patients with cluster-associated cases died, compared with 32% of patients with sporadic cases, but this difference was not significant ($p = 0.33$).

Intervention

In 32 (86%) of 37 encounters with nursing staff, the person contacted was not aware of a diagnosis of invasive GAS disease among their residents before our call. In addition, even when our contacts (usually either directors of nursing or nurses designated to oversee infection control for the facility) did know of the diagnosis, they generally had little knowledge about GAS disease. Before 2004, we noted that 9 of 12 nursing homes did not identify additional cases of invasive disease after our call. In 2004, we began notifying nursing homes after we identified single cases in their facilities. Since then, we are aware of only 1 facility with a cluster, and that facility had 2 cases 4 days apart.

We collected throat and skin lesion cultures from staff and residents for a unit with a cluster of invasive GAS disease at facility B and from staff and residents of the entire nursing home at facility G. At facility B, 2 (2.7%) of 75 throat cultures from staff and 2 (5.9%) of 34 throat cultures from residents were positive for GAS; 5 (6.2%) of 81 throat cultures from staff and 2 (4.5%) of 44 throat cultures of residents were positive for GAS at facility G. All of those with positive throat cultures were asymptomatic at the time of culture. All except 1 isolate from a staff person at facility G who did not provide direct patient care had PFGE patterns indistinguishable from those associated with the invasive cases at the facility. Those with positive cultures were each treated with a course of antimicrobial drugs, and no additional cases were detected at either facility.

Discussion

Minnesota has had a unique opportunity to conduct active, population-based surveillance for invasive GAS disease for >11 consecutive years with nearly complete case reporting plus further molecular characterization of associated GAS isolates. Findings from this statewide sur-

Table. Clusters of invasive group A streptococcal disease in nursing homes, Minnesota, April 1995–2006

Facility	Year(s)	Month of onset for first case	No. cases	Interval between first and second case	Interval between first and last case	*emm* type
A	1995	Apr	4	1 mo	7 mo	01
B	1996–1997	Jan	5	10 mo	16 mo	89
C	1998	Feb	2	5 mo	5 mo	01
D	2000	May	2	2 mo	2 mo	82
E	2001	Apr	2	3 wk	3 wk	01
F	2002	Feb	2	9 mo	9 mo	28
G	2002	Mar	3	3 wk	3 mo	01
H	2002–2003	Dec	3	3 mo	3.5 mo	03
I	2003	Jan	2	2 mo	2 mo	89
J	2003	May	2	1 wk	1 wk	28
K	2003	Feb	3	2 wk	9 mo	01
L	2003–2004	Dec	2	1 mo	1 mo	12
M	2004	Feb	2	4 d	4 d	05

veillance, our review of the strengths and weaknesses of GAS surveillance specific for nursing homes, and further evaluation of factors associated with clusters of GAS in this setting provide information to aid in the development of effective national guidelines for the prevention and control of GAS infections in this vulnerable population.

Although <2% of Minnesota's population resides in nursing homes, at least 7% of invasive GAS cases occurred among this population. As noted in other studies (*27*), we also found that the case-fatality rate was higher for nursing home residents than for the rest of the population. Much of this increase in illness is likely due to the frequency of risk factors for invasive GAS disease among this population (e.g., advanced age and underlying diseases such as diabetes and chronic obstructive pulmonary disease); however, the increase may also be due to difficulties of limiting the introduction and transmission of GAS in this or any institutional setting or in a closed population.

The true incidence of GAS disease in nursing homes and the occurrence of clusters are likely higher than detected by our surveillance system. Collection of specimens from febrile nursing home residents is limited when infections in nursing home residents are treated empirically. In addition, our early surveillance methods likely misclassified the residence of GAS case-patients. We found that the street addresses for patients that were obtained from hospital admission records were often not the addresses of the nursing homes where case-patients resided but were instead the home address of a spouse or other family member. The percentage of case-patients with invasive GAS disease identified as living in nursing homes rose markedly in 1998 when a specific question about LTCF residence was added to the ABCs case report form. In 2002, we began collecting the name of the facility where potential case-patients resided, enabling nursing home residence to be confirmed. Because of these improvements in methods over time, we cannot appropriately compare our early nursing home disease rates to those calculated from more recent data to draw conclusions about changes in trends.

Prevention and effective control of GAS infections in nursing home residents can be improved with changes in surveillance. Knowledge of the initial case in a facility may help prevent a second case through review and improvement of infection control in the facility, the identification of and treatment for a colonized or infected staff member, or segregation of infected patients. We found that nursing homes were frequently unaware that their hospitalized residents had invasive GAS disease until notified by public health officials. All GAS infections identified by referring hospitals must be reported back in a timely manner to the nursing homes from which a patient was transferred. Surveillance for noninvasive GAS infections may also be needed. Because these infections are not reportable, nursing home staff and public health personnel may not be aware of the first introduction of GAS into a facility or ongoing transmission when the onsets of invasive GAS cases are separated by long periods.

Given the current limitations of public health surveillance, nursing home staff, especially those responsible for infection control, must be educated specifically about GAS disease and its transmission. Hospital infection control practitioners may be in the best position to find cases and to inform nursing homes when they review culture results for hospital surveillance.

We found further characterization of GAS isolates helpful when confronted with multiple cases of GAS disease in a facility. Even if laboratory resources are scarce, nursing home isolates of GAS should be saved for future testing if additional cases occur. Both e*mm* typing and PFGE are useful tools when attempting to determine whether ≥2 cases are related. A high percentage of temporally related cases had isolates with indistinguishable PFGE patterns, which suggests that continued transmission of a single strain is occurring in a facility, although reintroduction of a similar strain from the community cannot be excluded. In half of the situations in which a nursing home had 2 invasive cases, additional cases occurred regardless of whether the GAS isolates from the first 2 case-patients had matching PFGE patterns, which suggests a failure of infection control in these facilities. Although knowledge of GAS strain relatedness identified through PFGE or *emm* typing can help identify the source of the GAS infection, circulating within a facility or introduced from the community, we conclude that a thorough investigation is warranted when >1 case has occurred in a facility within a few months. *Emm* typing is not readily available in most public health laboratories; however, the results of *emm* typing of GAS isolates from ongoing ABCs is important for researchers currently developing multivalent GAS vaccines. The types most common among our nursing home case-patients are included in a 26-valent vaccine that has completed a phase II trial (*28*).

Although most invasive GAS disease cases occurring in nursing homes are sporadic, our experience suggests that the time of first awareness of any GAS disease in a nursing home is also the time to assess the extent of spread and institute infection control measures. Clinical syndromes of GAS should be reviewed with staff, and the importance of excluding staff and visitors with illness should be emphasized. Hand hygiene among staff, visitors, and residents needs to be emphasized. We also recommend that surveillance for GAS disease, including noninvasive disease, be implemented and that cultures be obtained from patients with potential cases. If ongoing transmission and disease continue, additional measures, such as performing screening cultures for GAS, can be helpful.

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References

1. O'Brien KL, Levine OS, Schwartz B. The changing epidemiology of group A *Streptococcus* infections. Semin Pediatr Infect Dis. 1997;8:10–6.
2. Cone LA, Woodard DR, Schlievert PM, Tomory GS. Clinical and bacteriologic observations of a toxic shock-like syndrome due to *Streptococcus pyogenes*. N Engl J Med. 1987;317:146–9.
3. Stevens DL, Tanner MH, Winship J, Swarts R, Ries KM, Schlievert PM, et al. Severe group A streptococcal infections associated with a toxic shock-like syndrome and scarlet fever toxin A. N Engl J Med. 1989;321:1–7.
4. Factor SH. Invasive group A streptococcal disease: risk factors for adults. Emerg Infect Dis. 2003;9:970–7.
5. Davies HD, McGeer A, Schwartz B, Green K, Cann D, Simor AE, et al. Invasive group A streptococcal infections in Ontario, Canada. N Engl J Med. 1996;335:547–54.
6. O'Loughlin RE, Roberson A, Cieslak PR, Lynfield R, Gershman K, Craig A, et al. The epidemiology of invasive group A streptococcal infection and potential vaccine implications: United States, 2000–2004. Clin Infect Dis. 2007;45:853–62.
7. Reid RI, Briggs RS, Seal DV, Pearson AD. Virulent *Streptococcus pyogenes*: outbreak and spread within a geriatric unit. J Infect. 1983;6:219–25.
8. Ruben FLNC, Heisler B, Korica Y. An outbreak of *Streptococcus pyogenes* infections in a nursing home. Ann Intern Med. 1984;101:494–6.
9. Schwartz B, Ussery XT. Group A streptococcal outbreaks in nursing homes. Infect Control Hosp Epidemiol. 1992;13:742–7.
10. Smith A, Li A, Tolomeo O, Tyrrell GJ, Jamieson F, Fisman D. Mass antibiotic treatment for group A streptococcus outbreaks in two long-term care facilities. Emerg Infect Dis. 2003;9:1260–5.
11. Barnham M, Kerby J. *Streptococcus pyogenes* pneumonia in residential homes: probable spread of infection from the staff. J Hosp Infect. 1981;2:255–7.
12. McNutt L-A, Casiano-Colon AE, Coles FB, Morse DL, Menegus M, Groth-Juncker A, et al. Two outbreaks of primarily noninvasive group A streptococcal disease in the same nursing home, New York, 1991. Infect Control Hosp Epidemiol. 1992;13:748–51.
13. Harkness GA, Bentley DW, Mottley M, Lee J. *Streptococcus pyogenes* outbreak in a long-term care facility. Am J Infect Control. 1992;20:142–8.
14. Barnham M, Hunter S, Hanratty B, Kirby P, Tanna A, Efstratiou A. Invasive M-type 3 *Streptococcus pyogenes* affecting a family and a residential home. Commun Dis Public Health. 2001;4:64–7.
15. Pritchard VG, Kerry CS. Streptococcal outbreak. J Gerontol Nurs. 1988;14:19–23.
16. Centers for Disease Control and Prevention. Nursing home outbreaks of invasive group A streptococcal infections—Illinois, Kansas, North Carolina, and Texas. MMWR Morb Mortal Wkly Rep. 1990;39:577–9.
17. Greene CM, Van Beneden C, Javadi M, Skoff TH, Beall B, Facklam R, et al. Cluster of deaths from group A streptococcus in a long-term care facility—Georgia, 2001. Am J Infect Control. 2005;33:108–13.
18. Schwartz B, Elliot JA, Butler JC, Simon PA, Jameson BL, Welch GE, et al. Clusters of invasive group A streptococcal infections in family, hospital, and nursing home settings. Clin Infect Dis. 1992;15:277–84.
19. Rahman M. Outbreak of *Streptococcus pyogenes* infections in a geriatric hospital and control by mass treatment. J Hosp Infect. 1981;2:63–9.
20. Centers for Disease Control and Prevention. Active Bacterial Core surveillance methodology. 2007 [cited 2008 Mar 18]. Available from http://www.cdc.gov/ncidod/dbmd/abcs/meth-start.htm
21. Natarajan A, Boxrud D, Dunny G, Srienc F. Flow cytometric analysis of growth of two *Streptococcus gordonii* derivatives. J Microbiol Methods. 1999;34:223–33.
22. Centers for Disease Control and Prevention. *Streptococcus pyogenes emm* sequence database. 2005 [cited 2006 May 4]. Available from http://www.cdc.gov/ncidod/biotech/strep/strepindex.htm
23. Birkholz DE. Minnesota Group Quarters Population. Population notes 2002;OSD-02-97 [cited 2008 Mar 18]. Available from http://www.demography.state.mn.us/series.html?Id=18841
24. Tenover FC, Arbeit RD, Goering RV, Mickelson PA, Murray BE, Persing DH, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol. 1995;33:2233–9.
25. Danila RN, Besser J, Rainbow J, Lexau CA, Triden L, Sayler B, et al. Population-based active surveillance for invasive group A streptococcal (GAS) disease. International Conference on Emerging Infectious Diseases; 1998 March 8–11, Atlanta, Georgia, USA. Washington: American Society for Microbiology; 1998.
26. Rainbow J, LeDell K, Thompson E, Kupferschmidt G, Juni B, Triden L, et al. Outbreak of invasive group A streptococcal disease (GASD) in a long-term care facility (LTCF) in Minnesota. International Conference on Emerging Infectious Diseases; 2004 February 29–March 3, Atlanta, Georgia, USA. Washington: American Society for Microbiology; 2004.
27. Jordan HT, Richards CL, Burton DC, Thigpen MC, Van Beneden CV. Group A streptococcal disease in long-term care facilities: descriptive epidemiology and potential control measures. Clin Infect Dis. 2007;45:742–52.
28. McMillan D. StreptAvax (ID Biomedical). Curr Opin Investig Drugs. 2006;7:186–90.

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Public Response to Community Mitigation Measures for Pandemic Influenza

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We report the results of a national survey conducted to help public health officials understand the public's response to community mitigation interventions for a severe outbreak of pandemic influenza. Survey results suggest that if community mitigation measures are instituted, most respondents would comply with recommendations but would be challenged to do so if their income or job were severely compromised. The results also indicate that community mitigation measures could cause problems for persons with lower incomes and for racial and ethnic minorities. Twenty-four percent of respondents said that they would not have anyone available to take care of them if they became sick with pandemic influenza. Given these results, planning and public engagement will be needed to encourage the public to be prepared.

Scientists and policymakers are concerned about the emergence of an influenza pandemic for which we will have neither a strain-specific vaccine nor sufficient antiviral medications at the onset of the outbreak. In February 2007, the Community Strategy for Pandemic Influenza Mitigation was issued; it describes the early, targeted, and layered use of nonpharmaceutical interventions, coupled with specific uses of antiviral influenza medications, to reduce transmission of pandemic influenza and mitigate the disease (*1*).

Researchers differ over the potential effectiveness of such community mitigation measures. Evidence to determine the best strategies for protecting persons during a pandemic is limited. Several studies based on findings from mathematical models and historical analyses suggest that early implementation of multiple measures, such as social distancing, school closures, and isolation of sick persons, may be effective in reducing the transmission of the virus (*2*–*6*). Other researchers cite uncertainty (*7*) or believe such measures may not be effective (*8*,*9*).

Community mitigation interventions include 1) isolation and treatment with influenza antiviral medications of all persons with confirmed or probable pandemic influenza; 2) voluntary home quarantine of and provision of antiviral medications as prophylaxis to members of households with persons with confirmed or probable influenza (if sufficient quantities of antiviral medications exist and a feasible means of distribution is in place); 3) dismissal of students from schools and closure of childcare facilities along with preventing the recongregation of children and teenagers in community settings; and 4) social distancing of adults in the community and workplace, which may include cancellation of large public gatherings and possible alteration of workplace environments and schedules to decrease social density. A great deal of cooperation from the public would be required to successfully implement community mitigation measures during a pandemic. Public reaction to an unfamiliar crisis is obviously difficult to predict. However, by using surveys that describe hypothetical scenarios, we can elicit potential responses of persons in these situations. Public opinion and input can help inform policy decisions and provide information about realistic expectations for mitigation measures before a public health emergency arises (*10*). This survey was conducted to gauge public reaction to social distancing and other nonpharmaceutical interventions that may be used during a severe pandemic.

Methods

Data reported here are derived from a survey by the Harvard School of Public Health Project on the Public and Biological Security. The survey was ≈20 minutes long and

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consisted of 85 questions. International Communications Research conducted the survey from September 28 through October 5, 2006.

The survey was conducted in English and Spanish with a representative national sample of 1,697 adults ≥18 years of age, including an over-sample of adults who had children <18 years of age in their households. Altogether, 821 such adults with children were interviewed. In the overall results, this group was weighted to its actual proportion of the total US adult population (cooperation rate was 75%; response rate was 36% [*11*]). Common methods for media and preelection surveys were used, and relied on weighting of the data to ensure representativeness. More information about the survey methods and complete question wordings is available in the online Technical Appendix, available from www.cdc.gov/EID/content/14/5/778-Techapp.pdf.

Surveys like this one, and others that would be conducted as part of a series in the event of a pandemic influenza, can provide technical assistance to public health officials by monitoring the response of the public to the evolving health threat posed by such an outbreak. In a public health emergency, surveys would have to be conducted with short field periods to enable rapid measurement of how the public reacts to a particular set of circumstances. These rapid cycle surveys would make it possible to provide timely information to public health officials and to ensure a quick response.

Getting survey results to public health officials in real time creates a situation similar to that of preelection polling (i.e., specific events can change the behavior and beliefs of many persons in a relatively short timeframe). National polling organizations that engage in preelection surveys use shorter field periods, which provide more up-to-date information but yield lower response rates than surveys conducted over longer time periods (*12*). Forecasts of voters' choices in preelection polls have shown that outdated information may introduce more errors into predictions of results than low response rates do (*13*).

Independent studies have shown that the results of statistically weighted data from shorter duration surveys are similar to those based on the higher response rate in surveys of long duration and can be used without an unacceptable risk for bias (*14–18*). Nonresponse in telephone surveys produces some known biases in survey-derived estimates because participation tends to vary for different subgroups of the population. To compensate for these known biases, sample data are weighted to the most recent Census data available from the Current Population Survey for sex, age, race, region, and education (*19*). Other techniques, including random-digit dialing, replicate subsamples, callbacks staggered over times of day and days of the week, and systematic respondent selection within households, are used to ensure that the sample is representative.

Possible sources of nonsampling errors for this survey include nonresponse bias, as well as specific wording of questions and the order in which questions are asked. The margin of error for the total sample was ±2.4%. To examine differences among subgroups, we compared responses by testing for differences in proportions, taking into account the effect of the study's design (*20*).

Because many of the respondents may not have been familiar with pandemic influenza, they were first presented with a descriptive hypothetical scenario: "Now I want to ask you some questions about a possible outbreak in the United States of pandemic flu, a new type of flu that spreads rapidly among humans and causes severe illness. Currently there have not been any cases of pandemic flu in the United States. However, imagine that there was a severe outbreak in the United States, possibly in your community. A lot of persons were getting sick from the flu and the flu was spreading rapidly from person to person." This scenario was intentionally designed to describe a severe situation without being overly alarming. Respondents were then asked how they would respond to and be affected by the circumstances that would arise from such an outbreak. The small proportion of the respondents who said they would be unable to cooperate with public health authorities could be translated into millions of persons who would have difficulty.

Results

Familiarity with Pandemic Influenza

To determine whether respondents understood what was meant by pandemic influenza, the survey asked how familiar the respondents were with the term (it is unfamiliar to most Americans). Forty-one percent said they knew what the term meant. Thirty-three percent reported that they had heard of the term but did not know what it meant, and 25% had never heard of pandemic flu (online Technical Appendix).

Ability to Stay Home

Respondents were asked about their ability to comply with public health recommendations during an influenza pandemic; 94% said they would stay at home, away from others, for 7–10 days if they had pandemic flu. In addition, 85% said all members of their household would stay at home for the same period if a member of their household were sick (Table 1; online Technical Appendix).

Eighty-five percent said they would be able to take care of sick household members at home for 7–10 days. However, 76% of respondents worried about getting sick if they cared for a sick household member.

Seventy-three percent said that they would have someone available to take care of them at home if they became

Table 1. Responses to questions about ability to stay home during an influenza pandemic*

Question	Possible responses		
	Yes	No	Don't know/refused to answer
Would stay at home for 7–10 d if public health officials recommended because you had flu	94	4	2
You and all members of household would stay at home for 7–10 d if public health officials recommended because a member of household had flu	85	12	4
If public health officials recommended, would be able to take care of sick household member for 7–10 d at home	85	13	2
If stayed at home with sick household member, would be worried about getting sick yourself	76	22	2
Have someone who could care for you at home if you were sick	73	24	4
	Likely	Unlikely	Don't know/refused to answer/NA
You or a member of your household might lose pay and have money problems	48	50	1
You or a member of your household might have a hard time being stuck at home for so long	46	54	1
You might not be able to get baby formula, diapers, or other important things for a baby in your household†	45	53	1
You or a member of your household might be unable to get the health care or prescription drugs that you need	43	55	2
You might not be able to get care for a disabled person in your household‡	36	48	15
You might not be able to get care for an older person in your household§	35	51	15
You might have difficulty taking care of the (child/children) <5 y in your household¶	32	67	1
You or a member of your household might lose your job or business as a result of having to stay home#	27	71	2

*From the Harvard School of Public Health, Pandemic Influenza Survey, 2006. Numbers represent percentage of responses to each question. NA, not applicable.
†Among respondents with major responsibility for children ≤2 y (n = 174).
‡Among respondents in households with disabled person (n = 470).
§Among respondents in households with persons ≥65 y (n = 408).
¶Among respondents who have major responsibility for children <5 y (n = 262).
#Among employed respondents (n = 1,101).

sick with pandemic flu and had to remain at home for 7–10 days. However, 24% said they would not have someone available to take care of them. Persons living in households with only 1 adult are far more likely not to have someone available to take care of them (45%) compared with persons from households with >1 adult (17%). Approximately one third of low-income (36%), African-American (34%), disabled (33%), and chronically ill (32%) adults said that they would not have anyone who could take care of them. A substantial proportion of the respondents (from 48% to 71%, depending on the measure) believed that they or a household member would likely experience problems if they had to stay at home for 7–10 days and avoid contact with anyone outside their household (Table 1).

School Closings

Thirty-nine percent of respondents reported having children <18 years of age living in their household (*21*), including 16% with children 13–17 years of age in the household, 22% with children 5–12 years of age, and 14% with children <5 years of age. Of adults in households that had children <18 years of age, 91% said that they have major responsibility for the children in their household (online Technical Appendix).

Respondents were told that to keep pandemic influenza from spreading and to protect the safety of children, some communities might close schools and daycare facilities for some period of time. Although the Community Strategy for Pandemic Influenza Mitigation used the term dismissal from school, the survey used the term school closure. Respondents were also told that the length of time schools and daycares would remain closed would probably be tied to the severity of the pandemic influenza outbreak.

If schools and daycare were closed for 1 month, 93% of adults who have major responsibility for children <5 years of age in daycare or children 5–17 years of age and have at least 1 employed adult in the household thought they could arrange care so that at least 1 employed adult in the household could go to work. Eighty-six percent thought they would be able to do so for 3 months (Table 2). Of those who said they could arrange care for 1 month so that at least 1 adult would be able to work, 87% said they or another family member would be the primary caretakers for children if schools and daycares had to be closed. Of these adults, 64% said they would need little or no help even if children had to be kept at home for a long time. Of those who said they would need a lot or some help, 50% said they would rely most on help from family, 11% on friends or

neighbors, and 34% on outside agencies (including government agencies, church and community groups, or voluntary agencies).

However, 60% of adults who have major responsibility for children <18 years of age said that at least 1 employed person in the household would have to stay home from work. Of employed persons, 25% who have major responsibility for children <18 years of age in their household said that if schools and daycares closed for 1 month, they would be able to work from home and take care of the children.

If schools were closed for 3 months, 95% of adults with major responsibility for children 5–17 years of age said they would be willing to give school lessons at home. Of those who were willing to do so, 47% thought they would need a lot or some help, although 53% said they would need little or no help.

Among adults with major responsibility for children 5–17 years of age, 85% thought that if schools were closed for 3 months, they would be able to keep their children and teenagers from taking public transportation, going to public events, and gathering outside home while schools were closed. Of adults who have major responsibility for children <5 years of age in daycare or children 5–17 years of age in their household, 25% reported that a child in their household gets free breakfast or lunch at school or daycare. Asked specifically about an outbreak of pandemic influenza, 34% of those whose children get free meals at school (8% of the total who have responsibility for children in this age group) said that if schools and daycare were closed for 3 months, not getting the free meals would be a problem.

Ability to Stay Home from Work

Sixty-three percent of the US adult population was employed at the time of the survey (*22*). Employed respondents were asked about the problems they might face being out of work for various lengths of time. Most employed persons (74%) believed they could miss 7–10 days of work without having serious financial problems; 25% said they would face such problems. Fifty-seven percent thought they would have serious financial problems if they stayed home for 1 month. Of those surveyed, 76% believed they would have such problems if they stayed home from work for 3 months (Table 3; online Technical Appendix).

Of employed respondents, 29% said that they would be able to work from home if they were asked to stay home for 1 month because of a serious outbreak of pandemic flu. Of the low-income workers (<$25,000/y), 13% believe that they would be able to work from home for that long, compared with 44% of high-income workers (≥$75,000/y).

Employed respondents were also asked about their employers' plans and policies for dealing with an outbreak of pandemic flu. Few working persons (19%) were aware

Table 2. Responses to questions about school closings during an influenza pandemic*

Question	Possible responses		
	Yes	No	Don't know/ refused to answer
If schools/daycare closed for 1 mo, could arrange care so that at least 1 employed adult in household could go to work†	93	5	2
If schools/daycare closed for 3 mo, could arrange care so that at least 1 employed adult in household could go to work†	86	11	3
If schools/daycare closed for 1 mo, at least 1 employed person would have to stay home from work†	60	37	3
Among those who could arrange care so that at least 1 adult in household could go to work if schools closed for 1 mo:			
If schools were closed for 3 mo, would be willing to give school lessons at home‡	95	5	<0.5
Would need help giving school lessons at home	47	53	<0.5
If schools and daycare closed for 1 mo would be able to work from home and take care of children§	25	72	3
If public health officials recommended, could keep children from taking public transportation, going to public events and gathering outside home while schools closed for 3 mo‡	85	13	2
	A lot/some	Little/none	Don't know/ refused to answer
Would need outside help with problems of having to keep children at home†	35	64	1
Children in household get free breakfast or lunch at school or daycare¶	25	74	1
If school/daycare closed for 3 mo, would be problem that children could not get free meals¶	8	91	1

*From the Harvard School of Public Health, Pandemic Influenza Survey, 2006. Numbers represent percentage of responses to each question.
†Among respondents who have major responsibility for children <5 y in daycare or children 5–17 y in household and have at least 1 working adult in household (n = 634).
‡Among respondents with major responsibility for children 5–17 y in household (n = 610).
§Among employed respondents who have major responsibility for children <5 y in daycare or children 5–17 y in household (n = 537)
¶Among respondents who have major responsibility for children <5 y in daycare or children 5–17 y in household (n = 664).

Table 3. Responses to questions about staying home from work during an influenza pandemic*†

Question	Possible responses		
	Yes	No	Don't know/ refused to answer
Ever work from home†	27	73	<0.5
Would be a serious financial problem if had to stay home for work for 7–10 d†	25	74	1
Would be a serious financial problem if had to stay home for work for 1 mo†	57	41	2
Would be a serious financial problem if had to stay home for work for 3 mo†	76	22	2
If had to stay home for 1 mo, would be able to work from home for that long†	29	69	2
If had to stay home for 3 mo, would be able to work from home for that long†	19	78	3
Workplace has plan for outbreak of pandemic flu†	19	63	18
Includes encouraging sick to stay home	16		
Provides information about flu	14		
Provides information on what supplies to have at home	12		
Includes expanding options to work from home	6		
Would stay home if public health official said you should, even if employer told you to come to work†	57	35	9
Are you worried employer would make you go to work if sick during an outbreak†	22	77	2
Worried employer would make you go to work if sick during outbreak†	43	50	7
Would stay home if public health official said you should, even if employer told you to come to work†	57	35	9
If had to stay home from work, would still get paid†	35	42	22

*From the Harvard School of Public Health, Pandemic Influenza Survey, 2006. Numbers represent percentage of responses to each question.
†Among employed respondents (n = 1,101).

of any workplace plan to respond to a serious outbreak of pandemic flu.

Of employed adults, 57% said they would stay home from work if public officials said they should; 35% said they would go to work if their employers told them to report to their jobs. Of employed adults, 22% were worried that, in the event of a serious outbreak of pandemic flu in their community, their employer would make them go to work even if they were sick.

Of employed respondents, 50% believed that their workplace would stay open if there was a serious outbreak of pandemic flu, even if public health officials recommended that some businesses in the community should shut down. Forty-three percent thought that their workplace would shut down.

Of employed respondents, 35% thought that if they stayed home from work, they would still get paid; 42% thought that they would not get paid, and 22% did not know whether they would get paid. Low-income respondents (from households <$25,000/y) were significantly less likely than high-income respondents (from households ≥$50,000/y) to believe they would still get paid (Table 4).

Ability to Cooperate with Other Recommendations

Respondents were given a scenario about an outbreak of pandemic influenza and asked if they would cooperate if public health officials recommended that for 1 month they curtail various activities of their daily lives. The initial response between 79% and 93% (depending on the measure) was that they would cooperate (Table 5; online Technical Appendix).

Problems Responding to Recommendations

On several measures, more low-income Americans (those who come from households with an annual income <$25,000/y) than high-income Americans believed they would experience problems responding to public health recommendations. Similarly, on many of these measures a higher proportion of African Americans and Hispanic Americans than whites believed they would experience problems (Table 4). The same holds true for persons who described their own health status as fair or poor (Table 6; online Technical Appendix).

Conclusions

If community mitigation measures were instituted for a severe influenza pandemic, most respondents would comply with recommendations but would be challenged to do so if their income or job was severely compromised. Results from this survey were useful in shaping the Community Mitigation Guidance because important information was obtained about public acceptability and key public concerns and challenges.

During a severe pandemic, public health authorities are likely to recommend that all but the sickest persons remain home while ill. Strategic planning by home-health, faith-based, and community organizations; medical providers; and public health agencies about how to coordinate care for those who would have to stay home ill during a pandemic will be essential, particularly for those who live alone.

The resiliency of those who would need to stay home during a pandemic will depend on their level of preparedness. Previous studies on personal preparedness at home

have shown that respondents have concerns about having sufficient supplies if asked to stay quarantined at home for a prolonged period of time (*23*). Two recent surveys indicate that many Americans have made no preparations for a public health emergency and most have prepared less than they think they should (*24*,*25*). Careful community planning, including public education and engagement, will be needed to encourage the public to be prepared for an emergency like a pandemic.

Survey results also indicated that most persons were concerned about getting sick themselves if they had to stay at home to care for a household member who was ill with pandemic flu. The public must be given accurate information before and during a pandemic about how to provide

Table 4. Responses to questions about potential problems adhering to public health recommendations, by household income, and race/ethnicity*

Question	Total	Household income				Race/ethnicity		
		<$25K	$25–49.9K	$50–74.9K	≥$75K	White (non-Hispanic)	Black (non-Hispanic)	Hispanic
	All respondents							
	n = 1,697	n = 226	n = 366	n = 300	n = 501	n = 1,345	n = 133	n = 114
If public health officials recommended, would not be able to take care of sick household member for 7–10 d at home	13	19†	16†	6	6	12	19	15
Do not have someone who could care for you at home if you were sick	24	36‡	25§	22	15	23	34¶	20
If asked to stay home 7–10 d, likely that:								
You or a member of your household might lose pay and have money problems	48	57§	58§	49§	35	43	68#	66#
You or a member of your household might be unable to get the health care or prescription drugs that you need	43	57‡	43§	38	35	41	52#	49
You or a member of your household might lose your job or business as a result of having to stay home	27	41‡	30§	24§	14	20	41#	53#
	Employed respondents							
	n = 1,101	n = 91	n = 224	n = 224	n = 406	n = 855	n = 87	n = 79
Would be a serious financial problem if had to stay home from work for 7–10 d	25	56‡	29†	15	15	23	20	37#
Would be a serious financial problem if had to stay home from work for 1 mo	57	84‡	69†	50§	37	53	65#	68#
Would be a serious financial problem if had to stay home from work for 3 mo	76	93‡	84†	71	64	74	76	88#
If had to stay home for 1 mo, would not be able to work from home for that long	69	85†	79§	71§	55	67	77	77
If you had to stay away from work, you:								
Would still get paid	35	14	25	47**	51**	39††	29	22
Would not get paid	42	64†	57†	30	18	41	48	55
Don't know	22	22	18	22	23	20	22	23

*From the Harvard School of Public Health, Pandemic Influenza Survey, 2006. Numbers represent percentage responding "yes" to each question.
†Statistically higher proportion than $50–74.9K and ≥$75K.
‡Statistically higher proportion than $25–49.9K, $50–74.9K, and ≥$75K.
§Statistically higher proportion than ≥$75K.
¶Statistically higher proportion than whites and Hispanics.
#Statistically higher proportion than whites.
**Statistically higher proportion than <$25K and $25–49.9K.
††Statistically higher proportion than Hispanics.

Table 5. Responses to questions about other community mitigation strategies*

Question	Possible responses		
	Yes	No	Don't know/refused/ not applicable
Would follow recommendation if public health officials said for 1 mo you should:			
Avoid air travel	93	5	1
Avoid public events like movies, sporting events, or concerts	92	7	<0.5
Avoid going to malls and department stores	91	9	1
Limit your use of public transportation, buses and trains	89	7	4
Cancel doctor or hospital appointments that are not critical at the time	89	10	1
Reduce contact with people outside your own household as much as possible	88	11	1
Avoid going to church or religious services	82	16	1
Postpone family or personal events such as parties, weddings, or funerals	79	18	3
	Likely	Not likely	Don't know/refused
Would stay in town or city during serious outbreak if public health officials recommended you do so	90	9	<0.5

*From the Harvard School of Public Health, Pandemic Influenza Survey, 2006. Numbers represent percentage of responses to each question.

at-home care along with precautions that caretakers should follow to protect their own health.

Employers can enable employees to comply with public health recommendations during a pandemic (*26*,*27*). Sick leave and other policies (such as telecommuting, staggered shifts, and other strategies) should promote and create incentives for workers to stay home if they or a household member becomes sick during a severe pandemic or if well, to report to work. Well employees should report to work (especially those in health care and other critical infrastructure jobs) to ensure business continuity and the ability to provide care as needed (*28*). Workers should be aware of their employer's pandemic preparedness plans and other strategies that will promote social distancing at the workplace during a pandemic. Implementing these measures will help to ensure a safer workplace during a pandemic and will mitigate transmission of disease.

Among the key interventions for potentially reducing transmission of the influenza virus during a pandemic will be to dismiss students from schools, close childcare facilities, and keep children from re-congregating in the community. Depending on the severity of the pandemic, the duration of school dismissal could range from a few weeks up to 3 months. How families would cope with the cascading effects from prolonged cancellation of school classes is a concern. Families could face the problem of serious income loss.

Table 6. Responses to questions about potential problems adhering to public health recommendations by health, chronic illness, and disability status*

Question	Total (n = 1,697)	Health status		Chronic illness		Disabled	
		Fair/poor (n = 196)	Excellent/very good/good (n = 1,481)	Yes (n = 355)	No (n = 1,317)	Yes (n = 323)	No (n = 1,354)
If public health officials recommended, would not be able to take care of sick household member for 7–10 d at home	13	25†	11	16	12	21‡	10
Do not have someone who could care for you at home if you were sick	24	34†	23	32§	22	33‡	22
If asked to stay home 7–10 days, likely that:							
You or a member of your household might lose pay and have money problems	48	55	48	47	49	49	48
You or a member of your household might be unable to get the health care or prescription drugs that you need	43	55†	40	50§	40	48	41
You or a member of your household might lose your job or business as a result of having to stay home	27	38†	25	24	28	31	26

*From the Harvard School of Public Health, Pandemic Influenza Survey, 2006. Numbers represent percentage of responses to each question.
†Statistically higher proportion than Excellent/very good/good health status.
‡Statistically higher proportion than those who are not disabled.
§Statistically higher proportion than those who do not have a chronic illness.

Most respondents said that at least 1 employed person would have to stay home from work during a pandemic to care for children. Therefore, employers can identify employees who may need to stay home to care for children and determine in advance if those employees could work from home, work staggered shifts, or be trained to take on other responsibilities, or if other employees can be cross-trained to take on some of those job functions. Employers must be prepared for increased absenteeism related to childcare responsibilities.

Community mitigation measures could cause particular problems for persons from low-income families and for racial and ethnic minorities. With these problems in mind, communities should plan for the needs of vulnerable populations who may be adversely affected during a pandemic. Workers who do not have sick or other leave time available will need support if they have to stay home during a pandemic. Communities should explore alternative ways of replacing school-based services, such as free meals, if schools are unable to provide those services.

These findings can inform planners about what the public may do if a pandemic occurs. However, the public might react differently when the event actually occurs. These results should be interpreted with caution in advance of a severe pandemic that could cause prolonged disruption of daily life and widespread illness in a community. Adherence rates to recommendations might be high during the early stages of a pandemic but results may not be as predictive over the course of several months. We have more confidence in the predictive ability of the survey in areas in which the public has a greater amount of personal experience, e.g., workplace issues, income, and the need for assistance at home.

Willingness to adhere to community mitigation measures may be influenced by the severity of illness persons observe in the community relative to their need for income and the level of community, individual, and family disruption. In addition, public response is likely to be affected by the perceived effectiveness of government and voluntary agencies in dealing with crisis situations. Planning for implementation of community mitigation measures, as well as actions to reduce secondary consequences, are important steps in enhancing adherence to public health recommendations.

The communication resources of government can be scarce during a crisis. Such resources can be used most effectively if there are recent data about what the public needs to learn. This was seen in the cases of severe acute respiratory syndrome and anthrax (*29*). During a pandemic, short-duration rapid-turnaround public surveys can provide timely information to public health officials about the acceptability of recommendations and needed communication to the public if problems are found (*15*). Although the challenge is formidable, our best chances of protecting health and maintaining functioning communities during a pandemic rely on optimal adherence to public health measures and a coordinated response within and between communities.

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References

1. Centers for Disease Control and Prevention. Interim pre-pandemic planning guidance: community strategy for pandemic influenza mitigation in the United States. 2007 Feb [cited 2008 Jan 4]. Available from http://www.pandemicflu.gov/plan/community/commitigation.html
2. Hatchett RJ, Mecher CE, Lipsitch M. Public health interventions and epidemic intensity during the 1989 influenza pandemic. Proc Natl Acad Sci U S A. 2007;104:7582–7.
3. Bootsma MC, Ferguson NM. The effect of public health measures on the 1918 influenza pandemic in U.S. cities. Proc Natl Acad Sci U S A. 2007;104:7588–93.
4. Markel H, Lipman HB, Navarro JA, Sloan A, Michalsen JR, Stern AM, et al. Nonpharmaceutical interventions implemented by US cities during the 1918–1919 influenza pandemic. JAMA. 2007;298:644–54.
5. Ferguson NM, Cummings DA, Fraser C, Cajka JC, Cooley PC, Burke DS. Strategies for mitigating an influenza pandemic. Nature. 2006;442:448–52.
6. Glass RJ, Glass LM, Beyeler WE, Min HJ. Targeted social distancing design for pandemic influenza. Emerg Infect Dis. 2006;12:1671–81.
7. Institute of Medicine; Committee on Modeling Community Containment for Pandemic Influenza. Modeling community containment for pandemic influenza. A letter report. Washington: National Academies Press; 2006.
8. Inglesby TV, Nuzzo JB, O'Toole T, Henderson DA. Disease mitigation measures in the control of pandemic influenza. Biosecur Bioterror. 2006;4:366–75.
9. Toner E. Do public health and infection control measures prevent the spread of flu? Biosecur Bioterror. 2006;4:84–6.
10. Schoch-Spana M, Franco C, Nuzzo JB, Usenza C, Working Group on Community Engagement in Health Emergency Planning. Community engagement: leadership tool for catastrophic health events. Biosecur Bioterror. 2007;5:8–25.
11. American Association for Public Opinion Research. Final dispositions of case codes and outcome rates for surveys, 2006 [cited 2008 Jan 4]. Available from http://www.aapor.org/uploads/standarddefs_4.pdf
12. Brady HE, Orren GR. Polling pitfalls: sources of error in public opinion surveys. In: Mann TE, Orren GR, editors. Media polls in Americans politics. Washington: Brookings Institution; 1992.

13. Mosteller F, Hyman H, McCarthy PJ, Marks ES, Truman DB. The pre-election polls of 1948. New York: Social Science Research Council; 1949.
14. Keeter S, Kennedy C, Dimock M, Best J, Craighill P. Gauging the impact of growing nonresponse on estimates from a nation RDD telephone survey. Public Opin Q. 2006;70:759–79.
15. Blendon RJ, Benson JM, DesRoches CM, Weldon KJ. Using opinion surveys to track the public's response to a bioterrorist attack. J Health Commun. 2003;8(Suppl 1):83–92.
16. Keeter S, Miller C, Kohut A, Groves RM, Presser S. Consequences of reducing non-response in a national telephone survey. Public Opin Q. 2000;64:125–48.
17. Curtin R, Presser S, Singer E. The effect of response rate changes on the Index of Consumer Sentiment. Public Opin Q. 2000;64:413–28.
18. Traugott MW. Assessing poll performance in the 2000 campaign. Public Opin Q. 2001;65:389–419.
19. Voss DS, Gelman A, King G. A review: preelection survey methodology; details from eight polling organizations, 1988 and 1992. Public Opin Q. 1995;59:98–132.
20. Daniel WW. Biostatistics: a foundation for analysis in the health sciences. New York: John Wiley; 1991. p. 225–6.
21. US Bureau of the Census. Current Population Survey. 2006 March Supplement. [machine-readable dataset] [cited 2008 Jan 4] Available from http://www.census.gov/apsd/techdoc/cps/cpsmar05.pdf
22. US Department of Labor, Bureau of Labor Statistics. Employment status of the civilian population by sex and age. 2006 Oct [cited 2008 Jan 4]. Available from http://www.bls.gov/news.release/empsit.t01.htm
23. Chesser A, Ablah E, Hawley SR, Wolfe D, St Germain T, Grube CD, et al. Preparedness needs assessment in a rural state: themes derived from public focus groups. Biosecur Bioterror. 2006;4:376–83.
24. American Red Cross. Survey reveals Americans not as prepared as they think. 2006 May 23 [cited 2008 March 10]. Available from http://www.redcross.org/pressrelease/0,1077,0_489_5398,00.html
25. American Public Health Association. National opinion survey to determine levels of preparedness for a public health crisis, 2007 Feb [cited 2008 Jan 4]. Available from http://www.nphw.org/2007/Survey%20Report.pdf
26. Deloitte Center for Health Solutions. Year two pandemic preparedness survey, 2006 November–December [cited 2008 Jan 4]. Available from http://www.deloitte.com/dtt/cda/doc/content/us_chs_yeartwopandemicsurvey121806v1.pdf
27. Conference Board. Are businesses doing enough to prepare for a pandemic? 2006 Jul [cited 2008 Jan 4]. Available from http://www.conference-board.org/publications/describe_ea.cfm?id=1194
28. Irvin C, Cindrich L, Patterson W, Ledbetter A, Southall A. Hospital personnel response during a hypothetical influenza pandemic: will they come to work? Acad Emerg Med. 2007;14(Suppl 1):S13. Epub 2007 May 1.
29. Blendon RJ, Benson JM, DesRoches CM, Weldon KJ, Taylor-Clark K, Fleischfresser C. Research on Americans' response to bioterrorist threats and emerging epidemics. In: Borrelli JV, editor. Bioterrorism: prevention, preparedness and protection. New York: Nova Science; 2007.

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Spread of *Streptococcus suis* Sequence Type 7, China

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Streptococcus suis sequence type (ST) 7 has been spreading throughout China. To determine events associated with its emergence, we tested 114 isolates. In all 106 ST7 strains responsible for human outbreaks and sporadic infections, the tetracycline-resistance gene, *tetM,* was detected on the conjugative transposon Tn*916.* Horizontal transmission of *tetM* is suspected.

A large outbreak of *Streptococcus suis* serotype 2 infection emerged in the summer of 2005 in Sichuan Province, People's Republic of China, and resulted in 215 cases and 38 deaths among humans (*1*). Sporadic infections were identified in 4 other provinces. A smaller, previously overlooked, outbreak occurred in Jiangsu Province in 1998; 25 cases and 14 deaths were reported (*1,2*). The causative agent of the Sichuan and Jiangsu outbreaks was identified as a clone of *S. suis* sequence type (ST) 7 (*3*). ST7 was first identified in 1996 in a patient with meningitis in Hong Kong and later caused the 1998 outbreak in Jiangsu; it spread further to cause the largest outbreak in Sichuan in 2005 (*3,4*). The spread of *S. suis* ST7 across China underscores the need to better understand the genetic and ecologic events associated with its emergence as an important pathogen in humans.

The Study

Using the MICroSTREP Plus system (Dade Behring, Deerfield, IL, USA), we tested 114 ST7 isolates from China and found that all isolates were resistant to tetracycline and susceptible to 12 of 13 antimicrobial drugs. Of these 114, 6 were isolated in 2006, 84 were from human patients and 8 from diseased pigs in the 2005 Sichuan outbreak, 7 were from sporadic human cases and 3 from diseased pigs in other provinces in 2005, and 4 were from human patients and 2 from diseased pigs in the 1998 Jiangsu outbreak (Table 1). The isolates were susceptible to penicillin, ampicillin, cefotaxime, ceftriaxone, cefepime, meropenem, levofloxacin, chloramphenicol, erythromycin, azithromycin, clindamycin, and vancomycin. In contrast, 7 of 12 *S. suis* serotype 2 strains from other countries and 18 of 34 serotype reference strains were resistant to tetracycline; 3 tetracycline-resistant strains were also resistant to erythromycin, azithromycin, and clindamycin.

Multilocus sequence typing analysis showed that of the 114 isolates from China, 106 were typed as ST7: 98 from the Sichuan and Jiangsu outbreaks, 5 from sporadic infections in other provinces in 2005 and 2006, and 3 from diseased pigs from other provinces in 2005. Of the other 8 isolates from sporadic cases in 2005 and 2006, 7 were ST1 and 1 was untypeable. Of the 12 serotype 2 strains from other countries, 8 were ST1 and 4 were ST25. Of the 34 serotype reference strains, serotype 2 strain R735 was ST1, 10 serotypes were untypeable, and 22 STs were identified as ST6 (serotypes 17 and 19), ST35, ST53-55, ST68-73, ST75-82, ST87, or ST91-2. Serotype 17 and 19 strains were identified as ST76 (Table 1) (*5,6*).

PCR was used to screen all isolates for tetracycline resistance genes; primers specific for *tetABCDEGKLMOQS* were used (*7,8*). Of the 114 tetracycline-resistant isolates from China, 111 (all 106 ST7 strains and 5 of 7 ST1 strains) harbored the *tetM* gene. The *tetO* gene was carried by 1 ST1 and 1 sequence-untypeable strain. All 7 tetracycline-resistant serotype 2 strains from other countries and 16 of 18 tetracycline-resistant strains in the 34 reference serotypes carried the *tetO* gene (Table 1) (*5,6*). The only other *tetM*-positive strain was from serotype 13, an ST71 isolated from a diseased pig in Denmark (Table 1). The PCR results were confirmed by sequencing the PCR-synthesized fragments.

To further characterize the *tetM* genes, the open reading frame (ORF) was completely sequenced by using 16 selected strains: 9 isolates from humans and 1 from a pig from the Sichuan outbreak; 3 from sporadic infections in Guangxi, Jiangsu, and Guangdong; 1 from a diseased pig in Jiangxi Province in 2005; and 2 from the Jiangsu outbreak in 1998 (Table 1). Sequence alignments showed 2 groups (GenBank accession nos. EF101931, EF016118). The first group comprised 15 of the 16 isolates typed as ST7 (*3*). The second group had only 1 isolate, GX1, typed as ST1 (*3*). The sequences of *tetM* gene for ST7 (strain SC84) and ST1 (GX1) were 1,920 and 1,917 bp, respectively, with 90 nt variations between the 2 sequences leading to 32 aa changes. Comparison of the 53 *tetM* sequences with those from public databases showed that the *tetM* of *S. suis* SC84 was most related to *Enterococcus faecium* isolate 9830470-4 plasmid pYA470-4 (DQ223243) (*7*). The *tetM* sequence

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of *S. suis* ST1 strain GX1 was most closely related to *S. pneumoniae* Tn916-like/Tn2009 (AY466395) and to *Gardnerella vaginali* (U58986) (Figure 1) (*9*).

Because the gene *tetM* is reported to be associated with transposon Tn*916*, we designed 24 pairs of primers targeting its 24 ORFs based on published Tn*916* sequences (Table 2). The complete sequence of Tn*916* from SC84 was obtained by sequencing the PCR-synthesized fragments. Between Tn*916* of SC84 and plasmid pYA470-4 of *E. faecium*, we observed 133 nt variations, 91 of which were in the *tetM* gene, 3 in the integrase gene, 1 in excisionase gene, and 38 in 10 additional ORFs. PCR showed that 111 isolates from China and 1 from Denmark have intact Tn*916* (Table 1).

The most recognized virulence genes of *S. suis*, including *mrp*, *sly*, and *ef,* were detected by PCR in all 114 Chinese isolates tested in this study. Of the 12 serotype 2 strains from other countries, 6 of 8 ST1 strains were posi-

Table 1. Source, serotype, sequence type, and tetracycline-resistant genes in *Streptococcus suis* strains*

No. strains	Source (no.)	Place of isolation	Year of isolation (no.)	Serotype (no.)	ST (no.)	*tet* gene (no. positive)	Tn*916* (no.)	Virulence genes (no.)			
								cps2j	*mrp*	*sly*	*ef*
98 outbreak-associated ST7 strains in China											
84	Human patients	Sichuan, China	2005	2	ST7	*tetM* (84)	Intact (84)	+ (84)	+ (84)	+ (84)	+ (84)
8	Diseased pigs	Sichuan, China	2005	2	ST7	*tetM* (8)	Intact (8)	+ (8)	+ (8)	+ (8)	+ (8)
4	Human patients	Jiangsu, China	1998	4	ST7	*tetM* (4)	Intact (4)	+ (4)	+ (4)	+ (4)	+ (4)
2	Diseased pigs	Jiangsu, China	1998	2	ST7	*tetM* (2)	Intact (2)	+ (2)	+ (2)	+ (2)	+ (2)
8 ST7 strains isolated from sporadic cases in China											
5	Human patients	6 provinces, China	2005 (2) 2006 (3)	2	ST7	*tetM* (5)	Intact (5)	+ (5)	+ (5)	+ (5)	+ (5)
3	Diseased pigs	Jiangxi, China	2005	2	ST7	*tetM* (3)	Intact (3)	+ 3)	+ (3)	+ (3)	+ (3)
7 ST1 and 1 untypeable strain isolated from sporadic cases in China											
5	Human patients	Guizhou, Guangxi (4)	2005	2 (4) 14	ST1	*tetM* (4)	Intact (4)	+ (4)	+ (5)	+ (5)	+ (5)
3	Human patients	3 provinces	2006	2	ST1 (2) UT	*tetO* (2) *tetM*	Intact (1)	+ (3)	+ (3)	+ (3)	+ (3)
12 serotype 2 strains from other countries											
5	Human patients	Netherlands (2), France (3)	NA	2	ST1	*tetO* (3)		+ (5)	+ (5)	+ (5)	+ (4)
3	Diseased pigs	Netherlands, France, England	NA	2	ST1			+ (3)	+ (3)	+ (3)	+ (2)
4	Human patients (2), healthy pigs, diseased pigs	Canada (3), England	NA	2	ST25	*tetO* (4)		+ (4)	–	–	–
34 serotype reference strains											
1	Human patients	Netherlands	NA	14	ST6			–	–	+	–
1	Diseased pig	Denmark	NA	13	ST71	*tetM*	Intact	–	–	+	–
25	Diseased pigs	Canada (11), Denmark (5), Netherlands (9)	NA	1/2, 2–12, 15–16, 22–30, 32, 34	ST1, ST35, ST53–55, ST68, ST69, ST72–73, ST75, ST77–78, ST80–82, ST87, ST91–92, UT (7)	*tetO* (11)		+ (2)	+ (2)	+ (7)	+ (1)
2	Diseased calves	Canada, United States	NA	20, 31	ST70 (1), UT (1)	*tetO* (1)		–	–	–	–
1	Diseased lamb	Canada	NA	33	UT			–	–	–	–
4	Healthy pigs	Canada	NA	17–19, 21	ST76 (2), ST79, UT	*tetO* (4)		–	–	+ (2)	–

*ST, sequence type; *tet*, tetracycline; UT, untypeable by multilocus sequence typing; NA, not available.

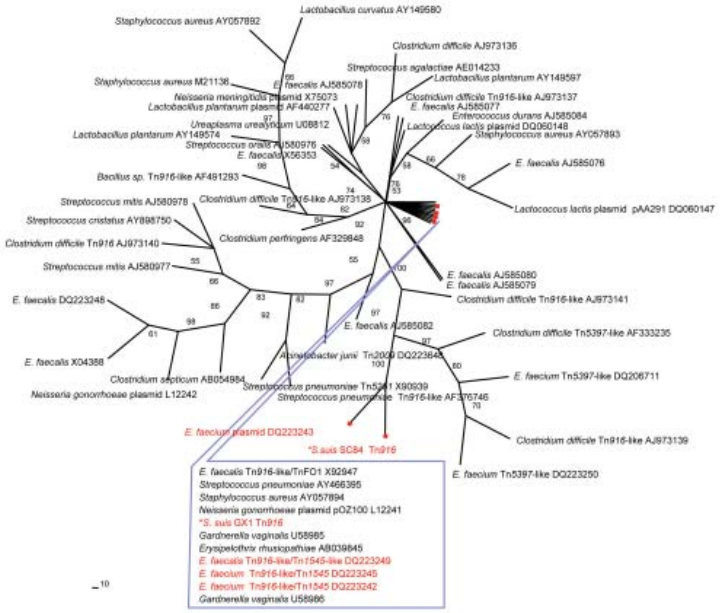

Figure 1. Phylogenetic relationship of the *tetM* sequences of *Streptococcus suis.* An unrooted maximum-parsimony tree was based on multiple aligned partial *tetM* sequences of 2 *S. suis* (asterisk) and 53 reference sequences retrieved from GenBank. The alignment length for the analysis was 1,415 bp. If available, the designation of the *tetM*-carrying plasmid or transposon is indicated, followed by the GenBank accession number. Percent bootstrap support at each internal node was based on 200 replicate trees. The sequences of known pig origin are marked in red.

tive for all 3 virulence genes. However, none of the 4 ST25 strains tested positive (Table 1). Of the 34 serotype reference strains, serotype 2 strain R735 was positive for *mrp* and *sly*. The *sly* gene was detected in 10 reference strains that were typed serotype 1/2 as untypeable, serotype 2 as ST1, serotype 4 as ST54, 5 as ST53, 7 as untypeable, 13 as ST71, 14 as ST6, 16 as ST73, 17 as ST76, and 18 as ST79 (Table 1).

Table 2. Primers used to detect *tet* genes and conjugative transposon Tn*916* in *Streptococcus suis**

Gene	Primers (5′ → 3′)	Product size, bp	Annealing temperature, °C
tetA	GCTACATCCTGCTTGCCTTC; CATAGATCGCCGTGAAGAGG	210	55
tetB	TTGGTTAGGGGCAAGTTTTG; GTAATGGGCCAATAACACCG	659	55
tetC	CTTGAGAGCCTTCAACCCAG; ATGGTCGTCATCTACCTGCC	418	55
tetD	AAACCATTACGGCATTCTGC; GACCGGATACACCATCCATC	787	55
tetE	AAACCACATCCTCCATACGC; AAATAGGCCACAACCGTCAG	278	55
tetG	GCTCGGTGGTATCTCTGCTC; AGCAACAGAATCGGGAACAC	468	55
tetK	TCGATAGGAACAGCAGTA; CAGCAGATCCTACTCCTT	169	55
tetL	TCGTTAGCGTGCTGTCATTC; GTATCCCACCAATGTAGCCG	267	55
tetM	GTGGACAAAGGTACAACGAG; CGGTAAAGTTCGTCACACAC	406	55
tetO	AACTTAGGCATTCTGGCTCAC; TCCCACTGTTCCATATCGTCA	515	55
tetQ	TTATACTTCCTCCGGCATCG; ATCGGTTCGAGAATGTCCAC	904	55
tetS	CATAGACAAGCCGTTGACC; ATGTTTTTGGAACGCCAGAG	667	55
ORF 24	ATGAGGTGTCTATTTTTTTA; TTATTGGCTGAATGAATGTT	120	52
ORF 23	AATTTGTGATTCCCAACATG; CGTCAGCATGTAAAAGGTAA	315	52
ORF 22	ATGATGAGATTAGCAAATGG; CTATTTGTCTTGTGTCGGTT	387	52
ORF 21	TTTCATTTTACGATAGCGTC; GTCGCCTGCGTGGACTGTCT	1,308	55
ORF 20	ATGCTGTTTGATTATGTAAG; TTATTTTTTTGTTGTTATCA	990	52
ORF 19	ATGAATTTTGGACAAAACCT; TTAAGCACCAATAATGCGAT	222	52
ORF 18	TTTAGGCAAATACAATGAGG; GATTGGTTGAGATAAACGTT	443	56
ORF 17	ATGGGATTTTTGAAATCGTC; TTAATTGGATATGCCATAAA	507	52
ORF 16	ATGGCATATCCAATTAAATA; TTACACCTCTTTTCGCACAG	2,448	52
ORF 15	ATGTGAAACCATCAATAGTA; TCATCTGAAAATAAAATGGC	2,265	52
ORF 14	ATGAAGTTGAAAACTTTAGT; TCATTGTTTGATTCGTCCTG	1,002	52
ORF 13	AGAAAAACAGATACCAAAGG; CGTTCTTTTCAAGTACCAAA	860	54
ORF 12-*tetM*	ATGCTTTGTATGCCTATGGT; CTAAGTTATTTTATTGAACA	2,022	54
ORF 5–10	ATTATAAACTACAAGTGGAT; TTCGTTTAATCTGAATACGA	2,233	52
Xis-Tn	ATGAAGCAGACTGACATTCC; TTCGTTTAATCTGAATACGA	204	52
Int-Tn	GACTGGAGAGAGCCAACGAA; CATCATGCCGTTGTAATCAC	925	54

*tet, tetracycline; ORF, open reading frame.

To determine the significance of horizontal gene transfer of Tn*916* with the *tetM* and virulence genes, we constructed a rooted phylogenetic tree by using the maximum-parsimony method. The sequence of *S. pneumoniae* R6 was chosen as the outgroup that is closely related to *S. suis* (*10*). The data suggest *S. suis* ST7 evolved originally from ST1 and ST48. The horizontal transfer of tested virulence genes and *tetM* occurred in various stages of the evolution of *S. suis* and played a major role in the emergence of ST1 and ST7 (Figure 2).

Conclusions

We report that *S. suis* ST7 was responsible for 2 large outbreaks and sporadic infections in several provinces of China and has recently acquired the tetracycline resistance gene, *tetM*, associated with the conjugative transposon Tn*916* (*3*,*7*). Horizontal transfer of Tn*916* with the *tetM* gene occurred in at least 3 STs located at various stages in the constructed phylogenetic tree and played a central role in the evolution of the epidemic *S. suis* ST7 clone. All 3 virulence genes tested in this study were shown to be transferred horizontally (*11*–*13*).

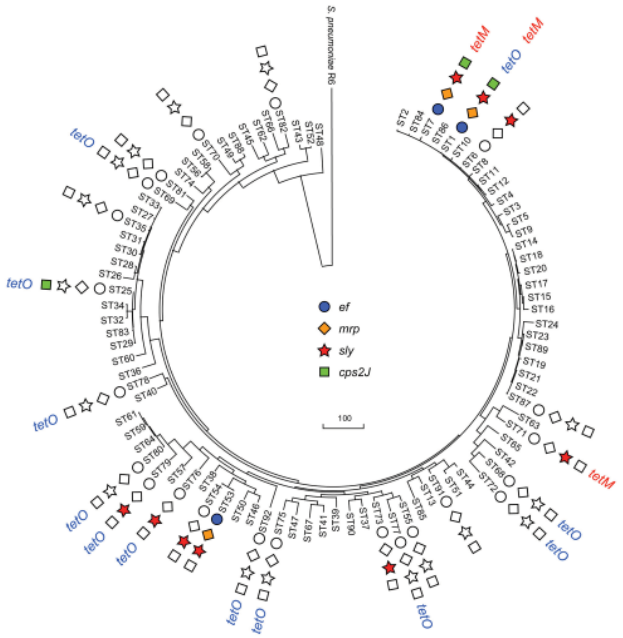

Figure 2. Horizontal transfer events of virulence genes and conjugative transposon Tn*916* with *tetM* in *Streptococcus suis* sequence types. The rooted maximum-parsimony tree was based on the concatenated sequences of 7 housekeeping genes used for multilocus sequence typing analysis of *S. suis* by using S. *pneumoniae* R6 as the outgroup. Virulence genes acquired by strains of various sequence types were from this study and other published papers. The colored labels indicate positive detection; uncolored labels indicate negative results for the virulence gene; no label indicates that the strain was not available for testing. The scale bar indicates a branch length corresponding to 100 character-state changes.

Our data support the contention that Tn*916* with *tetM* acts as an important selective factor that provides considerable advantages for the clone emergence and spread of *S. suis* ST7 (*3*). The widespread use of tetracycline in swine feed could provide the selective pressure for clone amplification and spreading, thus contributing to the outbreak of *S. suis* ST7 through Tn*916* (*14*). The country-wide spread of *S. suis* ST7-*tetM* represents a model of selective pressure leading to the emergence of a bacterium as a virulent pathogen in humans. The case of *S. suis* ST7 is a sign that pathogens present in food animals can result in substantial public health problems if no action is taken to prevent the indiscriminate use of antimicrobial drugs in animal feed (*15*).

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References

1. Yu H, Jing H, Chen Z, Zheng H, Zhu X, Wang H, et al. Human *Streptococcus suis* outbreak, Sichuan, China. Emerg Infect Dis. 2006;12:914–20.
2. Zhu F, Yang H, Hu X, Wang H, Wang G, Song Y, et al. Homogeneity study on the *Streptococcus suis* isolated from human and swine [in Chinese]. Zhonghua Liu Xing Bing Xue Za Zhi. 2000;21:427–9.
3. Ye C, Zhu X, Jing H, Du H, Segura M, Zheng H, et al. *Streptococcus suis* sequence type 7 outbreak, Sichuan, China. Emerg Infect Dis. 2006;12:1203–8.
4. King SJ, Leigh JA, Heath PJ, Luque I, Tarradas C, Dowson CG, et al. Development of a multilocus sequence typing scheme for the pig pathogen *Streptococcus suis:* identification of virulent clones and potential capsular serotype exchange. J Clin Microbiol. 2002;40: 3671–80.
5. Gottschalk M, Higgins R, Jacques M, Mittal KR, Henrichsen J. Description of 14 new capsular types of *Streptococcus suis.* J Clin Microbiol. 1989;27:2633–6.
6. Gottschalk M, Higgins R, Jacques M, Beaudoin M, Henrichsen J. Characterization of six new capsular types (23 through 28) of *Streptococcus suis.* J Clin Microbiol. 1991;29:2590–4.
7. Agerso Y, Pedersen AG, Aarestrup FM. Identification of Tn5397-like and Tn*916*-like transposons and diversity of the tetracycline resistance gene *tet(M)* in enterococci from humans, pigs and poultry. J Antimicrob Chemother. 2006;57:832–9.
8. Ng LK, Martin I, Alfa M, Mulvey M. Multiplex PCR for the detection of tetracycline resistant genes 20. Mol Cell Probes. 2001;15: 209–15.
9. Morse SA, Johnson SR, Biddle JW, Roberts MC. High-level tetracycline resistance in *Neisseria gonorrhoeae* is result of acquisition of streptococcal *tetM* determinant 4. Antimicrob Agents Chemother. 1986;30:664–70.
10. Hoskins J, Alborn WE Jr, Arnold J, Blaszczak LC, Burgett S, DeHoff BS, et al. Genome of the bacterium *Streptococcus pneumoniae* strain R6. J Bacteriol. 2001;183:5709–17.

11. Okwumabua O, O'Connor M, Shull E. A polymerase chain reaction (PCR) assay specific for *Streptococcus suis* based on the gene encoding the glutamate dehydrogenase. FEMS Microbiol Lett. 2003;218:79–84.
12. Silva LM, Baums CG, Rehm T, Wisselink HJ, Goethe R, Valentin-Weigand P. Virulence-associated gene profiling of *Streptococcus suis* isolates by PCR. Vet Microbiol. 2006;115:117–27.
13. Wisselink HJ, Joosten JJ, Smith HE. Multiplex PCR assays for simultaneous detection of six major serotypes and two virulence-associated phenotypes of *Streptococcus suis* in tonsillar specimens from pigs. J Clin Microbiol. 2002;40:2922–9.
14. Klare I, Konstabel C, Badstubner D, Werner G, Witte W. Occurrence and spread of antibiotic resistances in *Enterococcus faecium* 6. Int J Food Microbiol. 2003;88:269–90.
15. Yang H, Chen S, White DG, Zhao S, McDermott P, Walker R, et al. Characterization of multiple-antimicrobial-resistant *Escherichia coli* isolates from diseased chickens and swine in China. J Clin Microbiol. 2004;42:3483–9.

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Morbillivirus and Pilot Whale Deaths, Mediterranean Sea

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An outbreak of a lethal morbillivirus infection of long-finned pilot whales occurred in the Mediterranean Sea from the end of October 2006 through April 2007. Sequence analysis of a 426-bp conserved fragment of the morbillivirus phosphoprotein gene indicates that the virus is more closely related to dolphin morbillivirus than to pilot whale morbillivirus.

Morbilliviruses have emerged as serious pathogens of cetaceans and pinnipeds worldwide (*1*). The 2 cetacean morbilliviruses that have been identified are porpoise morbillivirus (PMV), isolated from harbor porpoises that died along the coast of Ireland, and dolphin morbillivirus (DMV), first identified in striped dolphins from the Mediterranean Sea (*1*,*2*). Although to our knowledge, morbillivirus outbreaks in pilot whales have not been previously reported, antibodies to morbilliviruses have been reported in 86% of 2 species of pilot whales (*Globicephala melas* and *G. macrorrhynchus*) in the western Atlantic (*3*). Barrett et al. found that 93% of stranded long-finned pilot whales (*G. melas*) were seropositive for morbillivirus, which provides more evidence that cetacean morbilliviruses are widespread (*4*). Molecular evidence from a pilot whale that was stranded on the coast of New Jersey, USA, and died from encephalitis, suggested that the long-finned pilot whale is host for a different, novel type of cetacean morbillivirus (pilot whale morbillivirus [PWMV]), which is distinct from PMV and DMV (*5*). We report an epizootic of lethal morbillivirus infection in long-finned pilot whales that occurred in the Mediterranean Sea.

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The Study

During a 6-month period (end of October 2006 through April 2007), >27 long-finned pilot whales were found stranded, 6 alive and 21 dead, along the southern Spanish Mediterranean coast and Balearic Islands. According to information from the Andalucia regional stranding network, CIRCE, (Conservation, Information, Research, Cetaceans), nongovernment organizations, and scientists working on that coastal area, 10 of these pilot whales were stranded in the Strait of Gibraltar area from the end of October 2006 through early February 2007. From January through April 2007, 7 of these whales were found stranded on the Almería coast, 6 on the Murcia coast, 2 on the Valencia coast; another 2 were found beached on the Baleares Islands. The Table compares the times and locations of these strandings with those of historical strandings.

Of these stranded whales, 18 were found in an advanced autolytic condition, but 9 were fresh or only moderately autolytic, of which complete necropsies were performed on 7, partial necropsies on 2, and samples were collected from all 9. Histologic and immunohistochemical examination of formalin-fixed tissues (mainly lymph node, brain, esophagus, liver, and kidney) was performed for 9 whales, and a virologic examination was performed on frozen tissues (mainly lymph node, lung, and brain) from 6.

According to biological and morphometric parameters, all stranded pilot whales were adults or subadults, except 2 that were juveniles. One female whale stranded off Baleares Islands was 7 months pregnant. For most of the stranded whales, the main macroscopic findings detected during the necrospsy were moderate to severe cachexia, represented by marked loss of volume of epaxial musculature. Stomachs were empty. In 3 whales, subcutaneous tissues were yellowish (icteric) and edematous. All necropsied whales had enlarged edematous lymph nodes, which showed parenchymal multifocal necrosis (especially digestive tract lymph nodes). Erosive stomatitis and erosive-to-ulcerative necrotizing esophagitis was detected in 3 whales. For 2 whales, the urinary bladder was empty and had thickened walls containing yellowish dense mucus in the lumen.

Microscopically, the main lesions were found in lymph nodes, which had a multifocal necrotizing lymphoadenitis and multinuclear syncytial cells. A nonpurulent encephalitis with syncitial cells and intranuclear inclusion bodies, intracytoplasmic inclusion bodies, or both, were detected in 6 whales from which neurologic tissues were analyzed microscopically. Mild interstitial pneumonia was detected in 4 whales, but inflammatory lesions of the lung were absent in the others. One whale, stranded in Murcia, had a focal pyogranulomatous pneumonia caused by *Aspergillus* sp. Mild to severe, erosive to ulcerative necrotizing esophagitis was detected microscopically in all analyzed whales found to have gross lesions in this organ.

Table. Pilot whale strandings, historical and epizootic, Mediterranean coastal area

Area, dates of historical records	No. stranded	Average no. strandings/y	No. historical strandings, 1998–2006 (dates)	No. epizootic strandings, 2006–2007 (dates)
Strait of Gibraltar, 1998–Sep 2006	8	0.9	3 (1998–2006 Oct –Feb)	10 (2006 Oct–2007 Feb)
Almería, 1998–Dec 2006	22	2.4	2 (1998–2006 Jan–Apr)	7 (2007 Jan–Apr)
Murcia, 2004–Dec 2006	12	4	1 (2004–2006 Jan–Apr)	6 (2007 Jan–Apr)
Baleares Islands, 1999–Dec 2006	2	0.25	Not known	2 (2007 Apr)
Total	44	7.55		25 (2006–2007 Oct–Apr)

Immunohistochemical staining, with a polyclonal antibody (*6*), showed morbillivirus antigen in bronchiolar epithelium, syncytial cells, monocyte-like cells, and cell debris of affected lymph nodes and brain; these tissues often showed a positive intracytoplasmic globular or granular immunoreaction. Morbillivirus antigen was detected in all whales for which an immunohistologic study was performed, mainly in the brain (n = 6), lymph nodes (n = 9), and lungs (n = 4) (Figure 1).

Reverse transcription–PCR (RT-PCR) to detect cetacean morbillivirus (CetMV) was performed for available samples of brain, lung, spleen, lymph node, liver, and kidney from 6 of the pilot whales and 1 fetus. Molecular detection of CetMV was performed by a 1-step RT-PCR of a 426-bp conserved region of the phosphoprotein gene, described previously (*7*). We conducted a BLAST (www.ncbi.nlm.nih.gov/blast/Blast.cgi) search to compare sequenced products with sequences described in the GenBank for morbillivirus. All sequences alignments were obtained, and p-distances were calculated by using MEGA version 3.1 (*8*).

Of those whales analyzed for virus (6 pilot whales and 1 fetal whale), a morbillivirus was detected by RT-PCR in the brains of 5, lymph nodes of 6, and the lungs of 4. All samples from the fetus (brain, lung, lymph nodes, liver, and kidney) were RT-PCR positive for morbillivirus. Sequencing showed the same sequence in all positive samples from animals stranded in different areas of the southern coast of Spain (Figure 2). The novel sequence obtained was closely related to DMV (p-distance 0.01–0.03) and less closely related (more divergent) to PWMV (p-distance 0.11).

Conclusions

The morbillivirus epizootic reported here induced high mortality rates among long-finned pilot whales in the Mediterranean Sea (Table). The epizootic had a spatiotemporal sequence, involving the long coast from southern Spain, beginning October–November 2006 in the Strait of Gibraltar, spreading eastward to Almería and finally northeast to Murcia; the last cases were detected in Valencia and the Balearic Islands in April 2007. High mortality rates among striped dolphins (*Stenella coeruleoalba*) have been noted since July 2007 in those coastal areas (currently under investigation along the coasts of Almería, Murcia, Valencia, and Catalunian) (data not shown). In our laboratories, a DMV has been isolated from 3 of those stranded striped dolphins (1 stranded along Murcia and 2 along the Almería coasts). This virus is molecularly almost identical to that reported here as affecting pilot whales (F. Esperón, pers. comm.).

The first morbillivirus epizootic described in cetaceans involved striped dolphins in the Mediterranean Sea in the 1990s when a DMV was described (*1*,*2*). Because the viruses isolated from those striped dolphins and these pilot whales are closely related phylogenetically, interspecies transmission should be considered. This epidemiologic point is reinforced by a new die-off event of striped dolphins in Mediterranean waters associated temporally and spatially with the pilot whale deaths caused by a DMV reported here. In the pilot whales the central nervous and lymphatic systems were the most severely affected tissues. Although pilot whales worldwide may be enzootically infected with morbillivirus (*9*), the virus involved in the present epizootic differs from PWMV (*5*), which supports previous evidence that different strains of CetMV may be infecting dolphins and whales (*10*).

Possible explanations for how and why the disease starts are, among others, pollutants (*11*), the high intensive chronic anthropogenic effects in the Strait of Gibraltar area, a DMV entering a naive pilot whale population, or a progressive decreasing of humoral immunity against the

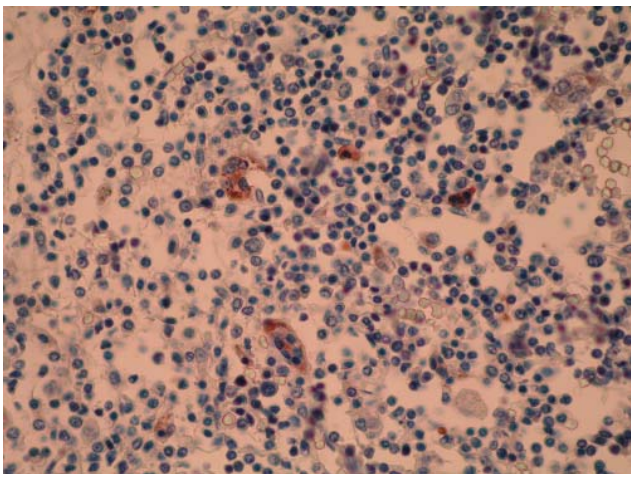

Figure 1. Lymph node of pilot whale. Positive intracytoplasmic immunoperoxidase staining of morbilliviral antigen in several syncytial cells and in monocyte-like cells. Avidin-biotin-peroxidase with Harris hematoxylin counterstain. Original magnification ×400.

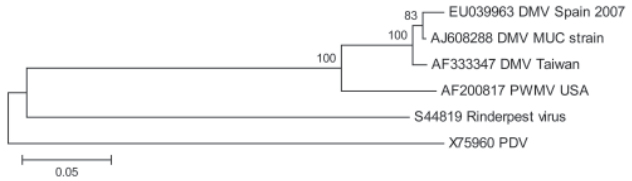

Figure 2. Neighbor-joining phylogram of 6 selected sequences from marine mammal morbilliviruses and Rinderpest virus. The name of the sequence indicates the GenBank accession number, virus species, and the country of the isolate. DMV, dolphin morbillivirus; PWMV, pilot whale morbillivirus; PDV, phocine distemper virus. The scale bar indicates the p-distance of the branches.

virus in these populations (*12*). Further research is needed to investigate the role of morbilliviruses on the health and massive deaths of pilot whales and other cetaceans.

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References

1. Barrett T, Visser IKG, Mamaev L, Goatley L, van Bressem MF, Osterhaus ADME. Dolphin and porpoise morbilliviruses are genetically distinct from phocine distemper virus. Virology. 1993;193:1010–2.
2. Domingo M, Visa J, Pumarola M, Marco A, Ferrer L, Rabanal R, et al. Pathologic and immunocytochemical studies of morbillivirus infection in striped dolphins (*Stenella coeruleoalba*). Vet Pathol. 1992;29:1–10.
3. Duignan PJ, House C, Geraci JR, Duffy N, Rima BK, Walsh MT, et al. Morbillivirus infection in cetaceans of the Western Atlantic. Vet Microbiol. 1995;44:241–9.
4. Barrett T, Blixenkrone-Moller M, Di Guardo G, Domingo M, Duignan P, Hall A, et al. Morbilliviruses in aquatic mammals: report on round table discussion. Vet Microbiol. 1995;44:261–5.
5. Taubenberger JK, Tsai MM, Atkin TJ, Fanning TG, Krafft AE, Moeller RB, et al. Molecular genetic evidence of a novel morbillivirus in a long-finned pilot whale (*Globicephalus melas*). Emerg Infect Dis. 2000;6:42–5.
6. Yang WC, Pang VF, Jeng CR, Chou LS, Chueh LL. Morbilliviral in a pigmy sperm whale (*Kogia breviceps*) from Taiwanese waters. Vet Microbiol. 2006;116:69–76.
7. Reidarson TH, McBain J, House C, King DP, Stott JL, Krafft A, et al. Morbillivirus infection in stranded common dolphins from the Pacific Ocean. J Wildl Dis. 1998;34:771–6.
8. Kumar S, Tamura K, Nei M. MEGA3. Integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief Bioinform. 2004;5:150–63.
9. Van Bressem M, Van Waerebeek K, Jepson PD, Raga JA, Duignan PJ, Nielsen O, et al. An insight into the epidemiology of dolphin morbillivirus worldwide. Vet Microbiol. 2001;81:287–304.
10. Taubenberger JK, Tsai M, Krafft AE, Lichy JH, Reid AH, Schulman FY, et al. Two morbilliviruses implicated in bottlenose dolphin epizootics. Emerg Infect Dis. 1996;2:213–6.
11. Aguilar A, Borrell A. Abnormally high polychlorinated biphenyl levels in striped dolphins (*Stenella coeruleoalba*) affected by the 1990–1992 Mediterranean epizootic. Sci Total Environ. 1994;154:237–47.
12. Van Bressem MF, Visser IK, Van de Bildt MW, Teppema JS, Raga JA, Osterhaus AD. Morbillivirus infection in Mediterranean striped dolphins (*Stenella coeruleoalba*). Vet Rec. 1991;129:471–2.

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Sandfly Fever Sicilian Virus, Algeria

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To determine whether sandfly fever Sicilian virus (SFSV) is present in Algeria, we tested sandflies for phlebovirus RNA. A sequence closely related to that of SFSV was detected in a *Phlebotomus ariasi* sandfly. Of 60 human serum samples, 3 contained immunoglobulin G against SFSV. These data suggest SFSV is present in Algeria.

Recent attention has been drawn to *Toscana virus* (family *Bunyaviridae*, genus *Phlebovirus,* species *Sandfly fever Naples virus*) in countries surrounding the Mediterranean because the virus causes meningitis during summer. *Sandfly fever Sicilian virus* (SFSV) is a distinct arthropodborne phlebovirus transmitted by sandflies, specifically by *Phlebotomus papatasi* (*1*). It was discovered in Italy (Palerma, Sicilia), where it affected troops of the World War II Allied Army Forces after the Sicily landings in 1943. SFSV infection produces a febrile illness during the warm season; in contrast with Toscana virus infection, SFSV infection is not associated with neurologic manifestations.

Human cases of SFSV infection have been reported from Italy, Egypt, Pakistan, Iran, and Cyprus (*1*,*2*). Seroprevalence studies performed with human or vertebrate serum indicate that SFSV, or a closely related virus, is circulating in Jordan (*3*), Israel (*4*), Sudan (*5*), Tunisia (*6*), Pakistan (*7*), Egypt (*8*), Bangladesh (*9*), and Iran (*10*). The most comprehensive study, initiated by Tesh et al. (*11*), did not find neutralizing antibodies reactive to SFSV in human serum from the Algerian populations of Tamanrasset and Djanet. Therefore, at the outset of this study, no evidence or data suggested the presence of SFSV in Algeria.

The Study

Over a 4-night period in July 2006, a total of 460 sandflies were trapped as described (*12*). Trapping was performed at Larbaa Nath Iraten (previously known as Fort National) in the Kabylian region of Algeria, near Tizi Ouzout (Figure 1). CDC Miniature Light Traps were adapted for sandfly capture by using an ultrafine mesh. Traps were hung 1–2 m above ground. They were placed during late afternoon in or near animal housing facilities (chickens, rabbits, goats, horses). Each morning, sandflies were collected, identified morphologically, and placed in 1.5-mL microfuge tubes. Captured sandflies belonged to 7 species: *P. perniciosus* (n = 364), *P. longicuspis* (n = 61), *P. sergenti* (n = 21), *P. ariasi* (n = 6), *P. perfiliewi* (n = 3), *P. papatasi* (n = 1), and *Sergentomyia minuta* (n = 1). They were organized into 24 pools, each containing up to 30 sandflies. Each pool was ground in RNA NOW chaotropic solution (Ozyme, Montigny le Bretonneux, France). RNA purification was performed according to the manufacturer's protocol. A total of 10 µL of RNA suspension was used for reverse transcription with random hexanucleotide primers with the Taqman Reverse Transcription Reagents (Applied Biosystems, Foster City, CA, USA) in a final volume of 50 µL, according to the manufacturer's recommended protocol. To test these specimens for Toscana virus RNA and phlebovirus RNA, we used 10 µL of cDNA in the previously described assays (*12*,*13*). Pool F tested positive with nested primers Phlebo2+/Phlebo2–. The PCR product was sequenced directly with primers used for PCR amplification. A 201-nt sequence (excluding primers) was obtained and submitted to the National Center for Biotechnology Information BLAST program, which retrieved a unique hit, consisting of Cyprus phlebovirus polymerase gene with a 92% identity score. Because *Cyprus virus* has been reported to be closely related to SFSV (*2*), we amplified and sequenced the homologous genome region of SFSV strain Sabin by using the primers described above. The same approach was applied to *Arbia virus,* a related phlebovirus isolated in Italy simultaneously with Toscana virus for

Figure 1. Map of Algeria showing where sandflies were trapped (■).

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comparative analysis. These 3 sequences were deposited in the GenBank database under accession nos. EU240880, EU266619, and EU266620. Laboratory contamination can be excluded because the sequence corresponding to SFSV-Algeria is divergent from its closest sequence (SFSV-Italy-Sabin) by 4%, which corresponds to 8 nt mutations; in addition, SFSV-Italy-Sabin has been manipulated after PCR amplification and sequencing of SFSV-Algeria to compare it genetically with the sequence obtained from Algerian sandflies.

Together with homologous sequences of selected phleboviruses, the 3 sequences determined in this study were used to perform genetic distance comparison and phylogenetic analysis. Nucleotide and amino acid distances are presented in the Table. Distance analysis unambiguously indicated that Algeria virus is a variant genotype of SFSV. The same conclusion applied to Cyprus phlebovirus. These 3 viruses exhibited amino acid and nucleotide distances of <9.3% and <7.5%, respectively. Phylogenetic analyses (Figure 2) indicated that Algeria formed a strong cluster (100% bootstrap support) with SFSV strain Sabin and the Cyprus phlebovirus. Therefore, we propose that they can be considered as 3 variant strains of the tentative species SFSV. Because sandfly material was stored in a chaotropic solution, virus isolation was not possible, which will necessitate field work with storage conditions suitable for virus isolation attempts.

Detection of SFSV RNA in sandflies led us to test human serum for SFSV antibodies. We tested 60 samples from healthy persons for SFSV immunoglobulin (Ig) G by indirect immunofluorescence assay as described (*14*) with minor modifications. Briefly, equal quantities of infected and uninfected Vero cells were mixed together and spotted onto 2-well glass slides through a 3-min cytospin-based centrifugation at 900 rpm. Samples were tested at a 1:20 dilution in phosphate-buffered saline. Three (5%) samples contained SFSV IgG but not Toscana virus IgG.

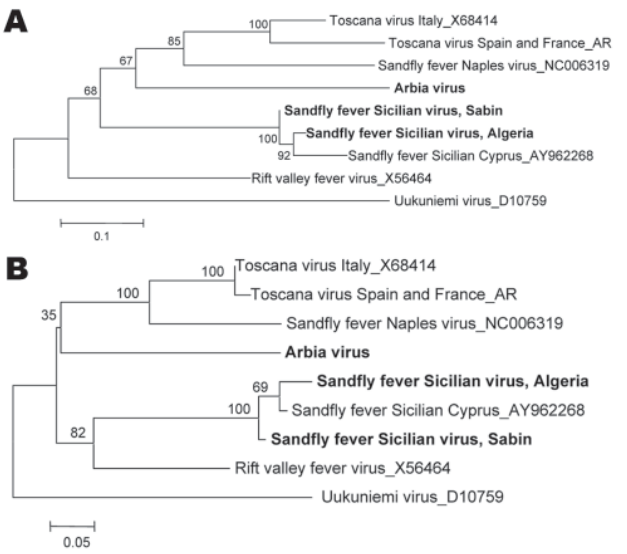

Figure 2. Phylogenetic analysis of *Sandfly fever Sicilian virus,* Algeria, based on A) 207-nt or B) 67-aa sequence in the polymerase gene. Distances and groupings were determined by the pairwise or Kimura 2-parameter algorithm and neighbor-joining method with the MEGA v2 software program (www.megasoftware.net). Bootstrap values are indicated and correspond to 500 replications**. Boldface** indicates virus names that correspond to sequences determined in this study. Scale bars indicate pairwise nucleotide distances (0.1 = 10%) and Kimura 2-parameter amino acid distances (0.05 = 5%).

Conclusions

Together, molecular and serologic data constitute evidence that SFSV is present in Algeria. Genetic analysis of a partial region of the polymerase gene (L genome segment) indicated that the Algerian, Italian, and Cypriot strains of SFSV are closely related. Another study performed with M RNA sequences showed that Italian and Cypriot strains of SFSV are closely related as well (*15*).

To our knowledge, SFSV has been previously isolated in *P. papatasi* flies only. In this study, detection of SFSV

Table. Pairwise genetic distances between Sandfly fever Sicilian virus sequence and homologous sequences of selected phleboviruses*

No.	Virus	1	2	3	4	5	6	7	8	9
										Nucleotide sequences
1	Sandfly fever Sicilian virus, Algeria	–	7.5	4.0	40.4	38.8	42.3	40.1	38.5	48.2
2	Sandfly fever Sicilian virus, Cyprus	4.5	–	6.5	42.7	41.3	39.3	43.3	38.5	53.0
3	Sandfly fever Sicilian virus, Italy	9.3	1.5	–	39.1	37.9	42.3	39.6	35.8	46.5
4	Arbia virus	54.7	52.2	50.7	–	38.2	42.2	40.9	40.4	53.8
5	Toscana virus, Italy	48.0	47.8	44.0	42.7	–	17.4	30.4	38.1	48.2
6	Toscana virus, France	46.3	47.8	47.8	44.8	1.5	–	34.8	42.0	52.0
7	Sandfly fever Naples virus, Sabin	52.0	50.7	48.0	52.0	25.3	25.4	–	41.2	51.3
8	Rift Valley fever virus	41.3	37.3	34.7	45.3	37.3	41.8	46.7	–	47.1
9	Uukuniemi virus	69.3	67.2	61.3	64.0	60.0	65.7	62.7	58.7	–
		Amino acid sequences								

*Sandfly fever Sicilian virus isolated from *Phlebotomus ariasi* sandflies in Algeria. Selected phleboviruses are those for which the corresponding sequence was available in the GenBank database.

RNA in 1 female *P. ariasi* sandfly must be interpreted with caution. In particular, this finding does not mean that *P. ariasi* is a vector of SFSV in the study region. The presence of SFSV RNA may result from mechanical transmission from a viremic vertebrate. Therefore, specific studies should be conducted to investigate vectors of SFSV in Algeria. Seroprevalence data demonstrate that SFSV or an SFSV-related virus can infect humans. Further studies are needed to determine whether the clinical picture is limited to self-resolving febrile illness, as previously reported in Italy.

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Dr Izri is an entomologist who is interested in sandflies, specifically in their role as vectors of viral diseases of human and veterinary interest.

References

1. Karabatsos N. International catalogue of arboviruses including certain other viruses of vertebrates. San Antionio (TX): American Society of Tropical Medicine and Hygiene; 1985.
2. Papa A, Konstantinou G, Pavlidou V, Antoniadis A. Sandfly fever virus outbreak in Cyprus. Clin Microbiol Infect. 2006;12:192–4.
3. Batieha A, Saliba EK, Graham R, Mohareb E, Hijazi Y, Wijeyaratne P. Seroprevalence of West Nile, Rift Valley, and sandfly arboviruses in Hashimiah, Jordan. Emerg Infect Dis. 2000;6:358–62.
4. Cohen D, Zaide Y, Karasenty E, Schwarz M, LeDuc JW, Slepon R, et al. Prevalence of antibodies to West Nile fever, sandfly fever Sicilian, and sandfly fever Naples viruses in healthy adults in Israel. Public Health Rev. 1999;27:217–30.
5. McCarthy MC, Haberberger RL, Salib AW, Soliman BA, El-Tigani A, Khalid IO, et al. Evaluation of arthropod-borne viruses and other infectious disease pathogens as the causes of febrile illnesses in the Khartoum Province of Sudan. J Med Virol. 1996;48:141–6.
6. Chastel C, Bach-Hamba D, Launay H, Le Lay G, Hellal H, Beaucornu JC. Infections à arbovirus en Tunisie : nouvelle enquête sérologique chez les petits mammifères sauvages. Bull Soc Pathol Exot. 1983;76:21–33.
7. Darwish MA, Hoogstraal H, Roberts TJ, Ghazi R, Amer T. A seroepidemiological sunvey for *Bunyaviridae* and certain other arboviruses in Pakistan. Trans R Soc Trop Med Hyg. 1983;77:446–50.
8. Darwish MA, Feinsod FM, Scott RMN, Ksiazek TG, Botros BAM, Farrag IH, et al. Arboviral causes of non-specific fever and myalgia in a fever hospital patient population in Cairo, Egypt. Trans R Soc Trop Med Hyg. 1987;87:1001–3.
9. Gaidamovich SY, Baten MA, Klisenko GA, Melnikova YE. Serological studies on sandfly fevers in the Republic of Bangladesh. Acta Virol. 1984;28:325–8.
10. Saidi S, Tesh RB, Javadian E, Sahabi Z, Nadim A. Studies on the epidemiology of sandfly fever in Iran. II. The prevalence of human and animal infection with five phlebotomus fever virus serotypes in Isfahan province. Am J Trop Med Hyg. 1977;26:288–93.
11. Tesh RB, Gajdamovic SJa, Rodhain F, Vesenjak-Hirjan J. Serological studies on the epidemiology of sandfly fever in the Old World. Bull World Health Organ. 1976;54:663–74.
12. Charrel RN, Izri A, Temmam S, Delaunay P, Toga I, Dumon H, et al. Cocirculation of 2 genotypes of Toscana virus, southeastern France. Emerg Infect Dis. 2007;13:465–8.
13. Sanchez-Seco MP, Echevarria JM, Hernandez L, Estevez D, Navarro-Mari JM, Tenorio A. Detection and identification of Toscana and other phleboviruses by RT-nested-PCR assays with degenerated primers. J Med Virol. 2003;71:140–9.
14. Fulhorst CF, Monroe MC, Salas RA, Duno G, Utrera A, Ksiazek TG, et al. Isolation, characterization and geographic distribution of Cano Delgadito virus, a newly discovered South American hantavirus (family *Bunyaviridae*). Virus Res. 1997;51:159–71.
15. Liu DY, Tesh RB, Travassos Da Rosa AP, Peters CJ, Yang Z, Guzman H, et al. Phylogenetic relationships among members of the genus *Phlebovirus* (*Bunyaviridae*) based on partial M segment sequence analyses. J Gen Virol. 2003;84:465–73.

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Lakes as Source of Cholera Outbreaks, Democratic Republic of Congo

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We studied the epidemiology of cholera in Katanga and Eastern Kasai, in the Democratic Republic of Congo, by compiling a database including all cases recorded from 2000 through 2005. Results show that lakes were the sources of outbreaks and demonstrate the inadequacy of the strategy used to combat cholera.

The association between *Vibrio cholerae* and aquatic environments has long been studied, but emphasis has been almost exclusively placed on coastal areas such as the Bay of Bengal, the point of origin of cholera. There, outbreaks are closely linked to estuarine areas, where environmental *V. cholerae* strains emerge and then spread in human communities during the monsoon season (*1*) by attaching themselves to surfaces provided by plants, algae, and zooplankton (*2*,*3*). Some recent studies have investigated environmental and climatic factors that may encourage the spread of cholera in African countries (*4*,*5*); these studies also focused on coastal areas. Except for 2 case–control studies performed in Burundi and Kenya (*6*,*7*), little is known about the epidemiology of cholera in inland areas of Africa. A recent article, based on the analysis of 632 reports of cholera outbreaks worldwide, has shown that 87.7% of cholera cases occurred in sub-Saharan Africa and that the highest concentration of outbreaks was in the eastern provinces of the Democratic Republic of Congo (DRC) (*8*). In this country, dozens of emergency programs have been implemented by humanitarian organizations, national health services, and international agencies; they have, however, failed to achieve long-term control of cholera epidemics. To search for environmental factors that could explain the recurrence of cholera outbreaks, we conducted an epidemiologic study in 2 inland provinces of the DRC severely hit by cholera.

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The Study

From 2002 through 2005, reports of cholera cases and deaths from cholera were collected weekly from each health district of Katanga (497,076 km^2, 9,598,380 inhabitants) and Eastern Kasai (170,103 km^2, 6,713,009 inhabitants) with the help of local and national staff of the DRC Ministry of Health. The definition of a case-patient was "any person 5 years of age or older in whom severe dehydration develops or who dies from acute watery diarrhea"; the age limit was lowered to 2 years for cases associated with confirmed cholera outbreaks, as recommended by the World Health Organization (WHO) (*9*). Each new outbreak was confirmed by culture and identification of *V. cholera* O1 from 5 to 10 stool samples.

For 2000 and 2001, only cumulative data collected weekly in each province were available; no detailed database was kept. However, data were completed with information from reports of epidemic investigations and interventions (105 reports filed from 1999 through 2005) and the testimonies of medical teams interviewed during field visits. A geographic information system was established, based on the data collected from the 106 health districts of the 2 provinces. Six health districts were removed from statistical analysis because >10% of weekly reports were missing (Figure 1). Using regression techniques (see online Technical Appendix, available from www.cdc.gov/EID/content/14/5/798-Techapp.pdf), we statistically examined the relationship between the number of cholera cases in each health district and the following list of geographic and environmental variables: area; population; and presence/absence of cities of >100,000 inhabitants, of railway stations, of harbors, of major tracks or roads, and of lakes.

A total of 67,738 cases and 3,666 deaths (case-fatality rate 5.4%) were reported from 2000 through 2005 in Katanga and Eastern Kasai, which corresponded to 8.4% of

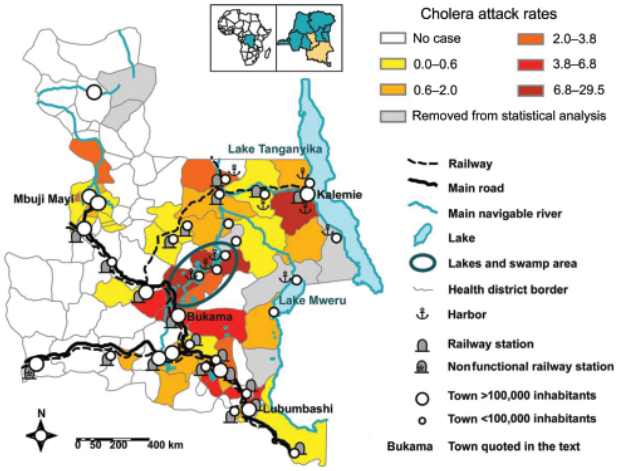

Figure 1. Katanga and Eastern Kasai, showing distribution of cholera attack rate from 2002 through 2005 and average attack rate of cholera per 10,000 inhabitants per health district.

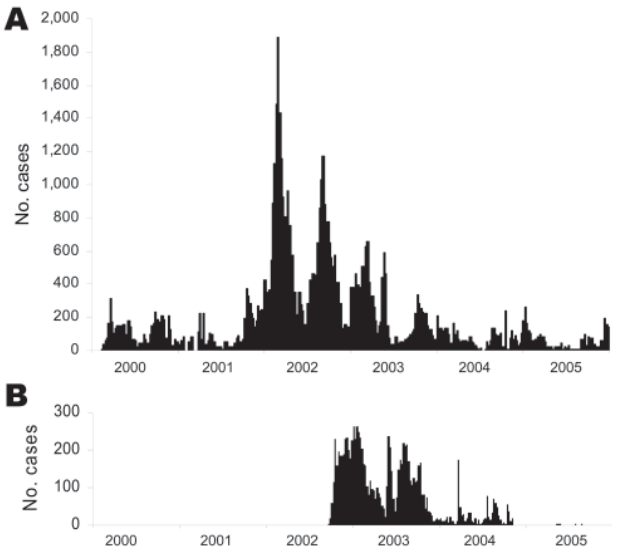

Figure 2. Weekly case incidence of cholera in Katanga (A) and Eastern Kasai (B) from 2000 through 2005.

cases and 19.6% of deaths worldwide recorded by WHO during the same period (*10–15*). Relatively calm periods were separated by episodes of exacerbation between 2001 and 2003 (Figure 2). In 2000, epidemics were reported only in the areas of Lake Tanganyika and Lake Mweru (on the eastern border of Katanga). Only a brief outbreak (752 cases) was reported in Lubumbashi, the capital of Katanga, located in the south. The first exacerbation began in the middle of 2001, when thousands of civilians, trying to escape civil war. fled from Kalemie (bordering Lake Tanganyika). From May to December 2001, cholera outbreaks were reported in various cities of Katanga, including Bukama (center of Katanga) and Lubumbashi. From Bukama, outbreaks spread to the lakes north of the city. A peak was reached in March 2002, followed by a period of marked decrease, during which cholera persisted only in lake areas. A second exacerbation began in Bukama in August 2002, when fishermen returned from the lakes to sell their catches. This outbreak rapidly spread to Mbuji Mayi in Eastern Kasai where, as discovered during the outbreak investigation, the first case was in a tradeswoman who had traveled by train to Bukama to buy fish. In Mbuji Mayi, the cholera epidemic lasted until June 2004 and accounted for 4,949 cases. Concomitantly, in September 2002, another outbreak started in Lubumbashi; it lasted 9 months and affected 4,288 people.

Since July 2004, cholera has persisted only in lake areas. In the north of Bukama, this persistence has been due to iterative outbreaks, successively affecting cities and villages on the lakeshores. Cholera has also persisted on the shores of Lake Tanganyika, especially in the health district of Kalemie, where no lasting intermission has been recorded.

In our study, most cases (60%) occurred in lake areas (13% of inhabitants of the 2 provinces) (Figure 1). The number of cholera cases was statistically significantly higher in the presence of a lake, a main road, a harbor, or a railway (Table). The analysis of reports filed after each intervention showed that they were not conducted in the same way in each area. For every cholera outbreak in Mbuji Mayi, Lubumbashi, and other main cities in southern Katanga, medical staffs were reinforced by humanitarian organizations to set up centers for cholera treatment and to implement public awareness and information campaigns. In contrast, in lake areas, humanitarian organizations intervened in only 17 of 54 outbreaks. Interventions almost exclusively targeted patient care and sensitization campaigns were rarely implemented. Mean duration of interventions was 6 weeks (range 2–20) in lake areas versus 20 weeks in big cities (range 10–30).

Conclusions

Our study shows that, despite the difficulties encountered in gathering reliable field data in a country with a civil war and a disorganized healthcare infrastructure, a consistent set of results that help to understand the epidemiology of cholera in the DRC could be obtained. Information pooled from maps of the spatial distribution of cases, statistical analyses, and screening of investigation reports shows that lake areas are the source of iterative outbreaks and that these sometimes spread to main cities hundreds of kilome-

Table. Model parameters and odds ratios of the negative binomial model selected for cholera cases in Katanga and Eastern Kasai, Democratic Republic of the Congo, 2000–2005*

Characteristic	Coefficient estimate	Standard error	t value	Pr(>|t|)	Odds ratio	95% CI
Intercept	5.50	1.04	5.27	8.92×10^{-7}		
Ln (area)	–0.28	0.11	–2.44	1.64×10^{-2}		
Population	1.97×10^{-6}	1.17×10^{-6}	1.69	9.46×10^{-2}		
Railway station	0.61	0.25	2.42	1.74×10^{-2}	1.8	1.12–3.02
Harbor	1.39	0.33	4.16	7.06×10^{-5}	4.0	2.09–7.75
Main road	1.43	0.41	3.50	7.09×10^{-4}	4.2	1.88–9.32
Lake	2.01	0.33	6.12	2.20×10^{-8}	7.5	3.92–14.23

*Coefficient estimate, regression coefficients (for discrete variables, their exponential gives the odds ratio); t value, value of the t distribution; Pr(>|t|), probability of the null hypothesis of a coefficient estimate not statistically different from zero; CI, confidence interval; intercept, average number of cases; Ln, natural logarithm.

ters away because of the movements of traders and other travelers. Although we fully acknowledge that the correlative nature of this study calls for further research to understand the details of the local natural history of cholera, our results show that the strategy used until now to combat cholera should be reexamined. Maximum effort must be concentrated on populations living around the lakes. Cholera prevention programs should be reinforced there, and safe water should be provided, especially during cholera outbreaks. Because the targets of the programs are relatively small populations living close to lakes, these programs could be more easily afforded than those implemented on a provincial or a national level. In this way, main cities, which are still under the threat of a new outbreak spreading from the lakes, would be protected.

Acknowledgments

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References

1. Colwell RR. Infectious disease and environment: cholera as a paradigm for waterborne disease. Int Microbiol. 2004;7:285–9.
2. Reidl J, Klose KE. *Vibrio cholerae* and cholera: out of the water and into the host. FEMS Microbiol Rev. 2002;26:125–39.
3. Islam MS, Drasar BS, Sack RB. The aquatic flora and fauna as reservoirs of *Vibrio cholerae*: a review. J Diarrhoeal Dis Res. 1994;12:87–96.
4. Mendelsohn J, Dawson T. Climate and cholera in KwaZulu-Natal, South Africa: the role of environmental factors and implications for epidemic preparedness. Int J Hygiene Environ Health; 2007 [cited 2008 Feb 18]. Available from http://www.sciencedirect.com
5. Constantin de Magny G, Guegan JF, Petit M, Cazelles B. Regional-scale climate-variability synchrony of cholera epidemics in West Africa. BMC Infect Dis. 2007;7:20.
6. Birmingham ME, Lee LA, Ndayimirije N, Nkurikiye S, Hersh BS, Wells JG, et al. Epidemic cholera in Burundi: patterns of transmission in the Great Rift Valley Lake region. Lancet. 1997;349:981–5.
7. Shapiro RL, Otieno MR, Adcock PM, Phillips-Howard PA, Hawley WA, Kumar L, et al. Transmission of epidemic control *Vibrio cholerae* O1 in rural western Kenya associated with drinking water from Lake Victoria: an environmental reservoir for cholera? Am J Trop Med Hyg. 1999;60:271–6.
8. Griffith DC, Kelly-Hope LA, Miller MA. Review of reported cholera outbreaks worldwide, 1995–2005. Am J Trop Med Hyg. 2006;75:973–7.
9. World Health Organization. Guidelines for cholera control. 2nd ed. Geneva: The Organization; 1996.
10. World Health Organization. Cholera, 2000. Wkly Epidemiol Rec. 2001;76:233–40.
11. World Health Organization. Cholera, 2001. Wkly Epidemiol Rec. 2002;77:257–64.
12. World Health Organization. Cholera, 2002. Wkly Epidemiol Rec. 2003;78:269–76.
13. World Health Organization. Cholera, 2003. Wkly Epidemiol Rec. 2004;79:281–8.
14. World Health Organization. Cholera, 2004. Wkly Epidemiol Rec. 2005;80:261–8.
15. World Health Organization. Cholera 2005. Wkly Epidemiol Rec. 2006;81:297–307.

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Serologic Evidence for Novel Poxvirus in Endangered Red Colobus Monkeys, Western Uganda

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Enzyme-linked immunosorbent assay, Western blot, and virus neutralization assays indicated that red colobus monkeys in Kibale National Park, western Uganda, had antibodies to a virus that was similar, but not identical, to known orthopoxviruses. The presence of a novel poxvirus in this endangered primate raises public health and conservation concerns.

The virus subfamily *Chordopoxviridae* contains emerging pathogens of considerable concern, both because of their historic impact on global human health and because of their zoonotic potential (*1*). Although certain poxviruses in this subfamily are among the most extensively studied viral pathogens (e.g., smallpox, vaccinia), the natural diversity of mammalian poxviruses remains poorly characterized. This fact is especially true in sub-Saharan Africa, where at least 1 zoonotic orthopoxvirus (monkeypox) has caused sporadic human outbreaks since 1970 (*2*), and infected rodents exported to the United States caused a highly publicized human outbreak in 2003 (*3*,*4*). In this study, we describe serologic evidence for a previously uncharacterized poxvirus in endangered red colobus monkeys (*Procolobus rufomitratus tephrosceles*) from Kibale National Park, western Uganda (*5*,*6*). Our results, based on postadsorption ELISA, Western blot, and virus neutralization assays, extend our understanding of the host range and diversity of poxviruses and raise public health and conservation concerns.

The Study

From June 12 to June 24, 2006, 31 red colobus (13 males, 18 females, all adult or subadult) were sampled from Kibale National Park, western Uganda (5,6). Animals were chemically immobilized in the field by intramuscular injection of tiletamine/zolezepam (4.6–9.6 mg/kg body weight) with 1.0- or 1.5-mL plastic darts with 5/8-inch needles shot from a variable-pressure compressed air rifle. After initial darting, animals were given tiletamine/zolezepam or ketamine HCl intravenously as needed. Blood samples were collected into vacutainers containing sodium-EDTA, plasma was separated in the field by centrifugation, and samples were stored in liquid nitrogen for transport to North America. Animals were placed in cloth bags to recover from anesthesia and were released near trees and vines that were easy to climb and within visual range of their social groups. All animals appeared healthy at the time of capture. Animal protocols were approved by the McGill University Animal Care Committee before data collection.

A vaccinia virus (VV) ELISA was used as an initial screening test to detect antipoxvirus antibodies. VV-reactive antibodies were detected with horseradish peroxidase (HRP)–conjugated polyclonal goat anti–rhesus macaque (RM; *Macaca mulatta*) immunoglobulin (Ig) G (Fc specific; Nordic Laboratories, Tilsbug, the Netherlands), which readily detected antibodies of other primate species, including red colobus monkeys and humans. By using this approach, samples from 8 (26%) of 31 red colobus plasma scored positive (>200 ELISA units [EU]), 21 (68%) of 31 scored seronegative (<100 EU), and 2 (6%) of 31 had equivocal results (100–200 EU) (online Appendix Figure, available from www.cdc.gov/EID/content/14/5/801-appG.htm).

Postadsorption ELISA tests (*7*) were used next to determine the specificity of the response to 1 of 3 orthopoxviruses: VV, monkeypox virus (MPV), or cowpox virus (CPV). Plasma samples were tested directly by ELISA or preadsorbed (1:30) for 30 minutes at 37°C with VV, MPV, or CPV lysate normalized to contain 6×10^8 PFU/mL before addition to virus-coated ELISA plates (Figure 1). The postadsorption ELISA test allows one to differentiate among closely related orthopoxviruses. For instance, VV-specific antibodies are best depleted by VV lysate assayed on a VV-coated ELISA plate (Figure 1, panel A). However, analysis of samples from 10 red colobus with detectable anti-VV antibody responses (online Appendix Figure) did not show a clear pattern following preadsorption with VV, MPV, or CPV antigens, which suggests a similar cross-reactivity to each of these 3 orthopoxviruses.

Western blot analysis was performed to characterize more fully the antipoxvirus response of the red colobus. Two micrograms of purified MPV, VV, and CPV viral proteins were separated by 4%–20% gradient sodium dodecyl sulfate–polyacrylamide gel electrophoresis, transferred to polyvinylidene difluoride membranes (Invitrogen, Carlsbad, CA, USA), and probed with plasma (1:10,000) before addition of HRP-conjugated polyclonal goat antihuman Ig

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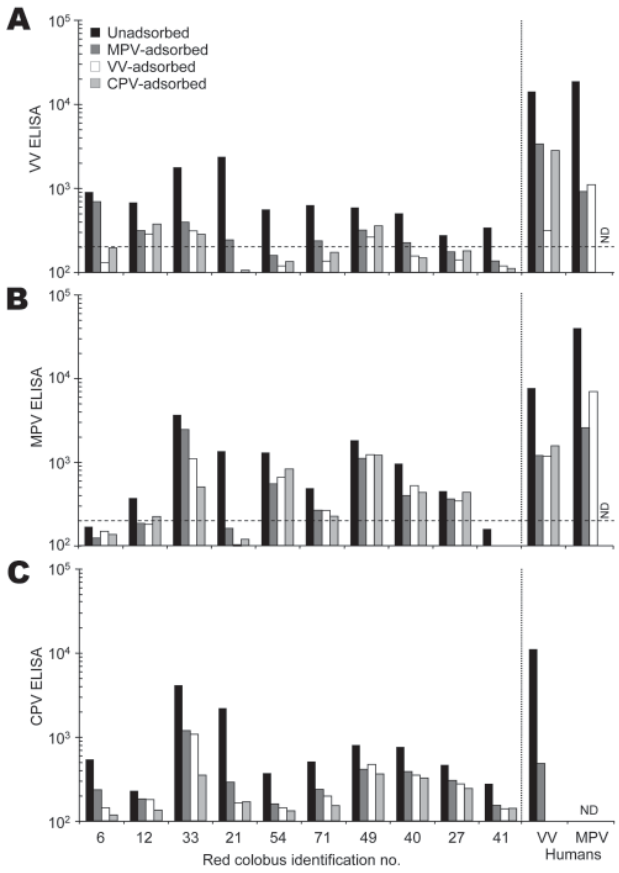

Figure 1. Serologic characterization of red colobus to *Orthopoxvirus* antigens. Plasma samples were collected from 31 red colobus, and 10 samples with detectable antibody responses to vaccinia virus (VV) antigens (online Appendix Figure, available from www.cdc.gov/EID/content/14/5/801-appG.htm) were chosen for further analysis. Plasma samples were tested for specificity by a postadsorption ELISA (*7*) in which samples were either unadsorbed or preadsorbed with monkeypox virus (MPV), vaccinia virus (VV), or cowpox virus (CPV) antigens prior to performing an ELISA on A) VV-, B) MPV-, or C) CPV-coated ELISA plates. The results obtained by using plasma from a VV-immune human study participant (VV human) and a MPV-immune participant (MPV human) are shown for comparison. The dashed line indicates the cut-off value for a seropositive antibody response (200 ELISA units). ND, not determined.

G (γ-specific, Jackson Immunolabs, Inc., West Grove, PA, USA) and chemiluminescent detection (Pierce SuperSignal West Dura Substrate, Rockville, IL, USA) (Figure 2). VV-immune human, MPV-immune human, MVA (modified vaccinia Ankara)-immune RM and MPV-immune RM plasma samples were included as positive controls to identify banding patterns typically observed in orthopoxvirus-immune humans and nonhuman primates. Western blot analysis proved more sensitive than anti-VV ELISA (online Appendix Figure), with plasma from 30 of 31 red colobus reacting with at least 1 protein band from MPV, VV, or CPV. However, unlike the orthopoxvirus-immune human and RM controls, samples from red colobus demonstrated fewer immunoreactive bands and different immunodominant banding patterns, suggesting infection with either a distantly related orthopoxvirus or a virus from a different genus in the *Poxviridae* family.

Members of the *Orthopoxvirus* genus elicit cross-neutralizing antibodies against other members of the same genus. To determine if the red colobus were infected with an orthopoxvirus or a more distantly related poxvirus, plaque reduction neutralization assays were performed using 100 PFU of MPV, VV, or CPV. Plasma samples from all 31 red colobus were tested, and all exhibited a neutralizing titer 50 of <20 against MPV, VV, or CPV (data not shown). These findings suggest that although the monkeys were infected with a poxvirus with serologic cross-reactivity to VV (online Appendix Figure), lack of a detectable neutralizing antibody response (<20) indicates that the animals may have been infected with a poxvirus that is not a member of the *Orthopoxvirus* genus.

Conclusions

Our results provide evidence that red colobus in Kibale National Park have been exposed to a previously uncharacterized poxvirus. Kibale red colobus may have been exposed to monkeypox or to a "monkeypox-like" virus, but we could not confirm this with our current serologic tools. On the other hand, other poxviruses, such as Tanapox virus and Yaba monkey tumor virus, have been identified in Africa, and infection by 1 of these poxviruses or a related virus cannot be ruled out. Future studies will require optimizing serologic diagnostics against these divergent poxviruses (with appropriate positive and negative controls) to determine the identity of the poxvirus/poxviruses that have infected the red colobus described here. In this light, we

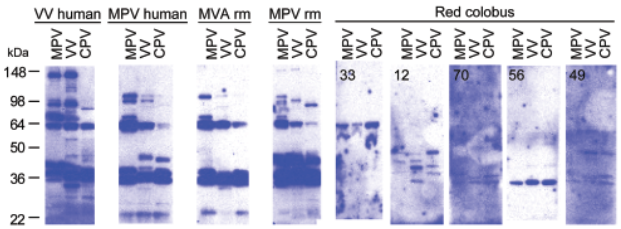

Figure 2. Western blot analysis of *Orthopoxvirus* (OPV)–reactive antibody responses in red colobus. Western blot analysis was performed to further characterize humoral immune responses against OPV antigens. Purified monkeypox virus (MPV), vaccinia virus (VV), or cowpox virus (CPV) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred to polyvinylidene difluoride membranes, and probed with plasma from a VV-immune human, MPV-immune human, MVA-immune RM, MPV-immune RM, and 5 representative red colobus. The red colobus animal identification number is shown in the upper left corner of each Western blot for comparison with the ELISA data for the same sample described in Figure 1.

note that tanapox, a zoonosis of suspected primate origin (*8*,*9*), derives its name from the Tana River, eastern Kenya, which supports an isolated population of red colobus closely related to those in Kibale (*P. r. rufomitratus*) (*10*,*11*).

A protracted outbreak of infectious disease occurred in Kibale red colobus from 1971 to 1981, where it caused a death rate up to nearly 10% in some social groups, apparently killing only adult male monkeys (*12*). Although neither formal clinical data nor biologic samples were collected, descriptions of lesions of affected monkeys suggested diffuse to multifocal areas of inflammation with gray mottling and epidermal crusts on the face (most commonly the lips), perineum, and inguinum, followed by alopecia and impaired locomotion. Monkeys sampled for the present study would almost certainly not yet have been born during this period, but these observations raise the possibility that outbreaks of disease at least outwardly consistent with poxvirus infection have occurred previously in Kibale red colobus.

Poxviruses are known for their potential to cross species barriers (*1*), and red colobus living in small, unprotected forest fragments outside of Kibale National Park interact aggressively and at high rates with local persons and their domestic animals (*13*). At the same time, persons in rural western Uganda already bear a high incidence of pathogens, including HIV (*14*), which renders a substantial proportion of the population immunocompromised and susceptible to opportunistic infections. Recent outbreaks of zoonotic poxviruses have not been documented in our study area, despite a local and regional healthcare system that would most likely have detected such events. However, the presence of a novel and potentially zoonotic poxvirus in red colobus should be viewed as a point of concern for the future of public health in this region and elsewhere.

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References

1. Regnery RL. Poxviruses and the passive quest for novel hosts. Curr Top Microbiol Immunol. 2007;315:345–61.
2. Di Giulio DB, Eckburg PB. Human monkeypox: an emerging zoonosis. Lancet Infect Dis. 2004;4:15–25.
3. Reed KD, Melski JW, Graham MB, Regnery RL, Sotir MJ, Wegner MV, et al. The detection of monkeypox in humans in the Western Hemisphere. N Engl J Med. 2004;350:342–50.
4. Hammarlund E, Lewis MW, Carter SV, Amanna I, Hansen SG, Strelow LI, et al. Multiple diagnostic techniques identify previously vaccinated individuals with protective immunity against monkeypox. Nat Med. 2005;11:1005–11.
5. Struhsaker TT. Ecology of an African rain forest: logging in Kibale and the conflict between conservation and exploitation. Gainesville (FL): University Press of Florida; 1997.
6. Chapman CA, Struhsaker TT, Lambert JE. Thirty years of research in Kibale National Park, Uganda, reveals a complex picture for conservation. Int J Primatol. 2005;26:539–55.
7. Dubois MD, Slifka MK. Retrospective analysis of monkeypox infection. Emerg Infect Dis. 2008 Apr; [Epub ahead of print].
8. Downie AW, Taylor-Robinson CH, Caunt AE, Nelson GS, Manson-Bahr PEC, Matthews THC. Tanapox: a new disease caused by a poxvirus. BMJ. 1971;1:363–8.
9. Jezek Z, Arita I, Szceniowski M, Paluku KM, Kalisa R, Nakano JH. Human tanapox in Zaire: clinical and epidemiological observations on cases confirmed by laboratory studies. Bull World Health Organ. 1985;63:1027–35.
10. Struhsaker TT. Vocalizations, phylogeny and palaeogeography of red colobus monkeys (*Colobus badius*). Afr J Ecol. 1981;19:265–83.
11. Ting N. Mitochondrial relationships and divergence dates of the African colobines: evidence of miocene origins for the living colobus monkeys. J Hum Evol. In press.
12. Struhsaker TT. Variation in adult sex ratios of red colobus monkey social groups: implications for interspecific comparisons. In: Kappeler PM, editor. Primate males: causes and consequences of variation in group composition. Cambridge (UK): Cambridge University Press; 2000. p. 108–19.
13. Goldberg TL, Gillespie TR, Rwego IB, Kaganzi C. Killing of a pearl-spotted owlet (*Glaucidium perlatum*) by male red colobus monkeys (*Procolobus tephrosceles*) in a forest fragment near Kibale National Park, Uganda. Am J Primatol. 2006;68:1007–11.
14. Joint United Nations Programme on HIV/AIDS (UNAIDS). AIDS epidemic update. Geneva: The Programme; 2007.

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Increase in West Nile Neuroinvasive Disease after Hurricane Katrina

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After Hurricane Katrina, the number of reported cases of West Nile neuroinvasive disease (WNND) sharply increased in the hurricane-affected regions of Louisiana and Mississippi. In 2006, a >2-fold increase in WNND incidence was observed in the hurricane-affected areas than in previous years.

Hurricane Katrina devastated portions of Louisiana and Mississippi on August 29, 2005. Previous reports of West Nile neuroinvasive disease (WNND) in this area after this hurricane did not examine any statewide increases in 2005 (*1*). However, this report did not show potential regional increases of WNND in areas that experienced substantial hurricane damage. Because West Nile virus (WNV) is now endemic in areas of the United States that are at risk for hurricanes, understanding effects of such events on WNV epidemiology is important for directing appropriate public health responses. The objective of this study was to determine whether cases of WNND increased regionally after Hurricane Katrina.

The Study

We used WNV human case data for Louisiana and Mississippi from the Centers for Disease Control and Prevention (CDC) (*2*); cases of meningitis, encephalitis, or meningoencephalitis reported to CDC were considered WNND cases. Cases are listed by date of onset of first symptoms and corresponding CDC week, and parish or county of residence at the estimated time of infection.

Affected parishes or counties were defined as those in which >50% of the total area was within 50 miles of the hurricane track coordinates (*3*) (ArcView 8.0; Environmental Systems Research Institute, Redlands, CA, USA). Eight of 64 parishes in Louisiana and 21 of 82 counties in Mississippi fit our definition of hurricane affected (Figure 1). Counties within the storm's track after its winds had diminished to <75 miles per hour were considered not affected.

We compared the number of WNND cases during the 3-week period before the storm with the number of cases in the 3-week period immediately after Hurricane Katrina to determine whether the number of WNND cases changed immediately after the storm in Louisiana and Mississippi. Because the hurricane-affected region experienced extensive migration of its residents and no valid population estimates exist for this period, the number of WNND cases reported was used. Landfall of Hurricane Katrina occurred at the beginning of CDC week 35, and news reports estimated that the final evacuation of persons from the New Orleans area occurred the following Sunday, September 4 (*4*), the beginning of CDC week 36. Because WNV infection has a 3–14-day incubation period (*5*), persons with storm-related exposures could have contracted WNV and become symptomatic as early as CDC week 35 or as late as the end of week 37. We considered WNND cases in which the reported onset of symptom dated from CDC weeks 35–37 as potentially influenced by the hurricane.

In Louisiana, no cases of WNND were reported in the 3 weeks before Hurricane Katrina (CDC weeks 32–34) in the 8-parish region affected by the storm. In the 3 weeks after the storm (CDC weeks 35–37), 11 WNND cases were reported in the affected region (Table 1). This increase in WNND cases in the hurricane-affected region was not observed during the same periods in 2002, 2003, 2004, or 2006. No increase was noted after the hurricane in unaffected parishes during the same periods.

A similar pattern was observed in Mississippi. In the 3 weeks after landfall, the affected region showed an increase from 0 to 10 WNND cases; the unaffected region of Mis-

Figure 1. Hurricane Katrina track and hurricane-affected Louisiana parishes and Mississippi counties. Affected parishes and counties (gray) were defined as those in which >50% of the total area was ≤50 miles of the hurricane track coordinates.

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Table 1. West Nile neuroinvasive disease (WNND) before and after Hurricane Katrina for the 3 years before the storm (2002–2004), the year of the storm (2005), and the year after the storm (2006) in Louisiana parishes and Mississippi counties*

	Affected areas				Unaffected areas			
	CDC weeks 32–34†		CDC weeks 35–37‡		CDC weeks 32–34†		CDC weeks 35–37‡	
State	WNND cases	95% CI	WNND cases	95% CI	WNND cases	95% CI	WNND cases	95% CI
Louisiana								
2002	22	14.6–33.3	8	4.1–15.8	37	26.9–51.0	20	13.0–30.9
2003	2	0.6–7.2	3	1.09–8.8	18	11.4–28.5	16	9.9–26.0
2004	1	0.2–5.6	1	0.2–5.6	23	15.4–34.5	11	6.2–19.7
2005	0	0–3.0§	11	6.2–19.7§	28	19.4–40.5	12	6.9–21.0
2006	11	6.2–19.7	8	4.1–15.8	13	7.7–22.2	16	9.9–26.0
Mississippi								
2002	13	7.7–22.2	12	6.9–21.0	38	27.7–52.2	21	13.8–32.1
2003	3	1.1–8.8	3	1.1–8.8	5	2.2–11.7	4	1.6–10.2
2004	1	0.2–5.6	2	0.6–7.2	12	6.9–21.0	4	1.6–10.2
2005	0	0–3.0§	10	5.5–18.4§	8	4.1–15.8	10	5.5–18.4
2006	12	6.9–21.0	9	4.8–17.1	14	8.4–23.5	9	4.8–17.1

*CDC, Centers for Disease Control and Prevention; CI, confidence interval (Poisson).
†3 weeks before landfall of hurricane.
‡3 weeks after landfall of hurricane.
§p<0.05.

sissippi showed only a minor increase in cases during the same periods (8 cases before and 10 cases after the storm).

To assess potential long-term effects of Hurricane Katrina on WNND incidence, we compared incidence rates of WNND for both states during 2006 with rates during the 4 years preceding the storm (2002–2005). Because the hurricane-affected region experienced population displacement, we used special population estimates from the US Census Bureau for rate estimations for 2006 (*6*). For unaffected parishes or counties that did not have an updated census estimate, we used the Census 2000 population estimate (*7*). Louisiana had population reductions of 398,853 persons (–28%) in hurricane-affected parishes and 17,521 persons (<–1%) in unaffected parishes. Mississippi had population reductions of 21,708 persons (–3%) in affected counties and 34,545 persons (–2%) in unaffected counties.

Despite losses in population, the affected parishes of Louisiana had an increase in the number of WNND cases from an average annual number of 30 cases in 2002–2005 to 45 cases in 2006. In the affected counties of Mississippi, WNND cases increased from an annual number of 23 cases in 2002–2005 to 55 cases in 2006. Incidence rate ratios and 95% confidence intervals were calculated for each state and region (affected and unaffected) (Table 2). Incidence rate ratios for 2006 were >2-fold higher in the hurricane-affected regions of both states than the mean historic incidence rates (2002–2005). Unaffected areas of both states showed decreased (Louisiana) and stable (Mississippi) WNND incidences compared with historical incidence. Figure 2 shows epidemic curves of 2005–2006 cases by week in affected and unaffected areas for both states.

Conclusions

Our evidence demonstrates that areas directly affected by Hurricane Katrina experienced increases in WNND cases after the storm compared with before the storm. Analyses of the immediate period after the storm indicate that the observed increase was unique both in time and to the affected region. WNND incidence in 2006 equaled or exceeded the incidence rates in both states during the

Table 2. Incidence rate ratios of West Nile neuroinvasive disease (WNND) in 2002–2005 and 2006 in Louisiana parishes and Mississippi counties*

	West Nile neuroinvasive disease incidence rate†						Incidence rate ratio (95% CI)
State, area	2002‡	2003‡	2004‡	2005‡	2002–2005§	2006¶	
Louisiana							
Affected	5.6	1.3	0.2	1.4	2.1	4.4	2.09 (1.48–2.94)
Unaffected	4.1	2.7	2.7	3.2	3.2	1.5	0.47 (0.35–0.64)
Mississippi							
Affected	6.1	2.1	0.8	1.5	2.6	6.5	2.45 (1.77–3.47)
Unaffected	5.5	1.7	1.2	1.3	2.4	1.7	0.71 (0.55–1.03)

*WNND incidence rates increased 2-fold in the hurricane-affected regions of both states. The unaffected regions showed a decrease in WNND incidence rates (Louisiana) and no change in incidence (Mississippi). CI, confidence interval.
†No. cases/100,000.
‡Population estimate based on 2000 US Census (*8*).
§Cumulative WNND incidence = (no. WNND cases 2002 + 2003 + 2004 + 2005) / cumulative population (Census 2000 [*8*] × 100,000).
¶Population estimate based on 2006 US Census estimate (*7*).

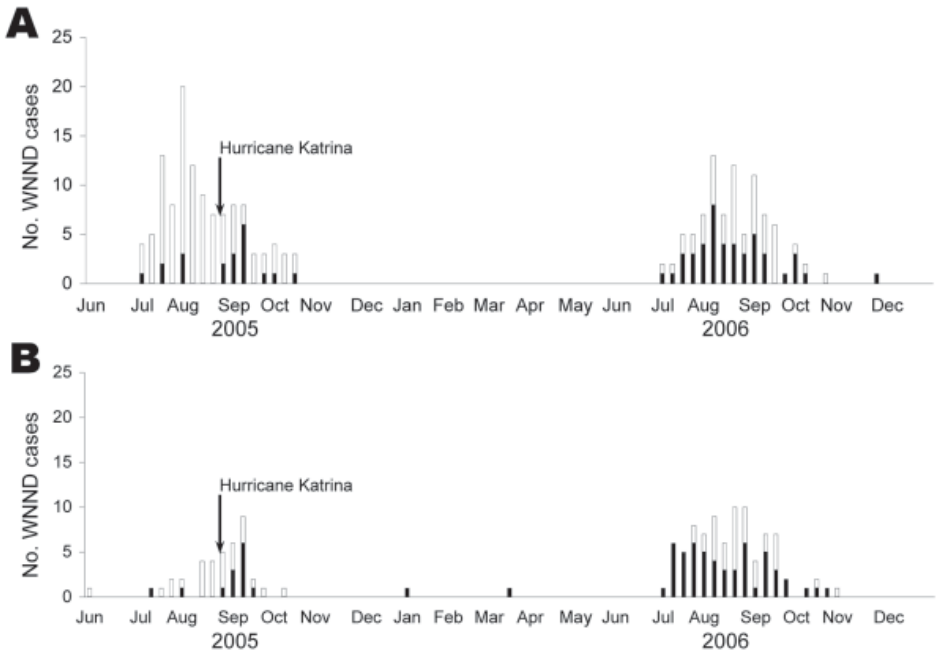

Figure 2. Cases of West Nile neuroinvasive disease (WNND) in Louisiana (A) and Mississippi (B), 2005–2006. Hurricane Katrina made landfall on August 29, 2005 (Centers for Disease Control and Prevention [CDC] week 35). An increase in WNND cases is noted in the hurricane-affected parishes and counties (black columns) during the 3 weeks after the storm (CDC weeks 35–37). Cases of WNND increased throughout the 2006 season in hurricane-affected parishes. Cases of WNND from unaffected parishes and counties are shown in white columns.

2002 epidemic. Because WNND complications are seen in ≈1% of WNV infections (*5*), a small increase in WNND cases represents a much larger increase in WNV human transmission.

Because our study is based on surveillance data, confounding factors that may bias our analysis should be considered. Although Lehman et al. (*1*) indicated that case reporting lagged in Louisiana after Hurricane Katrina, no evidence was provided to suggest that year-end case totals were affected. To account for potential interstate reporting differences, we have conducted separate analyses for each state. Creation of 3-week periods on the basis of the day (August 29, 2005) and week (CDC week 35) that the storm made landfall may also introduce bias. Some cases with reported onset dates in the 2 weeks after the storm may have resulted from transmission events before August 29th. However, storm-related exposure to mosquitoes began before the storm's landfall, when in preparation for the approaching storm, residents boarded windows and cleared yards. Despite these potential confounding factors, we believe the magnitude of the increase in WNND cases occurring immediately after Hurricane Katrina and the increases in WNND incidence in 2006, within the hurricane-affected region, is substantial enough to warrant further examination.

The immediate increase in cases may be attributed to increased human exposure to mosquitoes. Tens of thousands of persons in the hurricane-affected region were living in damaged housing or were waiting outside for days to be evacuated. The sudden decrease in WNND cases in the hurricane-affected areas 3 weeks after landfall could be attributed to reduced human exposure caused by eventual evacuation and aerial application of insecticides (*8*). The increase in WNND incidence in 2006 might also be due to increased human-mosquito exposure as a result of mosquito larval habitat creation (root ball voids from fallen trees, and flooded abandoned swimming pools), continued substandard living conditions, and increased outdoor reconstruction activities.

Hurricane Katrina was the first major tropical cyclone to make landfall in a large metropolitan area since the 1999 introduction of WNV into the United States. The scale of hurricane damage, especially to residences, may have contributed to increases in WNND. We recommend a region-specific, short- and long-term analysis of arboviral disease to accurately assess the public health effect of natural disasters. Prepositioning mosquito control assets and continuing to provide enhanced emergency assistance for surveillance and control could aid in inhibition of mosquito-transmitted diseases during the immediate period after a hurricane and throughout an extended recovery period.

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Dr Caillouët is a postdoctoral research fellow at Tulane University School of Public Health and Tropical Medicine. His research interests include vector-borne disease ecology and aquatic mosquito ecology.

References

1. Lehman JA, Hinckley AF, Kniss KL, Nasci RS, Smith TL, Campbell GL, et al. Effect of Hurricane Katrina on arboviral disease transmission. Emerg Infect Dis. 2007;13:1273–5.
2. Centers for Disease Control and Prevention. Neuroinvasive and non-neuroinvasive domestic arboviral diseases (includes diseases caused by California serogroup viruses; eastern and western equine encephalitis viruses; and Powassan, St. Louis encephalitis, and West Nile viruses). 2004 [cited 2007 Oct 23]. Available from http://www.cdc.gov/ncidod/dvbid/westnile/surv&control.htm#casedefinition
3. Knabb RD, Rhome JR, Brown DP. Tropical cyclone report: Hurricane Katrina, August 23–30, 2005 [cited 2008 Feb 19]. Available from http://www.nhc.noaa.gov/pdf/TCR-AL122005_Katrina.pdf
4. Final evacuation of New Orleans completed. Reuters [International Herald Tribune]. 2005 Sep 4.
5. Hayes EB, Komar N, Nasci RS, Montgomery SP, O'Leary DR, Campbell GL. Epidemiology and transmission dynamics of West Nile virus disease. Emerg Infect Dis. 2005;11:1167–73.
6. US Census Bureau. Special population estimates for impacted counties in the Gulf Coast area. 2007 [cited 2008 Feb 19]. Available from http://www.census.gov/Press-Release/www/emergencies/impacted_gulf_estimates.html
7. US Census Bureau. US census 2000 [cited 2008 Feb 19]. Available from http://www.census.gov/main/www/cen2000.html
8. Breidenbach M, Haagsma K, Olson S, Teig D, Spears B, McHugh C, et al. Air Force aerial spray operations to control adult mosquitoes following Hurricanes Katrina and Rita. Wing Beats. 2006;Summer:7–15.

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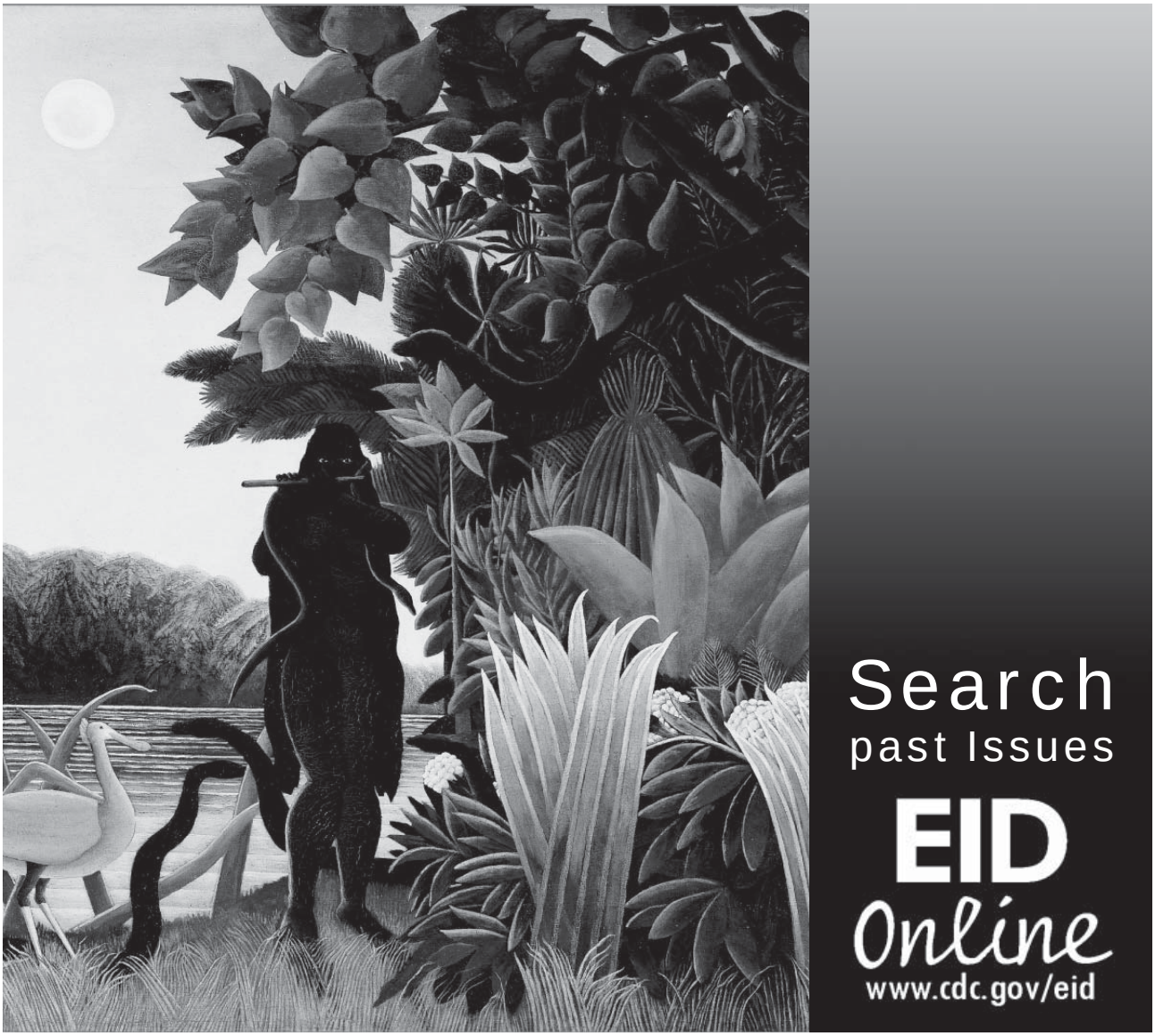

Outbreak of Puumala Virus Infection, Sweden

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An unexpected and large outbreak of Puumala virus infection in Sweden resulted in 313 nephropathia epidemica patients/100,000 persons in Västerbotten County during 2007. An increase in the rodent population, milder weather, and less snow cover probably contributed to the outbreak.

Members of the genus *Hantavirus* (family *Bunyaviridae*) are rodent-borne pathogens, and virus is transmitted to humans by inhalation of infected rodent excreta (*1*). In Sweden, Finland, Norway, Russia, and parts of central Europe, Puumala virus (PUUV) is endemic in bank voles (*Myodes glareolus*). PUUV infection in humans cause nephropathia epidemica (NE), a mild form of hemorrhagic fever with renal syndrome (HFRS). In Sweden ≈90% of all NE cases are found in the 4 northernmost counties. Västerbotten County (Figure 1) has the highest incidence of human hantavirus infection in Sweden and probably one of the highest worldwide. Historically, the incidence rate is 20 per 100,000 persons per year (*2*), but the true incidence is considered to be 7–8 times higher (*3*).

There is a 3–4-year periodicity in the number of NE cases that is linked to the bank vole population dynamics in northern Sweden (*2*). After inhaling infectious aerosols originating from rodent saliva, urine, or feces, the patient has a 1–5-week incubation period before onset of disease symptoms. The most common NE symptoms are fever, headache, nausea, abdominal and back pain, vomiting, myalgia, and visual disturbance. One third of the patients have mostly mild hemorrhagic manifestations (*4*,*5*). Renal failure is typical with initial oliguria during the acute phase and polyuria in the convalescence phase. Dialysis is sometimes needed and <0.5% of NE cases are fatal. There is no effective treatment or available vaccine.

The Study

The local University Hospital of Umeå is the reference center for diagnosis of NE serving the 4 northernmost counties of Sweden, and many patients with NE are hospitalized here. In 2007, a sudden and large outbreak of hantavirus infections occurred in northern Sweden. The outbreak peaked in January 2007 (Figure 1) with many NE patients who had a considerable effect on public health services. The NE outbreak continued in the following months, but with fewer cases than in early 2007 (Figure 1).

For NE diagnosis, we used an immunofluorescence assay to detect PUUV-reactive immunoglobulin (Ig) M and IgG antibodies in serum of all patients with clinically suspected NE (*6*). A real-time reverse transcription–PCR (*6*) was used to obtain an amplification product from 1 patient sample. This product was sequenced and the S-segment sequence obtained (GenBank accession no. EU177630) was highly homologous to those of other rodent PUUV isolates from the area.

NE is a reportable disease under the Swedish Communicable Diseases Act. The outbreak peaked during the first 3 months of 2007; 972 cases were recorded in Sweden and 474 cases in Västerbotten County. NE patients mostly showed classic HFRS symptoms and mild to severe disease requiring hospitalization and occasionally intensive care. Accordingly, as many as 30% of the patients whose conditions had been diagnosed as NE were hospitalized, and 2 known deaths (case-fatality rate 0.25%) in the 2 northernmost counties in Sweden were recorded during the first 3 months of 2007. No patient had to continue dialysis after the acute phase of the disease.

We detected PUUV RNA in the milk of 2 breastfeeding women with a diagnosis of NE. Their children did not show any clinical symptoms of NE. However, we did not have access to samples to analyze whether the children had asymptomatic infections. Three pregnant women also had received a diagnosis of NE, but no clinical evidence of transmission from mother to child was reported. Analyses of the placentas did not detect any PUUV RNA. Only maternal IgG antibodies to PUUV were found in blood from umbilical cords. One woman miscarried after 12 weeks of pregnancy 3 weeks before showing symptoms of clinical NE, and death of the fetus may have been caused by viremia during the incubation period. During the peak of the outbreak (December 2006–March 2007), 488 cases occurred in Västerbotten County, and, as expected, more men (58%, 281/488) than women (42%, 207/488) had NE; most cases (72%) were among persons 35–74 years of age (Table).

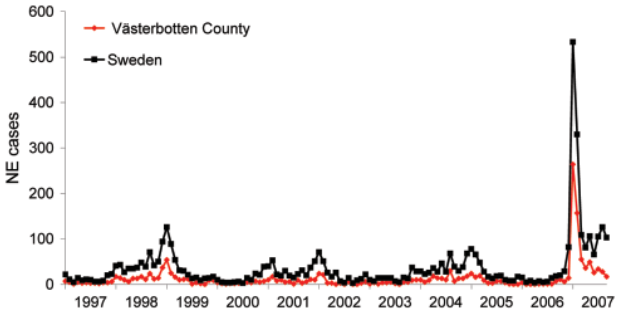

Figure 1. Monthly incidence of nephropathia epidemica (NE) in Sweden and Västerbotten County, Sweden, January 1997–September 2007.

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The incidence of NE in Västerbotten County was 313 diagnosed cases/100,000 persons in 2007 compared with 73/100,000 in 1999, 38/100,000 in 2002, and 61/100,000 in 2005 (Figure 1). The number of NE cases usually depends on the size of the vole population, which peaks every third to fourth year (*2*,*7*). An increase in the bank vole population was reported in northern Sweden in the fall of 2006, with a trap index of 7.64. This index is similar to those of 2 NE peaks in the fall of 1998–1999 and 2004–2005 when trap indices were ≈8 (*8*). Trapping indices represents the number of voles captured per 100 trapping nights, a reflection of the relative population size on each sampling occasion (*9*). Thus, the bank vole population was high, but not more than in previous peak years and could not explain the high number of NE cases in 2007.

We considered other possible factors influencing hantavirus transmission to humans. One factor is increased exposure of humans to infected rodent excreta. We had received several reports from inhabitants in areas where bank voles normally live that more bank voles were found in traps inside houses than usual. When we investigated the weather conditions during this period, December 2006 was exceptional with respect to the mild weather with no or little snow and hard ice cover in the coastal area of northern Sweden. In Västerbotten County, the average temperature in December was 6.0°C–9.0°C warmer than normal (normally the average temperature in Västerbotten County varies by –4°C along the coast and –13°C in the mountains) The average temperature in Sweden was 4.5°C–9.5°C warmer than normal in December 2006 (Figure 2). The snow cover during winter is important for bank vole survival because bank voles have access to food below the snow and hide from predators and the cold (*10*). During 2 previous NE peak periods (2001–2002 and 2004–2005), the ground was already covered with snow in early winter (Figure 2). For these reasons, during December 2006, when the ground had no snow cover for 25 of 31 days (Figure 2), bank voles may have sought refuge in barns and houses and other buildings, thereby increasing the exposure for the human population at risk. A concurrent epizootic may have occurred among bank voles, which resulted in larger numbers of infectious animals, as shown in previous rodent studies (*11*,*12*). However, we did not have access to rodents during this period and this hypothesis needs to be studied.

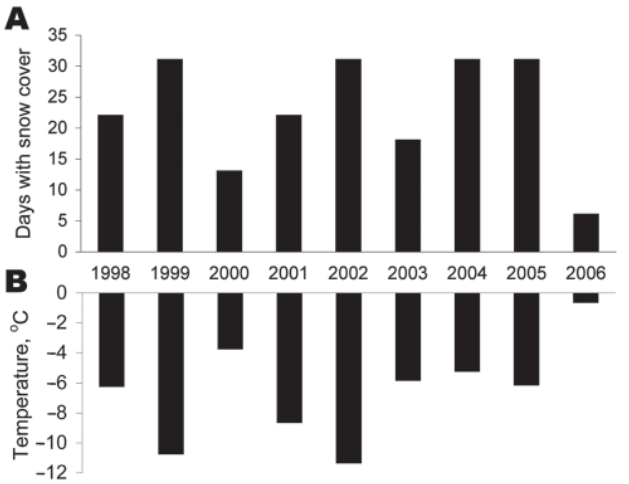

Figure 2. Climate conditions, December 1998–2006, in the nephropathia epidemica outbreak area of Västerbotten County, Sweden. A) Number of days with a snow cover. B) Average temperature. Snow cover was defined as a snow depth >0 cm. Measurements were made in locations ≈30 km from the coast. Data were obtained from the Swedish Meteorological and Hydrological Institute.

Table. Nephropathia epidemica cases in Västerbotten County, Sweden, December 1, 2006–March 31, 2007

Age group, y	No. (%) cases
<1–4	1 (0.20)
5–14	16 (3.3)
15–24	34 (7.0)
25–34	48 (9.8)
35–44	82 (17)
45–54	89 (18)
55–64	103 (21)
65–74	78 (16)
75–84	32 (6.6)
85–94	5 (1.0)
Total	488 (100)

Conclusions

This report shows how a zoonotic disease can suddenly result in an unexpected and large human outbreak. Presently, the numbers of NE cases in northern Sweden are still unusually high. Data indicate that the bank vole population during the fall of 2007 increased to an even higher level and a new outbreak is forecasted (*8*). However, the size of the rodent population is not the only factor that determines the size of a hantavirus epidemic. As shown in this report, climate factors may have contributed to the recent large outbreak in northern Sweden.

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References

1. Schmaljohn C, Hjelle B. Hantaviruses: a global disease problem. Emerg Infect Dis. 1997;3:95–104.
2. Olsson GE, Dalerum F, Hörnfeldt B, Elgh F, Palo TR, Juto P, et al. Human hantavirus infections, Sweden. Emerg Infect Dis. 2003;9:1395–401.

3. Ahlm C, Linderholm M, Juto P, Stegmayr B, Settergren B. Prevalence of serum IgG antibodies to Puumala virus (haemorrhagic fever with renal syndrome) in northern Sweden. Epidemiol Infect. 1994;113:129–36.
4. Settergren B, Juto P, Trollfors B, Wadell G, Norrby SR. Clinical characteristics of nephropathia epidemica in Sweden: prospective study of 74 cases. Rev Infect Dis. 1989;11:921–7.
5. Mustonen J, Brummer-Korvenkontio M, Hedman K, Pasternack A, Pietilä K, Vaheri A. Nephropathia epidemica in Finland: a retrospective study of 126 cases. Scand J Infect Dis. 1994;26:7–13.
6. Evander M, Eriksson I, Pettersson L, Juto P, Ahlm C, Olsson GE, et al. Puumala hantavirus viremia diagnosed by real-time reverse transcriptase PCR using samples from patients with hemorrhagic fever and renal syndrome. J Clin Microbiol. 2007;45:2491–7.
7. Brummer-Korvenkontio M, Vapalahti O, Henttonen H, Koskela P, Kuusisto P, Vaheri A. Epidemiological study of nephropathia epidemica in Finland 1989–96. Scand J Infect Dis. 1999;31:427–35.
8. Olsson GE, Hörnfeldt B, Hjertkvist M, Lundkvist Å. Nephropathia epidemica: high risk in Norrland during winter [in Swedish]. Lakartidningen. 2007;104:3450–3.
9. Olsson GE, White N, Ahlm C, Elgh F, Verlemyr AC, Juto P, et al. Demographic factors associated with hantavirus infection in bank voles (*Clethrionomys glareolus*). Emerg Infect Dis. 2002;8:924–9.
10. Hansson L, Hentonnen H. Gradients in density variations of small rodents: the importance of latitude and snow cover. Oecologia. 1985;67:394–402.
11. Ahlm C, Alexeyev OA, Elgh F, Aava B, Wadell G, Tärnvik A, et al. High prevalence of hantavirus antibodies in bank voles (*Clethrionomys glareolus*) captured in the vicinity of households afflicted with nephropathia epidemica. Am J Trop Med Hyg. 1997;56:674–8.
12. Escutenaire S, Chalon P, Verhagen R, Heyman P, Thomas I, Karelle-Bui L, et al. Spatial and temporal dynamics of Puumala hantavirus infection in red bank vole (*Clethrionomys glareolus*) populations in Belgium. Virus Res. 2000;67:91–107.

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Human Infections with *Plasmodium knowlesi*, the Philippines

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Five human cases of infection with the simian malaria parasite *Plasmodium knowlesi* from Palawan, the Philippines, were confirmed by nested PCR. This study suggests that this zoonotic infection is found across a relatively wide area in Palawan and documents autochthonous cases in the country.

Human malaria is commonly caused by *Plasmodium falciparum, P. vivax, P. malariae,* and *P. ovale*. However, a large focus of human infections with the simian malaria parasite, *P. knowlesi* (*1*), has recently been reported in Malaysian Borneo (*2*), and single case reports of infections acquired in Thailand (*3*) and Myanmar (*4*) have been documented. The diagnosis of *P. knowlesi* in humans may be missed by microscopy since the early blood stages of *P. knowlesi* morphologically resemble *P. falciparum*; the mature blood stages and gametocytes are similar to those of *P. malariae* (*2*).

The Study

Palawan is an island province lying southwest of the main islands of the Philippines. One of its smaller islands, Balabac, located off the southern tip, is separated from Borneo by the Balabac Strait (Figure). Malaria transmission occurs in all 19 municipalities of the province throughout the year. The *Anopheles flavirostris* mosquito is the reported primary vector in the area (*5*). Based on national control program data in 2005, a total of 16,339 malaria cases were reported from Palawan, accounting for 35% of the country's total. Of these, 11,580 (≈71%) were *P. falciparum*, 4,194 (26%) were *P. vivax*, 430 (3%) were *P. malariae*, and the remainder (135, <1%) were mixed-species infections.

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Blood films of 2 patients (A and B), whose condition was diagnosed by microscopy as *P. malariae* at a local laboratory in Palawan, were sent to the malaria national reference laboratory of the Research Institute for Tropical Medicine (RITM) in Manila. Microscopy showed mature trophozoites indistinguishable from *P. malariae* and young ring forms of *P. falciparum.* This observation, and the fact that macaques in the Philippines can harbor *P. knowlesi* (*6*,*7*), raised the possibility that these 2 patients were infected with *P. knowlesi*. Therefore, replicate slides were sent to the Malaria Research Centre, University of Malaysia Sarawak (UNIMAS), for confirmation of the identity of the *Plasmodium* species by molecular methods. DNA was extracted from the 2 slides as detailed previously (*8*) and examined by nested PCR assays for *P. falciparum, P. vivax, P. malariae, P. knowlesi*, and *P. ovale* as described by Singh and co-workers (*2*). One sample was positive for *P. knowlesi* mono-infection; the other was a mixed infection of *P. falciparum, P. malariae*, and *P. knowlesi*. PCR results were confirmed on subsequent testing at the malaria reference laboratory, London School of Hygiene & Tropical Medicine, United Kingdom.

The blood films had been collected in January 2006 from 2 men (>40 years of age) who lived in the villages of Tagbarungis and Bacungan near Puerto Princesa City, Pal-

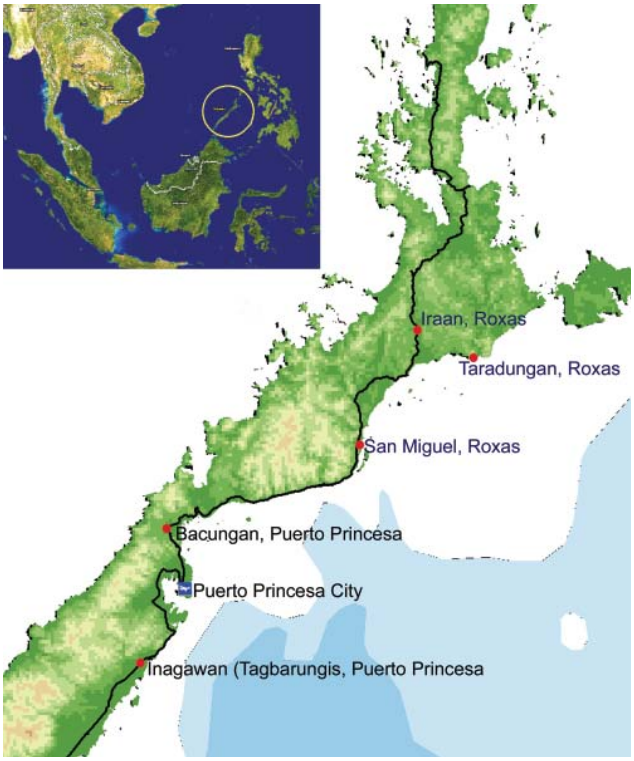

Figure. Map of Palawan, the Philippines, showing areas (red dots) where human *Plasmodium knowlesi* infections were confirmed (obtained from ESRI ArcGis 9 Media Kit and Provincial Development Office, Palawan, the Philippines).

awan (Figure). The patients were interviewed in July 2006 after their blood films were found to contain *P. knowlesi.* Both were subsistence farmers who had engaged in livelihood activities at night (charcoal making) in forested areas within a 50-km radius from their homes; neither had traveled out of Palawan within the past year. Before seeking medical treatment at the Palawan provincial malaria laboratory, they experienced chills, minor headaches, and daily low-grade fever, consistent with the reported quotidian fever pattern of this infection (*1*). *P. malariae* infection was diagnosed in both men by the local microscopist; each claimed to have responded well to chloroquine and primaquine drug therapy. The usual treatment regimen for *P. falciparum*/*P. malariae* was followed: 4 tablets of chloroquine (150 mg base/tablet) on days 1 and 2, and 2 tablets on day 3; 3 tablets of primaquine (15 mg/tablet) on day 4). The farmers reportedly stayed overnight before onset of their illness in forested foothills that contained many breeding sites ideal for *A. flavirostris* mosquitoes. Long-tailed macaques (*Macaca fascicularis*), the natural hosts for *P. knowlesi*, were observed to be roaming freely in the area. An additional 9 samples (D,E, G, H, I, J, K, O, and P), consisting of 5 blood films and 4 blood spots on filter paper, were obtained from patients at Bataraza and Roxas municipalities (also in Palawan) and *P. malariae* infection was diagnosed by the local microscopists. These samples were subsequently examined by nested PCR assays at UNIMAS after DNA extraction. Three were identified as *P. knowlesi*, 4 as *P. malariae*, and the remaining 2 as mixed species infections (Table). The findings of autochthonous *P. malariae* infections further compounded the problem of accurate diagnosis of *P. knowlesi* by microscopy. The *P. knowlesi* patients came from 3 different villages in Roxas, 80–100 km north of where the original 2 *P. knowlesi* case-patients resided, near Puerto Princesa (Figure). This suggests that human *P. knowlesi* infections are found across a relatively wide area in Palawan. PCR examination of more blood samples in other areas where *P. malariae* infections have been diagnosed by microscopy are necessary to determine the geographic distribution and public health importance of human knowlesi infections in the Philippines.

Conclusions

This report extends the geographic range of human *P. knowlesi* infections from Thailand (*3*), Myanmar (*4*), peninsular Malaysia (*8*), and Malaysian Borneo (*2*) to Palawan Island in the Philippines. Although the parasite has been isolated from local macaques in the Philippines in 1961 (*6*) and 1978 (*7*), this report documents autochthonous human cases in the country. Major progress in malaria control has been achieved in many malarious areas in the Philippines (*9*). However, *P. knowlesi* forms a previously unrecognized pool of infections that may be maintained in forested areas through its presence in a simian reservoir, despite control efforts in the human population. Current data suggest that human knowlesi malaria is strictly a zoonotic disease. To confirm this theory, further knowledge of the dynamics of human infection is needed.

Acknowledgments

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The malaria reference laboratory is supported by the Health Protection Agency, UK.

Ms Luchavez works in the Parasitology Department of the Research Institute for Tropical Medicine in the Philippines. Since 2003, her major research interests have included the development of quality assurance processes for malaria rapid diagnostic tests. She is one of the leading investigators in the WHO/Tropical Disease Research–coordinated network to develop a global quality assurance and product testing scheme for malaria diagnostics.

Table. Microscopy and PCR results of blood samples from Palawan, the Philippines

Patient	Age, y/sex	Location	*Plasmodium* species	
			Microscopy	PCR
A	50/M	Bacungan, Puerto Princesa	*P. falciparum* (gametocytes), *P. malariae*	*P. falciparum, P. malariae, P. knowlesi*
B	49/M	Inagawan, Tagbarungis, Puerto Princesa	*P. falciparum, P. malariae*	*P. knowlesi*
D	55/F	Caibulo, Iraan, Roxas	*P.malariae*	*P. knowlesi*
E	3/M	Balogo, San Miguel, Roxas	*P.malariae*	*P. knowlesi*
G	6/M	Maninguin, Iraan, Roxas	*P. malariae*	*P. malariae*
H	25/M	Minara, Roxas	*P. malariae*	*P. malariae*
I	10/F	Taradungan, Roxas	*P. malariae*	*P. knowlesi*
J	5/M	Bono-Bono, Bataraza	*P. vivax, P. malariae*	*P. falciparum, P. vivax, P. malariae*
K	14/F	Bono-Bono, Bataraza	*P. malariae*	*P. malariae*
O	9/M	Inogbong,Bataraza	*P. malariae*	*P. malariae*
P	5/F	Inogbong, Bataraza	*P. falciparum, P. malariae*	*P. falciparum, P. vivax*

References

1. Garnham PCC. Malaria parasites and other haemosporidia. Oxford: Blackwell Scientific Publications; 1966. p. 317–32.
2. Singh B, Kim Sung L, Matusop A, Radhakrishnan A, Shamsul SS, Cox-Singh J, et al. A large focus of naturally acquired *Plasmodium knowlesi* infections in human beings. Lancet. 2004;363:1017–24.
3. Jongwutiwes S, Putaporntip C, Iwasaki T, Sata T, Kanbara H. Naturally acquired *Plasmodium knowlesi* malaria in humans, Thailand. Emerg Infect Dis. 2004;10:2211–3.
4. Zhu HM, Li J, Zheng H. Human natural infection of *Plasmodium knowlesi.* Chinese Journal of Parasitology and Parasitic Diseases. 2006;24:70–1.
5. Oberst R, Schultz G, Laughlin L, Sy N, Santos M, Casimiro C. Epidemiological study of malaria in Palawan. The Philippine Journal of Microbiology and Infectious Diseases. 1988;17:41–8 [cited 2008 Mar 8]. Available from http://www.psmid.org.ph/index.php?fid=journals/Volume17Number2
6. Lambrecht FL, Dunn FL, Eyles DE. Isolation of *Plasmodium knowlesi* from Philippine macaques. Nature. 1961;191:1117–8.
7. Tsukamoto M, Miyata A, Miyagi I. Surveys on simian malaria parasites and their vectors in Palawan Island, the Philippines. Institute of Tropical Medicine, Nagasaki University, Japan. 1978;20:29–50 [cited 2008 Mar 8]. Available from http://naosite.lb.nagasaki-u.ac.jp/dspace/handle/10069/4234
8. Cox-Singh J, Davis TME, Lee KS, Shamsul SSG, Divis PCS, Matusop A, et al. *Plasmodium knowlesi* malaria in humans is widely distributed and potentially life threatening. Clin Infect Dis. 2008;46:165–71.
9. National Objective for Health. Philippines, 2005–2010 [cited 2008 Jan 7]. Available from www2.doh.gov.ph/noh2007/NOHWeb32/NOHperSubj/Chap4/Malaria.pdf

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Naturally Acquired Human *Plasmodium knowlesi* Infection, Singapore

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We report a case of naturally acquired *Plasmodium knowlesi* in Singapore, a malaria-free country. Diagnosis was confirmed by PCR with validated species-specific primers. In industrialized countries, free-ranging primates are a potential source of *P. knowlesi* human infection. *P. knowlesi* infection is a differential diagnosis of febrile illness acquired in Singapore.

Plasmodium knowlesi is one of the simian malarias that causes human infection (*1*,*2*). All 6 published reports of naturally acquired *P. knowlesi* infection were in rural settings with the largest case series being reported from East Malaysia (*3*–*8*). *P. knowlesi* is commonly misidentified as *P. malariae* since the blood stages are morphologically similar on microscopy, and molecular methods of detection are necessary for accurate diagnosis (*5*,*8*).

Singapore is an urban city-state, which was declared free of human malaria by the World Health Organization in 1982 (*9*). However, we report a case of locally acquired *P. knowlesi* malaria, which indicates that this emerging zoonotic parasite should be considered as an etiologic agent of acute febrile illness acquired in Singapore, the southernmost locale reported thus far.

The Case

A previously healthy 20-year-old soldier in the Singapore Army sought treatment on April 28, 2007. He had had a fever for 4 days, along with myalgia, anorexia, nausea, and occasional vomiting. For a year leading up to his illness, he had trained in a forested area inhabited by the long-tailed macaque (*Macaca fascicularis*) in Lim Chu Kang, northwestern Singapore. His only travel out of Singapore was a 3-week training visit to a non–malaria-endemic foreign country in September 2006 and another visit to Bukit Batok Nature Reserve in western Singapore, an area with monkeys (*M. fascicularis*) 1 month before onset of symptoms. On initial examination, his temperature was 39.5°C with a pulse rate of 106 beats/min. He was lethargic with tender hepatomegaly. Laboratory investigations showed thrombocytopenia (platelet count 66 × 10^9/L), hyperbilirubinemia (bilirubin 33 mmol/L [reference level 7–31 mmol/L]), and mild transaminitis (alanine transaminase 64 U/L [reference level 17–63 mmol/L] and aspartate transaminase 67 U/L [reference level 15–41 mmol/L]).

Initial diagnosis was dengue fever, which is endemic in Singapore. The patient experienced daily fever spikes from 39.5°C to 40.4°C (Figure 1). When fever persisted (40.4°C on day 6 of his illness, hospital day 3), the clinical picture was atypical for dengue fever. Blood films for malaria parasites were ordered, because introduced cases of malaria have been reported in Singapore (*10*). Microscopy showed *Plasmodium* parasitemia of 0.2% (equivalent to 7,700 parasites/mmol/L blood) with morphologic features consistent with *P. malariae*. Results of dengue reverse transcription–PCR (RT-PCR) on serum, 2 sets of blood cultures, and *Rickettsia typhi* serologic testing were negative. Results of a chest radiograph and ultrasound of the abdomen were normal.

Oral chloroquine was started with an initial dose of 600 mg base, followed by 300 mg base 6 h later and another 2 doses over the next 2 days. He defervesced rapidly; blood smears were negative 3 days after chloroquine therapy. At 2 weeks follow-up, he was clinically well.

Because *P. malariae* infection was not consistent with the clinical findings of the initial examination, we investigated further to determine the etiology of this case. Endpoint nested *Plasmodium* genus- and species-specific nested PCR carried out on DNA extracted from whole blood samples were positive for *Plasmodium* sp. but negative for the 4 species that cause human malaria (Table) (*11*). Similarly, the sample was negative on real-time PCR for the 4 human parasites (*12*). *P. knowlesi* species-specific PCR resulted in a 153-bp fragment indicative of *P. knowlesi* (*5*). This 153-bp PCR product was directly sequenced and verified in the BLAST database (www.ncbi.nlm.nih.gov/blast/

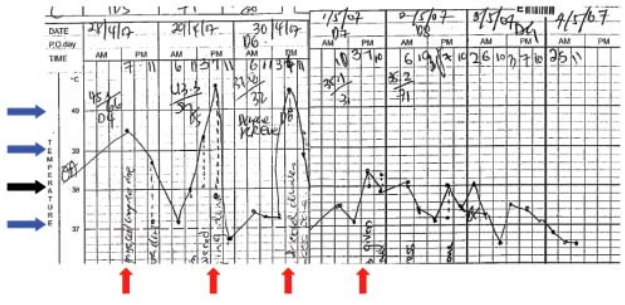

Figure 1. Patient's temperature chart showing fever spikes 24 h apart at approximately 7 PM daily (red arrows). The black arrow denotes 38°C, and each blue arrow denotes a difference of 1°C from the neighboring arrow.

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Table. Primers used for the PCR investigation of the clinical sample from Singapore*

Primers	Forward	Sequence (5′ → 3′)	Reverse	Sequence (5′ → 3′)	Results
Nest 1, genus specific primers					
Genus–specific (*11*)	rPLU 1	TCA AAG AAT AAG CCA TGC AAG TGA	rPLU 2	TAC CCT GTT GTT GCC TTA AAC TCC	+
Nest 2, genus- and species-specific primers					
Genus-specific (*11*)	rPLU 3	TTT TTA TAA GGA TAA CTA CGG AAA AGC TGT	rPLU 4	TAC CCG TCA TAG CCA TGT TAG GCC AAT ACC	+
Plasmodium knowlesi–specific (*5*)	Pmk8	GTT AGC GAG AGC CAC AAA AAA GCG AAT	Pmkr9	ACT CAA AGT AAC AAA ATC TTC CGT A	+
P. vivax–specific (*11*)	rVIV1	CGC TTC TAG CTT AAT CCA CAT AAC TGA TAC	rVIV2	ACT TCC AAG CCG AAG CAA AGA AAG TCC TTA	–
P. falciparum–specific (*11*)	rFAL1	TTA AAC TGG TTT GGG AAA ACC AAA TAT ATT	rFAL2	ACA CAA TGA ACT CAA TCA TGA CTA CCC GTC	–
P. malariae–specific (*11*)	rMAL1	ATA ACA TAG TTG TAC GTT AAG AAT AAC CGC	rMAL2	AAA ATT CCC ATG CAT AAA AAA TTA TAC AAA	–
P. ovale–specific (*11*)	rOVA1	ATC TCT TTT GCT ATT TTT TAG TAT TGG AGA	rOVA2	GGA AAA GGA CAC ATT AAT TGT ATC CTA GTG	–

*PCR was carried out at the Environmental Health Institute and cycling conditions used were as described in the references shown in parentheses.
+, positive; –, negative.

Blast.cgi) to match only *P. knowlesi* small subunit ribosomal RNA (SSU rRNA).

We confirmed the pathogen by using previously described approaches to compare the sequences of the 5′ and 3′ ends of the circumsporozoite protein (csp) gene (*13*), as well as the gene encoding of the SSU rRNA (*5*) in our case sample, to other *Plasmodium* parasites. Sequences were obtained by direct sequencing of PCR products and aligned by using the ClustalW method (EMBL-EBI, Hixton, Cambridge, UK); we constructed phylogenetic trees by using the MegAlign software (DNASTAR Inc, Madison, WI, USA). The case sample (denoted as SingPk1) clustered with other *P. knowlesi* isolates and is clearly distinct from other *Plasmodium* species (Figure 2).

Conclusions

We describe an unequivocal case of *P. knowlesi* infection supported by clinical findings and laboratory diagnostics classic for this pathogen. Similar to our patient, the classic scenario that raises the suspicion of *P. knowlesi* infection is a blood smear consistent with *P. malariae* but with parasitemia exceeding 5,000 per mmol/L blood, daily fever spikes, and pronounced symptoms, features atypical for *P. malariae* infection (*1*,*5*). The daily fever spike is due to the *P. knowlesi* 24-hour asexual life cycle, the shortest of all primate malarias (*8*). *P. malariae* has a 72-hour asexual life cycle and manifests as chronic, asymptomatic infection with low level parasitemia (*5*).

As in this case, *P. knowlesi* is commonly mistaken for *P. malariae* by microscopy due to similarity of the blood stages (*5*). *P. knowlesi* can be misidentified as *P. falciparum* if only ring forms are identified (*5*). The *P. knowlesi*–specific primers used by both independent laboratories have previously been shown not to detect any of the 4 *Plasmodium* species that cause human infection or the 3 agents that cause simian malaria: *P. cynomolgi*, *P. fieldi,* and *P. fragile* (*5*). PCR detection using *P. knowlesi*–specific primers, followed by sequencing and phylogenetic analyses of the csp and SSU rRNA genes confirmed *P. knowlesi* infection in our patient. This report extends the range of natural *P. knowlesi* human infection from East Malaysia, peninsular Malaysia, Thailand, and Myanmar to Singapore, an industrialized country that had been declared malaria-free by WHO (*3*–*8*).

Our patient likely acquired the infection in the forested area in Lim Chu Kang where he had been training for the entire year before his illness. Experimental *P. knowlesi* studies show a prepatent period of 9–12 days in humans (*14*). *P. knowlesi* has no liver hypnozoite stage and does not cause relapse (*1*). The patient's pevious overseas travel 7 months before and his visit to Bukit Batok Nature Reserve a month before onset of illness are beyond the incubation period.

P. knowlesi's natural hosts are the macaques, *M. fascicularis* and *Macaca nemestrina* (*1*). Notably, the first studies on *P. knowlesi* were on a parasite isolated from a macaque imported into India from Singapore (*2*). *M. fascicularis* and *Presbytis femoralis* are the 2 native monkeys in Singapore, with *M. fascicularis* being the only species in Lim Chu Kang and Bukit Batok Nature Reserve (*15*). Mosquitoes of the *Anopheles leucophyrus* group have been identified as vectors of *P. knowlesi* and are present in surrounding countries in southeast Asia (*1*,*8*). Studies are ongoing to determine potential mosquito vectors and whether macaques are hosts of *P. knowlesi* in Singapore.

Our patient's condition was diagnosed within 6 days of illness, and the infection responded rapidly to oral chloroquine. Although most patients' infections respond well to antimalarial agents, 4 fatal cases of *P. knowlesi* infection were reported recently in patients ages 39 to 69 years, whose conditions were all diagnosed within 7 days of

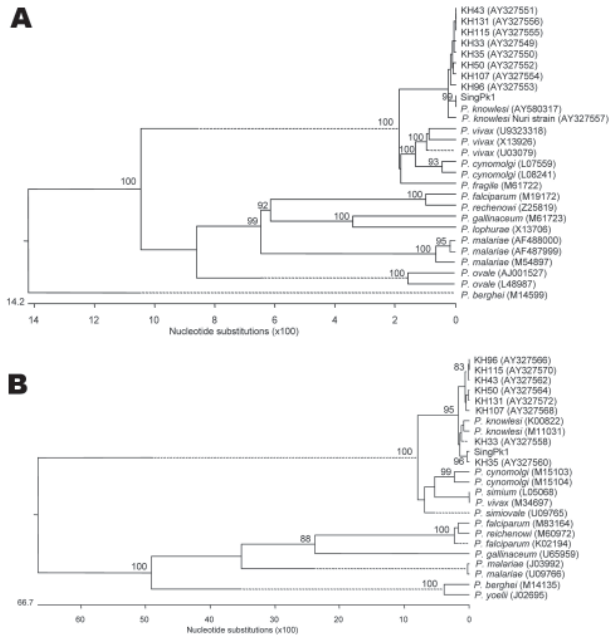

Figure 2. Phylogenetic trees comparing our case sample (denoted as SingPk1) with other *Plasmodium* species, based on SSU rRNA (A) and *csp* (B) sequences. Species and sequences used were selected to match those previously reported (*5*). Figures on the branches are bootstrap percentages based on 1,000 replicates, and only those above 80% are shown. GenBank accession numbers are in parentheses.

symptom onset (*8*). Common clinical features included fever, abdominal pain, thrombocytopenia (platelet count <30 $\times 10^9/\mu L$), renal impairment, and jaundice. All of the patients received a misdiagnosis of *P. malariae* infection.

P. knowlesi infection should be considered as an etiologic agent of malaria acquired in Singapore, particularly in cases with daily fever spikes and blood smears suggestive of *P. malariae*. Epidemiologic studies into the parasite's reservoir and mosquito vector will be important in the prevention of this emerging zoonotic disease.

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References

1. Coatney GR, Collins WE, Warren M, Contacos PG. The primate malarias [CD-ROM; original book published 1971]. Version 1.0. Atlanta: Centers for Disease Control and Prevention; 2003.
2. Knowles R, Das Gupta BM. A study of monkey-malaria and its experimental transmission to man. Ind Med Gaz. 1932;67:301–20.
3. Chin W, Contacos PG, Coatney GR, Kimball HR. A naturally acquired quotidian-type malaria in man transferable to monkeys. Science. 1965;149:865.
4. Fong YL, Cadigan FC, Coatney GR. A presumptive case of naturally occurring *Plasmodium knowlesi* in man in Malaysia. Trans R Soc Trop Med Hyg. 1971;65:839–40.
5. Singh B, Kim Sung L, Matusop A, Radhakrishnan A, Shamsul SS, Cox-Singh J, et al. A large focus of naturally acquired *Plasmodium knowlesi* infections in human beings. Lancet. 2004;363:1017–24.
6. Jongwutiwes S, Putaporntip C, Iwasaki T, Sata T, Kanbara H. Naturally acquired *Plasmodium knowlesi* malaria in human, Thailand. Emerg Infect Dis. 2004;10:2211–3.
7. Zhu HM, Li J, Zheng H. Human natural infection of *Plasmodium knowlesi*. Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi. 2006;24:70–1.
8. Cox-Singh J, Davis TME, Lee KS, Shamsul SSG, Matusop A, Ratnam S, et al. *Plasmodium knowlesi* malaria in humans is widely distributed and potentially life threatening. Clin Infect Dis. 2008;46:165–71.
9. Goh KT. Eradication of malaria from Singapore. Singapore Med J. 1983;24:255–68.
10. Chiam PT, Oh HM, Ooi EE. Localised outbreak of Falciparum malaria in Singapore. Singapore Med J. 2003;44:357–8.
11. Singh B, Bobogare A, Cox-Singh J, Snounou G, Abdullah MS, Rahman HA. A genus- and species-specific nested polymerase chain reaction malaria detection assay for epidemiologic studies. Am J Trop Med Hyg. 1999;60:687–92.
12. Rougemont M, Van Saanen M, Sahli R, Hinrikson HP, Bille J, Haton K. Detection of four *Plasmodium* species in blood from humans by 18S rRNA gene subunit-based and species specific real-time PCR assays. J Clin Microbiol. 2004;42:5636–43.
13. McCutchan TF, Kissinger JC, Touray MG, Rogers MJ, Li J, Sullivan M, et al. Comparison of circumsporozoite proteins from avian and mammalian malarias: biological and phylogenetic implications. Proc Natl Acad Sci U S A. 1996;93:11889–94.
14. Chin W, Contacos PG, Collins WE, Jether MH, Alpert E. Experimental mosquito-transmission of *Plasmodium knowlesi* to man and monkey. Am J Trop Med Hyg. 1968;17:355–8.
15. Yang CMMK. Yong K, Lim KKP. Wild mammals of Singapore. In: Chou LM, PKL Ng. Essays in zoology, papers commemorating the 40th anniversary of the Department of Zoology. Singapore: National University of Singapore; 1990. p. 1–24.

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Rickettsia slovaca in *Dermacentor marginatus* and Tick-borne Lymphadenopathy, Tuscany, Italy

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Of 263 patients in Tuscany, Italy, from whom ticks were removed during July 2005–May 2007, five showed signs of tick-borne lymphadenopathy. Of the ticks, 17 were *Dermacentor marginatus*; 6 (35.3%) of these were identified by sequence analysis as containing *Rickettsia slovaca*. Tick-borne lymphadenopathy occurs in this area.

Rickettsia slovaca was first isolated in Czechoslovakia from the tick vector *Dermacentor marginatus* in 1968 (*1*) and was subsequently detected in several European countries. It was recognized as the causative agent of tick-borne lymphadenopathy (*2*–*4*) and *Dermacentor* spp.–borne necrosis-erythema-lymphadenopathy (*5*). Typical clinical signs of infection include skin lesions at the tick bite site and regional, often painful, lymphadenopathy (*2*,*3*). Acute disease can be followed by residual alopecia at the bite site (*2*). This disease is considered a mild rickettsiosis, but severe symptoms have been described, especially in untreated patients (*2*).

D. marginatus is the only member of the species *Dermacentor* reported in Italy; it is widely distributed in prairies and steppes up to 2,500 m above sea level, including the northern Apennines (*6*). Adults are active within a temperature range of 4°C to 16°C (*7*,*8*). Temperature influences the seasonality of tick-borne lymphadenopathy, which has a higher incidence during cold months (*4*,*9*). We describe results from a tick-borne zoonoses surveillance system that was implemented in 2002 at the Lucca local health unit (ASL 2) in Tuscany, Italy.

The Study

Patients admitted to emergency units in Tuscany, Italy, for tick removal were followed up for 40 days. Epidemiologic and clinical data were collected for each patient by using a standardized questionnaire. History of allergic reactions or hypersensitivity to tick bites was considered and evaluated to avoid mistakes in case definition.

Ticks were classified by using standard identification keys (*8*) and stored in 70% ethanol until DNA extraction. *D. marginatus* females were measured, and the degree of engorgement (tick engorgement index [TEI]) was visually estimated. Ticks were ranked by 3 TEI levels: 1 = completely unengorged, 2 = intermediate (idiosoma length ≈2× twice the scutum width), and 3 = engorged (idiosoma length >2× the scutum width). Association between TEI levels and occurrence of clinical symptoms was evaluated by using the Fisher exact test. All statistical analyses were conducted by using R statistical software (*10*). Arcview 3.3 (Environmental Systems Research Institute Inc., Redlands, CA, USA) was used to map the geographic distribution of cases in the study area (Figure 1).

For pathogen detection by PCR, ticks were individually homogenized with a pestle in microcentrifuge tubes and DNA was extracted with the DNeasy Blood and Tissue Kit (QIAGEN, Hilden, Germany). Negative controls (distilled water) were used to check for contamination of samples during this phase. Success of DNA extraction was verified by using PCR for tick mitochondrial 16S rDNA (*11*). Two PCR assays, targeting citrate synthase A (*gtl*A) and outer membrane protein A (*omp*A) genes, were used to identify spotted fever group rickettsiae as described (*12*).

Figure 1. Distribution of tick-borne lymphadenopathy cases in Tuscany, Italy. Circles indicate cases, squares indicate patients bitten by *Dermacentor marginatus* who were not classified as case-patients, and triangles indicate emergency units. Negative (white symbols) and positive (dark symbols) PCR results for spotted fever group rickettsiae are indicated.

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Cases of abnormal tickbite reaction were observed only in patients bitten by *D. marginatus* (Table 1). From July 2005 through May 2007, information on 263 patients was recorded in the surveillance system. Removed ticks were classified as *Ixodes ricinus* (n = 187), *Rhipicephalus sanguineus* (n = 6), or *D. marginatus* (n = 17); 53 were unclassified (lost or disrupted).

Five patients (29.4%) (2 adults and 3 children) showed clinical signs typically related to tick-borne lymphadenopathy. We defined tick-borne lymphadenopathy case-patients as those with skin lesion (eschar) at the tick bite site and regional lymphadenopathy (*2*). The 5 patients were examined by physicians at least twice. Each patient had enlarged lymph nodes (Figure 2 , panel A) at the second examination. Crusted scalp lesions (Figure 2, panel B) ranged in diameter from 8 mm to 25 mm. One patient was surgically treated to remove necrotic tissue from a large tache noire; he showed alopecia >8 months after acute episode (Figure 2, panel C). Three cases were recorded in spring, 1 in autumn, and 1 in winter (Table 2). Association between *Dermacentor* TEIs and occurrence of symptoms was not statistically significant (p = 0.13). Six (35.3%) of 17 ticks were positive by PCR for either *gtl*A or *omp*A. The *ompA* gene sequences of all positive samples showed similarity of 100% with the *R. slovaca* (GenBank accession no. U43808).

Conclusions

Until recently, Mediterranean spotted fever (MSF) caused by *R. conorii* and transmitted by the brown dog tick, *R. sanguineus*, was considered the only tick-borne rickettsiosis in Italy. Local investigations at ASL 2 showed a decrease in MSF incidence during the past 10 years and fewer *Rhipicephalus* spp. bites than *Ixodes* spp. and *Dermacentor* spp. bites. In the past 2 years, no MSF was officially recorded at ASL 2, and our results suggest an emerging role of *R. slovaca* as a tick-borne pathogen in the area.

Most bitten patients showed specific clinical manifestations (fever, itching, rash, weariness, and myalgia) and 5 (29.4%) had typical signs of tick-borne lymphadeno-

Table 1. Patients bitten by *Dermacentor mariginatus* and admitted to emergency units, Tuscany, Italy

Patient no.	Year of birth	Sex	Site of tick bite	TEI*	Symptoms	Therapy 1†	Therapy 2‡	PCR result§	Tick-borne lymphadenopathy
31	2005	F	Head	1	Small nodules		No		
60	1937	M	Trunk	2	Itching	Yes			
89	2003	M	Head	1	No		No	*Rickettsia slovaca*	
117	1943	F	Trunk	3	Wet painful rash (20 mm) over 12 mo	Yes			
121	1954	F	Arm	M	None		No		
138	1968	M	Head	1	None		No		
145	2001	F	Head	3	Enlarged cervical lymph node, painful lymph node, fever, alopecia		15	*R. slovaca*	Yes
149	1939	F	Head	2	Fever		10	*R. slovaca*	
154	1930	M	Trunk	1	None				
155	1968	F	Head	2	Enlarged cervical lymph node, painful lymph node, fever, weariness		15		Yes
159	1950	M	Trunk	M	Itching		No		
175	2000	M	Head	2	Enlarged cervical lymph node, tache noire, alopecia		15		Yes
252	1968	F	Head	1	Enlarged cervical lymph node, painful lymph node, weariness, myalgia		15	*R. slovaca*	Yes
254	1949	F	Head	3	None				
256	1949	F	Trunk	2	Tache noire, itching, small nodules		15	*R. slovaca*	
263	2002	F	Head	M	Fever, headache		28¶ + 15#		
266	2001	M	Head	3	Pain at tick bite site, enlarged, painful cervical lymph node		21¶ + 12#	*R. slovaca*	Yes

*TEI, tick engorgement index based on visual evaluation for female ticks: 1, completely unengorged; 2, intermediate (idiosoma length ≈2× scutum width); 3, engorged (idiosoma length >2× scutum width). M, male tick.
†Therapy administered (doxycycline) when patients were discharged from emergency unit.
‡Therapy administered (doxycycline) when disease was diagnosed; numbers indicate duration of treatment in days.
§Pathogen identification by outer membrane protein A gene sequencing.
¶Amoxicillin.
#Clarithromycin.

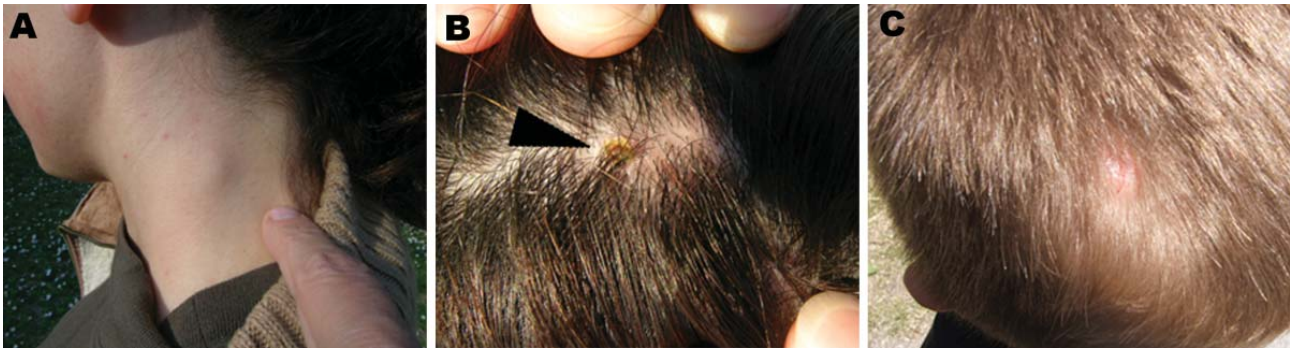

Figure 2. Enlarged lymph nodes (A), tache noire (arrowhead) (B), and alopecia (C) in patients admitted to the Lucca local health unit, Tuscany, Italy.

pathy. Pathogen identification in ticks agreed with the case definition in 50% of cases. Infected ticks were removed from 3 patients not considered to have lymphadenopathy; 1 patient showed no symptoms, probably because the tick was not attached long enough to enable pathogen transmission (TEI = 1).

A study reported that children and women have a higher risk than men for infection caused by *R. slovaca* (*4*); all of our patients with tick-borne lymphadenopathy were children or women. This result reflects the higher risk for bite by *Dermacentor* spp.; 6 (35.3%) of the 17 patients were <10 years of age and 10 (58.8%) were female.

Raoult et al. reported that all cases of tick-borne lymphadenopathy recorded in their study were found from October through May (*4*), confirming the seasonal incidence of tick-borne lymphadenopathy in cold months (*2*). The activity of *D. marginatus* adults in cold months has been reported (*7*,*8*). Our results confirm this trend, although the absence of cases of tick-borne lymphadenopathy in winter can be explained by the lower human frequency of high-risk areas (Table 2).

Cases reported in this study were concentrated in the northern part of ASL 2, despite the lower population density (Figure 1). Local investigations showed that wild boars, which seem to be important in the epidemiology of *R. slovaca* (*13*), are found in this area. In the northern part of this area, woodland habitat and human habits increase the risk for human contact with vectors. TEI did not show any association with cases but study of feeding duration is so far strictly applied to the transmission of *Borrelia burgdorferi* s.l. by *I. ricinus* (*14*).

In recent years, many efforts have been made to characterize distinct tick-borne diseases. A diagnostic approach that includes surveillance of patient symptoms and vectors can be helpful in identifying cases of tick-borne lymphadenopathy (*15*). Tick identification is also important in diagnosis (*4*).

Our data indicate a high prevalence of *R. slovaca* in *D. marginatus* collected from patients. On the basis of these results, all patients bitten by *D. marginatus* should be observed to determine whether specific treatments are required. The new surveillance system in Lucca will provide real-time data that will be useful for evaluation of patient health problems.

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References

1. Rehacek J. *Rickettsia slovaca*, the organism and its ecology. Acta Scientifica National Academy Scientifica Bohemoslovacae Brno. 1984;18:1–50.
2. Lakos A. Tick-borne lymphadenopathy (TIBOLA). Wien Klin Wochenschr. 2002;114:648–54.
3. Lakos A. Tick-borne lymphadenopathy—a new rickettsial disease? Lancet. 1997;350:1006.
4. Raoult D, Lakos A, Fenollar F, Beytout J, Brouqui P, Fournier PE. Spotless rickettsiosis caused by *Rickettsia slovaca* and associated with *Dermacentor* ticks. Clin Infect Dis. 2002;34:1331–6.
5. Oteo JA, Ibarra V, Blanco JR, Martinez de Artola V, Marquez FJ, Portillo A, et al. *Dermacentor*-borne necrosis erythema and lymphadenopathy: clinical and epidemiological features of a new tick-borne disease. Clin Microbiol Infect. 2004;10:327–31.
6. Mannelli A, Tolari F, Pedri P, Stefanelli S. Spatial distribution and seasonality of ticks (Acarina: Ixodidae) in a protected area in the northern Apennines. Parassitologia. 1997;39:41–5.

Table 2. Temporal distribution of *Dermacentor* spp. bites and tick-borne lymphadenopathy cases, Tuscany, Italy, 2006

Characteristic	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Dermacentor spp. tick bite	0	0	0	4	2	0	3	0	1	4	1	2
Tick-borne lymphadenopathy cases	0	0	0	2	1	0	0	0	0	1	0	1

7. Rukhkian MIa. Vertical migrations of hungry imagoes of the tick *Dermacentor marginatus* [in Russian]. Parazitologiia. 1987;21:680–3.
8. Manilla G. Acari, Ixodida (fauna d'Italia 36): Bologna (Italy): Edizioni Calderoni; 1998.
9. Raoult D, Weiller PJ, Chagnon A, Chaudet H, Gallais H, Casanova P. Mediterranean spotted fever: clinical, laboratory and epidemiological features of 199 cases. Am J Trop Med Hyg. 1986;35:845–50.
10. R Development Core Team. R: a language and environment for statistical computing. 2007 [cited 2008 Jan 31]. Available from http://www.R-project.org
11. d'Oliveira C, van der Weide M, Jacquiet P, Jongejan F. Detection of *Theileria annulata* by the PCR in ticks (Acari:Ixodidae) collected from cattle in Mauritania. Exp Appl Acarol. 1997;21:279–91.
12. Bertolotti L, Tomassone L, Tramuta C, Grego E, Amore G, Ambrogi C, et al. *Borrelia lusitaniae* and spotted fever group rickettsiae in *Ixodes ricinus* (Acari: Ixodidae) in Tuscany, central Italy. J Med Entomol. 2006;43:159–65.
13. Ortuno A, Quesada M, Lopez-Claessens S, Castella J, Sanfeliu I, Anton E, et al. The role of wild boar (*Sus scrofa*) in the eco-epidemiology of *R. slovaca* in northeastern Spain. Vector Borne Zoonotic Dis. 2007;7:59–64.
14. Gray J, Stanek G, Kundi M, Kocianova E. Dimensions of engorging *Ixodes ricinus* as a measure of feeding duration. Int J Med Microbiol. 2005;295:567–72.
15. Brouqui P, Bacellar F, Baranton G, Birtles RJ, Bjoersdorff A, Blanco JR, et al. Guidelines for the diagnosis of tick-borne bacterial diseases in Europe. Clin Microbiol Infect. 2004;10:1108–32.

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Fatal *Rickettsia conorii* subsp. *israelensis* Infection, Israel

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Underdiagnosis of fatal spotted fever may be attributed to nonspecific clinical features and insensitive acute-phase serologic studies. We describe the importance of molecular and immunohistochemical methods in establishing the postmortem diagnosis of locally acquired Israeli spotted fever due to *Rickettsia conorii* subsp. *israelensis* in a traveler returning to Israel from India.

Rickettsia conorii subspecies *israelensis,* the cause of Israeli spotted fever (ISF), has been described in Israel, Italy, and Portugal. ISF is characterized by fever, headache, and rash after a tick bite (*1*,*2*). Since nonspecific clinical symptoms occur during disease onset, and no eschar is present in most Israeli cases, ISF, like other rickettsioses, may be misdiagnosed. Fatal outcome has been described in previously healthy children and adults, particularly when appropriate and timely antimicrobial drug treatment was not administered (*2*).

Serologic tests are the most widely available diagnostic tools for spotted fever, but they are less than optimal for the diagnosis of rickettsial diseases in the acute phases (*3*). Autopsy findings may be nondiagnostic unless specialized molecular and immunohistochemical techniques or cell culture-based methods are used to detect rickettsiae (*3*–*5*). We report a confirmed case of fatal spotted fever in Israel due to *R. conorii* subsp. *israelensis*.

The Case

A 51-year-old previously healthy Israeli man was admitted to Assaf Harofeh Medical Center in Israel for febrile illness 1 month after he had returned from a trip to India. The patient lived in an urban environment in Israel and owned a dog. He had made two 1-month long business trips to India in March and in August 2005. He had been vaccinated against hepatitis A and typhoid fever, but had not taken antimalarial prophylaxis.

The patient's symptoms started on September 23, 2005, with fever ≈39°C accompanied by headache, weakness, and frequent urination. After he was given cefuroxime sodium, a generalized rash developed. He was then referred to the hospital on September 26. On admission, he was febrile (38.9°C), and a physical examination showed diffuse macular rash on the trunk, extremities, the palms of his hands, and the soles of his feet. An allergy to cephalosporins was suspected and cefuroxime was discontinued. Results of the following studies were nondiagnostic: routine blood and urine cultures; blood smears for malaria parasites; and serologic tests for HIV, hepatitis A, B, and C, West Nile virus, dengue virus, *Leptospira* spp., cytomegalovirus, and Epstein-Barr virus. The patient's condition worsened; on day 4 of hospitalization, severe muscle pain, tachycardia (189/min), tachypnea (40/min), oliguria, and generalized convulsions had developed. Intravenous piperacillin-tazobactam (4.5 g 3 times a day) plus oral doxycycline (100 mg twice a day) were initiated. Later that day the patient experienced respiratory failure and was transferred to the intensive care unit. During the days that followed, the patient was in a deep coma with decerebrate posture and multiorgan system failure. The skin rash became overtly petechial, with areas compatible with purpura fulminans. Because intravenous doxycycline is not available in Israel, doxycycline tablets were administered through the nasogastric tube, combined with intravenous meropenem. The patient died on October 2, 2005, on day 11 of illness (day 8 of hospitalization, day 5 of doxycycline therapy). An autopsy was performed and serum samples and tissue from various organs were preserved at –70°C for further study.

At autopsy, jaundice and edema with diffuse hemorrhagic rash, including the conjunctivae, were evident. Internal organs were congested, and moderate amounts of pleural fluid and ascites were noted. A pressure mark was evident on the left cerebellar tonsil, which indicated increased intracranial pressure. The cerebral cortex showed perivascular hemorrhages. Inflammatory cell infiltrates and occasional thrombi in the alveolar capillaries and arterioles were present in the lungs. Results of staining with silver-methenamine and periodic acid-Schiff were negative for pathogens.

Immunohistochemical staining performed at the Centers for Disease Control and Prevention (Atlanta, GA, USA) (*3*) showed spotted fever group rickettsiae in the vascular endothelial cells of the patient's brain and kidney (Figure 1). Serologic tests for *R. conorii* from day 7 of illness yielded negative results (both immunoglobulin [Ig] M and IgG). On day 11 of illness, IgG results remained negative and IgM results were borderline positive (Table).

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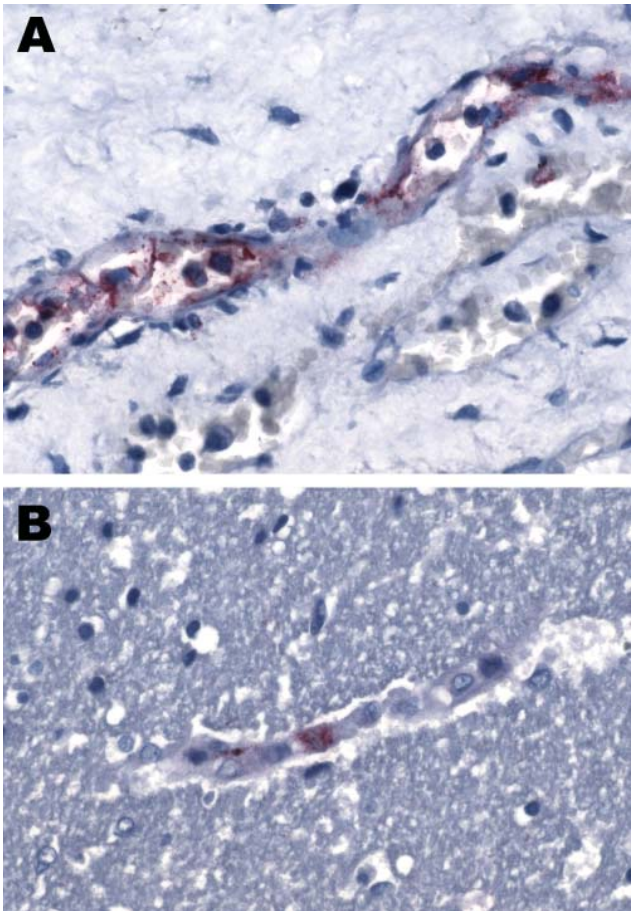

Figure 1. A small vessel in the kidney (A) and a capillary in the cerbral cortex (B) positive with immunohistochemical stain specific for spotted fever group rickettsiae**.** Original magnification ×158.

Results of nested PCR tests for spotted fever group rickettsiae (SFGR), performed at the Israeli National Reference Laboratory for Rickettsial Diseases on DNA samples prepared from serum collected on day 7 of illness (*8*), were negative. These tests were also applied to autopsy tissue samples (liver, muscle, skin, lung, kidney) and yielded a 214-bp amplicon from the 17-kDa protein gene of the SFGR (Figure 2). *Bfa*I restriction profile of 17-kDa protein gene amplicons consisted of 2 fragments, 50 and 164 bp that were identical to that of *R. conorii* subsp. *conorii* and *R. conorii* subsp. *israelensis* (*8*). A 208-bp fragment of the conserved 17-kDa *Rickettsia* spp. antigen gene was amplified at CDC from a DNA specimen obtained from a serum sample collected during the autopsy, and indicated SFGR DNA in the patient's bloodstream. An outer membrane protein A (ompA) gene fragment (70–602 nt) was amplified from the positive serum sample extracted at CDC and from skin, liver, and muscle samples extracted at the Israel Institute for Biological Research as described (*9*). Nucleotide sequences of each of the 4 *ompA* amplicons (GenBank accession no. EU122392) and *R. conorii* subsp. *israelensis* (U43797) were identical.

Conclusions

This case underscores the difficulties involved in establishing the diagnosis of ISF during the acute phase of the illness. It also emphasizes the importance of considering that returning travelers may have acquired the illness locally. Although rickettsial infections can be acquired by travelers to India (*10*), the long incubation time (1 month) and the positive diagnosis of the etiologic agent as *R. conorii* subsp. *israelensis* makes this possibility unlikely. An endemic ISF case due to dog ownership is the more likely scenario. On the other hand, physicians caring for travelers returning from Mediterranean countries such as Italy, Portugal, and Israel should be alert to the possibility of ISF in febrile patients. Absence of eschar in ISF may be an obstacle to the correct diagnosis as exemplified by a recent case of a UK traveler to Portugal (*11*).

Israeli spotted fever is endemic in Israel (*2*). National surveillance data have been available only since the early

Table. Diagnostic tests performed to identify spotted fever in the patient*

Day after disease onset	Assay	Specimen(s) tested	Result	Laboratory
7	IFA	Serum	IgM<100, IgG<100	IIBR†
	PCR	Serum	Negative	IIBR
11 (autopsy)	IFA	Serum	IgM = 64, IgG<32	CDC‡
	PCR	Serum sediment	*Rickettsia conorii* subsp. *israelensis*	CDC
		Liver, muscle, skin, lung, kidney	Spotted fever group rickettsiae	IIBR§
		Liver, muscle, skin	*R. conorii* subsp. *israelensis*	CDC¶
	IHC stain#	Brain, kidney	Positive	CDC
	Cell culture**	Liver, lung	Negative	IIBR

*IFA, immunofluorescent assay; Ig, immunoglobulin; IIBR, Institute for Biological Research; CDC, Centers for Disease Control and Prevention; IHC, immunohistochemical.
†IFA performed (*6*). Cutoff values for IgM and IgG are 100.
‡IFA performed (*7*). Cutoff values for IgM and IgG are 64.
§Nested PCR for 17-kDa protein gene (*8*).
¶Nested and semi-nested PCR for 17 kDa protein gene and recombinant outer membrane protein A gene fragment, respectively, followed by sequencing (*9*).
#Three-micron sections cut from formalin-fixed, paraffin-embedded brain and kidney tissue samples were stained by using an immunoalkaline phosphatase technique with a hyperimmune rabbit anti–*Rickettsia rickettsii* antibody at a dilution of <500 (*3*).
**Homogenized samples from lung and liver were applied by centrifugation onto monolayer of Vero cells culture in 24-well plates and incubated for 2 weeks at 35°C in 5% CO_2 atmosphere incubator.

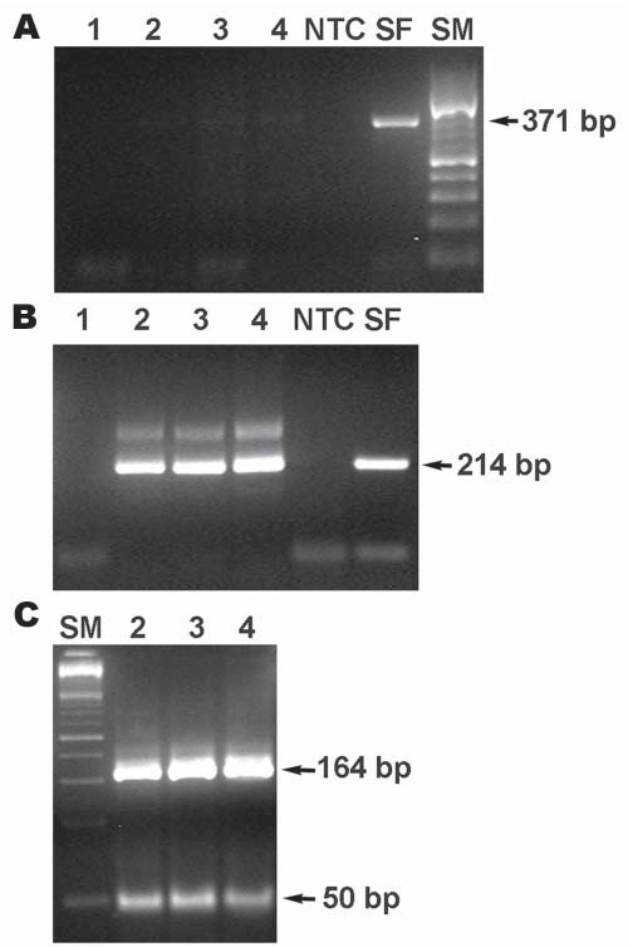

Figure 2. PCR product from the 17-kDa protein antigen gene obtained from DNA extracted from necropsied tissues of the patient. Primary PCR (A), nested PCR (B), and BfaI restriction enzyme pattern of the 17-kDa protein gene amplicon (C). Lane 1, reagent control; 2, skin; 3, liver; 4, lung; NTC, nontemplate control; SF, *Rickettsia conorii* DNA control; SM, size markers.

1970s (*10*). The incidence ranged from 0.7 to 10.3/100,000 (20–370 annual cases) from 1971 through 1980; it has declined steadily since 1980, reaching a nadir of 0.29/100,000 (20 cases) in 2004. The highest annual incidence reported was among children <10 years of age (10.5/100,000), and the lowest among persons >65 years (2/100,000) (*12*). A strong seasonal pattern exists, with the highest incidence occurring between June and October (*12*).

The case-fatality rate in Israel from 1971 through 1998 ranged from 0% to 3.5%, with an average rate of 0.7% (*12*). This rate may be an underestimate because seronegative fatal cases may not have been routinely investigated. Several examples of postmortem diagnoses in seronegative patients have been reported. In 1993, Yagupsky and Wolach described 2 children, whose postmortem diagnosis of ISF was established by using cell culture methods and animal inoculation studies (*5*). In 1997, 2 cases of unexplained deaths in young adults (31 and 38 years of age) were diagnosed after immunohistochemical detection of rickettsial antigen in paraffin-embedded tissue obtained at autopsy (*4,12*). Finally, nested PCR applied to sera and tissue in several serologically unconfirmed fatal cases of *R. conorii* infections was shown to be effective in establishing the correct diagnosis (*8,13,14*). PCR performed on whole blood or skin biopsy specimens of rash collected before treatment offers the possibility of improved early and rapid laboratory diagnosis of ISF and other rickettsial infections (*8,14,15*).

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References

1. Zhu Y, Fournier PE, Eremeeva M, Raoult D. Proposal to create subspecies of *Rickettsia conorii* based on multi-locus sequence typing and an emended description of *Rickettsia conorii*. BMC Microbiol. 2005;5:11.
2. Mumcuoglu KY, Keysary A, Gilead L. Mediterranean spotted fever in Israel: a tick-borne disease. Isr Med Assoc J. 2002;4:44–9.
3. Paddock CD, Greer PW, Ferebee TL, Singleton J Jr, McKechnie DB, Treadwell TA, et al. Hidden mortality attributable to Rocky Mountain spotted fever: immunohistochemical detection of fatal, serologically unconfirmed disease. J Infect Dis. 1999;179:1469–76.
4. Paddock CD, Childs JE, Zaki SR, Berger SA. Reply. Mortality in serologically unconfirmed Mediterranean spotted fever. J Infect Dis. 2000;181:810–2.
5. Yagupsky P, Wolach B. Fatal Israeli spotted fever in children. Clin Infect Dis. 1993;17:850–3.
6. Goldwasser RA, Steiman Y, Klingberg W, Swartz TA, Klingberg MA. The isolation of strains of rickettsiae of the spotted fever group in Israel and their differentiation from other members of the group by immunofluorescence methods. Scand J Infect Dis. 1974;6:53–62.
7. McDade JE, Fishbein DB. Rickettsiaceae: the rickettsiae. In: Lynnette EH, Halonen P, Murphy FA, editors. Laboratory diagnosis of infectious diseases: principles and practice. Viral, rickettsial, and chlamydial diseases. New York: Springer-Verlag, 1998. p. 864–89.
8. Leitner M, Yitzhaki S, Rzotkiewicz S, Keysary A. Polymerase chain reaction-based diagnosis of Mediterranean spotted fever in serum and tissue samples. Am J Trop Med Hyg. 2002;67:166–9.
9. Rozental T, Eremeeva ME, Paddock CD, Zaki SR, Dasch GA, Lemos ER. Fatal case of Brazilian spotted fever confirmed by immunohistochemical staining and sequencing methods on fixed tissues. Ann N Y Acad Sci. 2006;1078:257–9.
10. Walker DH. Rickettsial diseases in travelers. Travel Med Infect Dis. 2003;1:35–40.
11. Chai JTY, Eremeeva ME. Borland CDR, Karas JA. Fatal Israeli spotted fever in a UK traveler to South Portugal. J Travel Med. 2008;15:122–3.

12. Aharonowitz G, Koton S, Segal S, Anis E, Green MS. Epidemiological characteristics of spotted fever in Israel over 26 years. Clin Infect Dis. 1999;29:1321–2.
13. Schattner A, Leitner M, Keysary A, Geltner D. Case report: fatal seronegative rickettsial infection diagnosed by the polymerase chain reaction. Am J Med Sci. 1992;303:392–4.
14. Keysary A, Potasman I, Itzhaki A, Finkelstein R, Yitzhaki S, Strenger C, et al. Clusters of Mediterranean spotted fever in Israel. Vector Borne Zoonotic Dis. 2007;7:143–6.
15. Paddock CD, Koss T, Eremeeva ME, Dasch GA, Zaki SR, Sumner JW. Isolation of *Rickettsia akari* from eschars of patients with rickettsialpox. Am J Trop Med Hyg. 2006;75:732–8.

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Social Support and Response to AIDS and Severe Acute Respiratory Syndrome

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Negative public reactions to emerging infectious diseases can adversely affect population health. We assessed whether social support was associated with knowledge of, worry about, and attitudes towards AIDS and severe acute respiratory syndrome. Our findings suggest that social support may be central to our understanding of public responses to emerging infectious diseases.

The ability of public health institutions to effectively manage emerging infectious diseases (EIDs) and mitigate their consequences is partly a function of public reaction to an epidemic. Negative reactions may vary from denial to panic to stigmatization. Denial or minimization of the threat of an EID by the population at risk can impede prevention efforts and increase transmission. Alternatively, an overreaction to the threat of an EID can overwhelm public health systems and resources, divert resources from effective disease control activities, and lead to severe economic losses in areas affected by the disease. Stigmatization can increase problems of persons with the disease and facilitate transmission because persons with or at risk for the disease may avoid seeking healthcare and because governments may attempt to suppress information about EIDs, considering their potentially severe economic consequences. From a public health policy perspective, identifying personal characteristics and resources that predict responses of persons to EIDs is important to improve the ability of the public to learn about, rationally appraise the threat of, and minimize stigmatization of EIDs.

Determinants of a person's knowledge, worry, and stigma about EIDs are poorly understood. Although the quality of information conveyed to the public by various sources (e.g., healthcare providers, media) and epidemiologic characteristics of the disease (e.g., mode of transmission, case fatality rate) are important, early empirical work suggests that responses to EIDs are influenced by characteristics that include a greater stock of personal resources. We recently reported that less education was associated with being poorly informed about severe acute respiratory syndrome (SARS), very worried about AIDS, and having more stigmatizing attitudes toward AIDS and SARS (*1*,*2*). Social relationships may also be important. Helleringer et al. reported that social interactions among friends, peers, relatives, and community members may influence perceptions of HIV/AIDS risk and serve "as a resource for individuals to learn about and evaluate new behavioral strategies in the face of the epidemic" (*3*).

In this study, we assessed whether social support was independently associated with knowledge of, worry about, and attitudes toward AIDS and SARS in a representative sample of persons living in the New York City metropolitan area. Social support is defined as the functional aspect of social relationships (*4*).

The Study

Data for this study came from a cohort of persons ≥18 years of age who were living in the New York City metropolitan area on September 11, 2001. The cohort was recruited through a random digit dial telephone survey from March 25 through June 25, 2002. Contact information was obtained and follow-up interviews were conducted from September 25, 2002, through January 31, 2003, and from September 24, 2003, through February 29, 2004. Data regarding knowledge of, worry about, and attitudes toward AIDS and SARS were collected among a randomly selected subset of participants in the second cohort follow-up. The total sample included in this analysis, for whom data about AIDS, SARS, and social support were incomplete, consisted of 914 persons; response rate for eligible participants was 56%. Each participant received a $10 incentive. Additional details on sampling are provided elsewhere (*5*). The Institutional Review Board of the New York Academy of Medicine reviewed and approved this study.

We collected information about respondent sex, race/ethnicity, age, educational attainment, marital status, and household income at baseline. Social support was assessed by using a 5-item modified version of the Medical Outcomes Study social support scale (*6*); this abbreviated scale has a Cronbach α of 0.90 (*7*). Social support was categorized as low, medium, or high, on the basis of tertiles of support reported in the sample. We assessed respondents' knowledge of, worry about, and attitudes toward AIDS and SARS (Table 1). Further details on measures are provided elsewhere (*1*,*2*,*5*).

We described sociodemographic characteristics and level of social support of the respondents and created multivariable models predicting knowledge of, worry about, and

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Table 1. Measures of knowledge of, worry about, and stigmatization of AIDS and SARS*

Measure	Question and response options	Outcome definition
Knowledge of AIDS/SARS	Have you heard about AIDS (SARS) a great deal, some, not much, or not at all?	Binary: poorly informed (not much or not at all) versus not poorly informed (some or a great deal)
Worry about AIDS/SARS	Are you very, somewhat, or not at all worried about contracting AIDS (SARS)?	Binary: very worried versus not very worried (somewhat or not at all)
Stigmatization of AIDS/SARS	Do you agree strongly, agree somewhat, disagree somewhat, or disagree strongly with the following statements about controlling AIDS (SARS)? Requiring Americans with AIDS (SARS) to wear identification tags The government announcing it will execute persons who knowingly spread AIDS (SARS) Quarantining or separating all persons with AIDS (SARS) from others in the United States Avoiding areas in the United States that are heavily populated by gay men (Chinese) Forcing all gay men (Chinese) to be medically checked for AIDS (SARS) Not allowing gay men (Chinese) to enter the United States	Continuous: a summary stigmatization scale was constructed for each disease by adding responses to each of the 6 stigma questions; Cronbach α was 0.80 for the AIDS stigmatization scale and 0.72 for the SARS stigmatization scale

*SARS, severe acute respiratory syndrome.

stigmatizing attitudes toward AIDS and SARS, including covariates thought to potentially confound the relationship between social support and each outcome. Analyses were weighted to correct potential selection bias related to the number of household telephones, persons in the household, and oversampling, as well as to make the sample demographically similar to the New York City metropolitan area population according to US Census 2000 estimates. We used SUDAAN software to estimate standard errors and adjust analyses for the weighting (*8*).

Of the 914 participants, 45.4% were male, 54.1% white, 7.6% Asian or other race, 18.5% black, and 19.8% Hispanic (Table 2). In multivariable models controlling for sociodemographic variables (online Appendix Table, available from www.cdc.gov/EID/content/14/5/825-appT.htm), persons with low or medium levels of social support were significantly more likely than those with high levels of social support to report being poorly informed about AIDS ($p = 0.004$ for low support, $p = 0.038$ for medium support), being very worried about AIDS ($p = 0.010$ for medium support) and SARS ($p = 0.010$ for medium support), and to express more stigmatizing attitudes toward SARS ($p = 0.020$ for low support).

Conclusions

Reporting higher levels of social support was independently associated with greater knowledge of AIDS but not of SARS. Lower levels of social support were associated with more stigmatizing attitudes toward SARS but not AIDS. These patterns may be explained by differences in the epidemiology of AIDS and SARS in New York City. AIDS has been associated with tremendous illness and mortality rates in New York City (*9*,*10*). In contrast, the SARS epidemic was declared globally contained by the time of data collection (*11*). The psychological pathways through which social support influences responses to EIDs may be dependent on the effect of the epidemic on the local population. For example, greater social support may be associated with greater knowledge of AIDS because, relative to SARS, AIDS is more prevalent and persons with greater social support, through their social relationships, may have greater access to information that influences development of knowledge about EIDs.

Social support was inversely associated with worry about both AIDS and SARS, which suggested that association between social support and AIDS and SARS my persist despite differences in the epidemiology of EIDs. These findings are consistent with studies showing that social interactions are associated with perceptions of the risk for HIV/AIDS (*3*,*12*). Several biological and psychological mechanisms have been theorized to mediate the relationship between social support and health (*4*). Self-efficacy, defined as the degree of confidence persons have in their ability to perform specific tasks, is hypothesized to be one of the primary pathways through which social support operates (*4*). Therefore, social support may act as a buffer against worry about EIDs.

This study has several limitations. First, covariates were treated as time-fixed, which may have resulted in residual confounding. Second, social support is a multidimensional construct; specific dimensions may differentially influence knowledge of, worry about, and stigmatizing attitudes toward EIDs. Finally, for a cross-sectional analysis, it is difficult to establish temporality between levels of social support and knowledge of, worry about, and attitudes toward AIDS and SARS. Observed associations between social support and responses to EIDs may be explained by a common unmeasured covariate.

Table 2. Sociodemographic characteristics of 914 study participants, New York, New York, metropolitan area

Characteristic	No. (%)
Sex	
M	395 (45.4)
F	519 (54.6)
Race/ethnicity	
White	570 (54.1)
Asian/other	71 (7.6)
Black	130 (18.5)
Hispanic	131 (19.8)
Age, y	
18–34	245 (37.3)
35–54	393 (38.2)
≥55	267 (24.5)
Education	
Some college	642 (65.4)
High school or equivalent	182 (25.0)
Less than high school	88 (9.6)
Marital status	
Married	403 (53.0)
Divorced/separated/widowed	210 (15.6)
Never married/unmarried couple	295 (31.4)
Income	
≥$75,000	259 (33.9)
$40,000–$74,999	214 (27.5)
$20,000–$39,999	157 (23.5)
<$20,000	129 (15.2)
Social support	
Low	28 (30.2)
Medium	272 (30.5)
High	361 (39.3)

Emerging and reemerging infectious diseases will continue to profoundly affect human health. The ability to effectively mitigate disease consequences will depend, in part, on minimizing negative public responses. We showed that social support may be central to public responses to EIDs. Further research investigating the pathways linking social support to responses to EIDs may inform interventions that help guide public health and official responses to these diseases.

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References

1. Des Jarlais DC, Stuber J, Tracy M, Tross S, Galea S. Social factors associated with AIDS and SARS. Emerg Infect Dis. 2005;11:1767–9.
2. Des Jarlais DC, Galea S, Tracy M, Tross S, Vlahov D. Stigmatization of newly emerging infectious diseases: AIDS and SARS. Am J Public Health. 2006;96:561–7.
3. Helleringer S, Kohler HP. Social networks, perceptions of risk, and changing attitudes towards HIV/AIDS: new evidence from a longitudinal study using fixed-effects analysis. Population Studies. 2005;59:265–82.
4. Berkman LF, Glass T, Brissette I, Seeman TE. From social integration to health: Durkheim in the new millennium. Soc Sci Med. 2000;51:843–57.
5. Galea S, Vlahov D, Resnick H, Ahern J, Susser E, Gold J, et al. Trends of probable post-traumatic stress disorder in New York City after the September 11 terrorist attacks. Am J Epidemiol. 2003;158:514–24.
6. Sherbourne CD, Stewart AL. The MOS social support survey. Soc Sci Med. 1991;32:705–14.
7. Ahern J, Galea S, Fernandez WG, Koci B, Waldman R, Vlahov D. Gender, social support, and posttraumatic stress in postwar Kosovo. J Nerv Ment Dis. 2004;192:762–70.
8. Shah B, Barnwell B, Bieler G. SUDAAN user's manual, release 7.5. Research Triangle Park (NC): Research Triangle Institute; 1997.
9. Fordyce EJ, Singh TP, Vazquez FM, McFarland J, Thomas P, Forlenza S, et al. Evolution of an urban epidemic: The first 100,000 AIDS cases in New York City. Population Research Policy Review. 1999;18:523–44.
10. Centers for Disease Control and Prevention. HIV/AIDS surveillance report [cited 2007 Jul 19]. Available from http://www.cdc.gov/hiv/stats/hasr1301.htm
11. World Health Organization. China's latest SARS outbreak has been contained, but biosafety concerns remain [cited 2007 Jul 19]. Available from http://www.who.int/csr.don/2004_05_18a/en
12. Kohler HP, Behrman JR, Watkins SC. Social networks and HIV/AIDs risk perceptions. Demography. 2007;44:1–33.

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Acute Encephalitis Caused by Intrafamilial Transmission of Enterovirus 71 in Adult

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Enterovirus 71 (EV71) is a common cause of hand, foot, and mouth disease and sometimes causes severe neurologic complications, mainly in children. We report a case of adult-onset encephalitis caused by intrafamilial transmission of a subgenogroup C4 strain of EV71. This case elucidates the risk for EV71 encephalitis even in adults.

In children, enterovirus 71 (EV71) is a common cause of hand, foot, and mouth disease (HFMD), and most patients recover within 4–6 days. However, severe neurologic complications, such as acute encephalitis and poliolike paralysis, develop in some patients with EV71 infection. In the largest and most severe EV71-associated HFMD outbreak occurring in Taiwan in 1998, 405 children had severe neurologic complications, pulmonary edema, or both; 78 children died (*1*). In adults, transmission of EV71 within households is common, but EV71 infection is commonly limited to mild illness, and neurologic complications are uncommon in adults (*2*–*4*). We report a case of acute EV71 encephalitis in a mother and cases of HFMD in her 3 sons due to intrafamilial transmission of EV71.

The Case

In November 2006, a 37-year-old woman without serious past illness sought treatment at our hospital with hand tremor, unsteadiness, and a 2-day history of headache (day 1). Examination showed high fever (39.3°C), neck stiffness, intentional tremor of bilateral upper extremities, and truncal ataxia. Brain magnetic resonance images (MRI) and results of laboratory blood tests were normal. A cerebrospinal fluid (CSF) tap showed 305 leukocytes/mm^3 (82.5% polymorphonuclear leukocytes and 17.5% lymphocytes) and total protein concentration of 62 mg/dL with normal glucose levels. Empirical therapy with acyclovir and cefotaxime was initiated. On day 4, the patient reported diplopia and slurred speech. Ocular movements were not obviously restricted, and the extremities showed ataxia without weakness. She could not sit on the bed without support because of severe unsteadiness. Deep tendon reflexes were absent, and the patient had no pathologic reflexes. Brain MRI showed hyperintense lesions in the tegmentum of the medulla oblongata, pons, and midbrain in T2-weighted and fluid attenuated inversion recovery images (Figure 1). No abnormalities of the cervical spinal cord were detected on MRI. Results of nerve conduction studies were within normal ranges except for the absence of an F-wave in the median and ulnar nerves. Methylprednisolone (1 g/day) was administered for 3 days. From day 5 and forward, the patient gradually improved. A CSF tap on day 15 showed 14 leukocytes/mm^3 (100% lymphocytes) and a total protein concentration of 50 mg/dL. On day 22, MRI showed that the brain had normalized. Three months after the onset of disease, she had completely recovered.

During the illness, CSF was negative for bacteria and viruses. Enterovirus-specific RNA was detected from a stool sample on day 16 by a seminested reverse transcription–PCR (RT-PCR) with consensus-degenerative primers from Nix et al. (*5*); the virus was identified as EV71 by sequence analysis of the partial VP1 region (*5*). Serum neutralizing antibody titer against EV71 increased, from 8 on day 1 to 128 on day 15. There was no increase in serum antibodies against other viruses, including herpes simplex virus, cytomegalovirus, varicella-zoster virus, Epstein-Barr virus, rubella virus, and mumps virus by enzyme immunoassay, and against Japanese encephalitis virus by hemagglutination-inhibition test. Results for antinuclear antibody and antiganglioside antibodies were also negative.

Three days before the patient sought treatment, her 1-year-old son was affected with HFMD. This disease also developed in her other 2 sons, 5 and 7 years of age, on day 2. Her 3 children recovered within several days without any neurologic complications. Enterovirus-specific RNA was also detected in the stool samples from the 3 children by seminested RT-PCR (*5*), and all 3 viruses were identified as EV71 by sequence analysis. In addition, EV71 was isolated in Vero cells from stool samples from 2 of the 3 sons with HFMD. Stool and other clinical samples from the mother were all negative for virus isolation on Vero and RD cells.

The partial VP1 sequences (150 bp) of the PCR products directly amplified from stool samples of all 4 cases were 100% identical. The entire VP1 sequences (891 bp) of the EV71 isolates from 2 of her sons with HFMD (07-Ishikawa and 08-Ishikawa) were also 100% identical. Phy-

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logenetically, by using VP1-based genetic classification, the isolates were classified as subgenogroup C4 (Figure 2) (*6*). The subgenogroup C4 of EV71 has recently been identified in Japan and might have emerged in the surrounding countries, mainland People's Republic of China and Taiwan (*6*–*9*). The 07-Ishikawa strain shows a close genetic relationship to recent subgenogroup C4 strains in mainland China (97.4% nt identity to the SHZH04–38 strain) and those in Japan (97.0% nt identity to the 2779-Yamagata strain, Figure 2) (*7*,*9*).

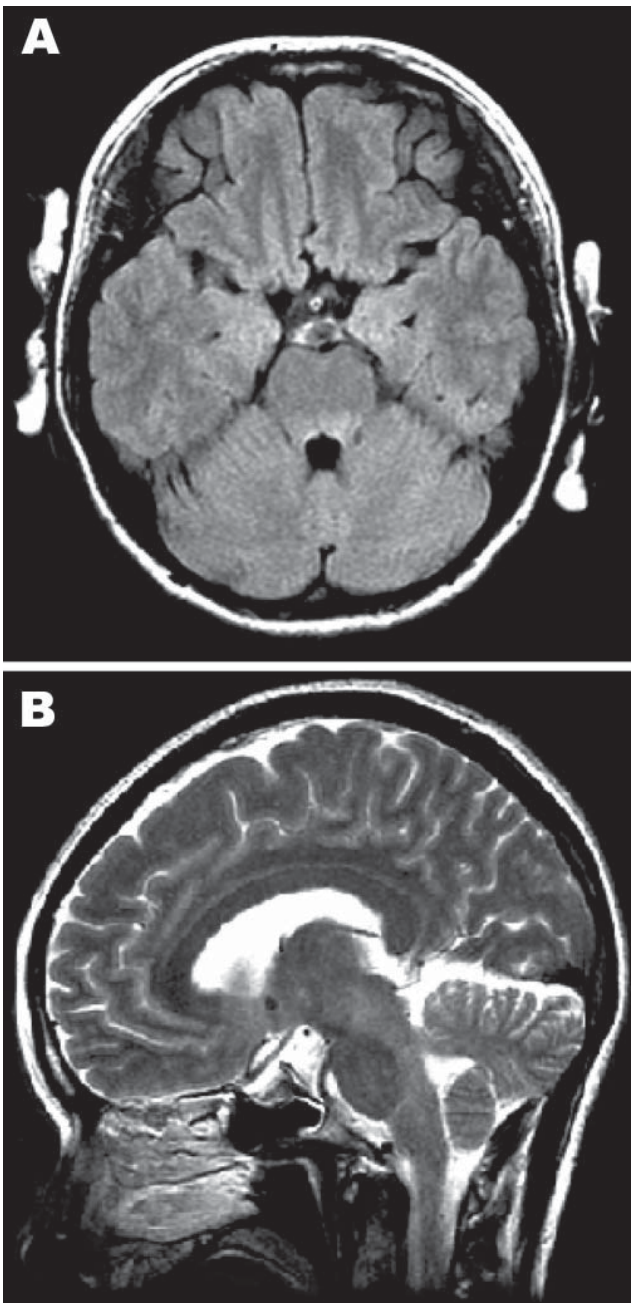

Figure 1. Magnetic resonance images of the brain. A) Hyperintense lesions in the tegmentum of the pons in the axial section of the fluid-attenuated inversion recovery image. B) In the sagittal section of the T2-weighted image, hyperintense lesions are present in the tegmentum of the midbrain, pons, and medulla oblongata.

Conclusions

Several EV71 outbreaks have been documented throughout the world, and clinical manifestations of EV71 infections can be diverse, including HFMD, herpangina, central nervous system (CNS) complications, and pulmonary edema. Recently, EV71-associated HFMD outbreaks with severe CNS complications have frequently been reported, especially in the Asian-Pacific region (*2*,*3*). In children, the CNS complications associated with EV71 manifest clinically in various ways, such as aseptic meningitis, acute flaccid paralysis, and rhombencephalitis; rhombencephalitis is one of the most common severe neurologic symptoms (*2*).

We diagnosed the mother's illness as EV71 encephalitis because the clinical features were similar to those of EV71 rhombencephalitis in children (*2*,*10*), although there has not previously been a detailed case report of adult-onset EV71 encephalitis. EV71 was not identified in the mother's CSF sample by virus isolation or direct molecular detection by RT-PCR, but EV71 was identified in her stool sample. We could not exclude the possibility of para- or post-infectious encephalitis during the initial stage of her illness. However, rhombencephalitis subsequently developed in this patient, which is common in children with EV71 encephalitis but is far less common from other infections and para- or post-infectious encephalitis. Clinical symptoms, MRI, and CSF findings of her illness were similar to those reported in children with EV71 encephalitis.

Several previous studies have demonstrated a rather low virus isolation rate in CNS specimens compared with that in other clinical samples, such as throat swab, rectal swab, and stool samples from EV71-associated cases with HFMD, encephalitis, or both (*2*,*3*,*11*). Along with the identification of EV71, the increase in serum neutralizing antibody titer against EV71 supports the diagnosis of acute EV71 infection. In addition, the lack of abnormalities of the spinal cord on MRI, the absence of an F-wave on nerve conduction study (a possible sign of radiculopathy), and the absence of deep tendon reflexes without weakness support the possibility of radiculitis as a complication. In a previous report, some patients with rhombencephalitis showed hyporeflexia or areflexia, but nerve conduction study findings were not reported (*2*).

Genetic analysis among 4 different EV71 isolates from the patients indicated probable intrafamilial transmission of EV71. In a recent study, EV71 transmission rate to household contacts was 52%, and the transmission rate from children to parents was 41% (*4*). Twenty-one percent of EV71-infected children experienced serious complications,

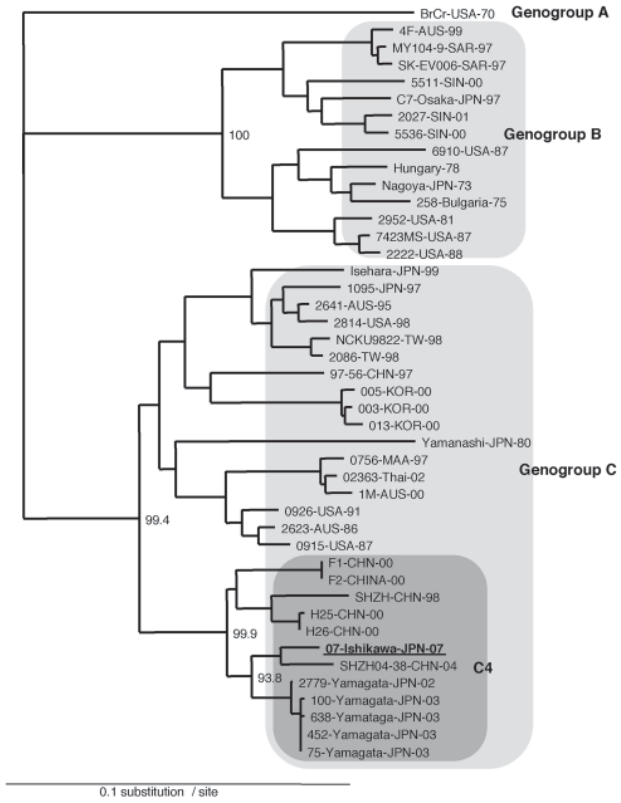

Figure 2. Phylogenetic analysis of EV71 based on the entire VP1 sequences. The tree was prepared by the neighbor-joining method by using the EV71 strains in the world as described previously (*6*) and newly identified subgenogroup C4 strains (*7*,*8*) were also included in the analysis.

including CNS or cardiopulmonary failure. By contrast, 53% of adults were asymptomatic, and all symptomatic adults recovered completely from uncomplicated illnesses (*4*). Considerable attention has been paid to EV71 infection in children because young age was considered the major risk factor associated with severe CNS complications, such as encephalitis resulting in severe neurologic sequelae and deaths (*2–4*,*12*). Thus, less attention has been paid to the adult-onset EV71 encephalitis. Our patient showed a good prognosis; however, a 19-year-old man died from EV71 encephalitis in Singapore (*3*). More careful disease surveillance, even for adults, will be needed during EV71-associated HFMD outbreaks.

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References

1. Ho M, Chen ER, Hsu KH, Twu SJ, Chen KT, Tsai SF, et al. An epidemic of enterovirus 71 infection in Taiwan. N Engl J Med. 1999;341:929–35.
2. Huang CC, Liu CC, Chang YC, Chen CY, Wang ST, Yeh TF. Neurologic complications in children with enterovirus 71 infection. N Engl J Med. 1999;341:936–42.
3. Chan KP, Goh KT, Chong CY, Teo ES, Lau G, Ling AE. Epidemic hand, foot, and mouth disease caused by human enterovirus 71, Singapore. Emerg Infect Dis. 2003;9:78–85.
4. Chang LY, Tsao KC, Hsia SH, Shih SR, Huang CG, Chan WK, et al. Transmission and clinical features of enterovirus 71 infections in household contacts in Taiwan. JAMA. 2004;291:222–7.
5. Nix WA, Oberste MS, Pallansch MA. Sensitive, seminested PCR amplification of VP1 sequences for direct identification of all enterovirus serotypes from original clinical specimens. J Clin Microbiol. 2006;44:2698–704.
6. Shimizu H, Utama A, Onnimala N, Li C, Li-Bi Z, Yu-Jie M, et al. Molecular epidemiology of enterovirus 71 infection in the Western Pacific Region. Pediatr Int. 2004;46:231–5.
7. Mizuta K, Abiko C, Murata T, Matsuzaki Y, Itagaki T, Sanjoh K, et al. Frequent inportation of enterovirus 71 from surrounding countries into the local community of Yamagata, Japan, between 1998 and 2003. J Clin Microbiol. 2005;43:6171–5.
8. Lin KH, Hwang KP, Ke GM, Wang CF, Ke LY, Hsu YT, et al. Evolution of EV71 genogroup in Taiwan from 1998 to 2005: an emerging of subgenogroup C4 of EV71. J Med Virol. 2006;78:254–62.
9. Li L, He Y, Yang H, Zhu J, Xu X, Dong J, et al. Genetic characteristics of human enterovirus 71 and coxsackievirus A16 circulating from 1999 to 2004 in Shenzhen, People's Republic of China. J Clin Microbiol. 2005;43:3835–9.
10. Shen WC, Chiu HH, Chow KC, Tsai CH. MR imaging findings of enteroviral encephalomyelitis: an outbreak in Taiwan. AJNR Am J Neuroradiol. 1999;20:1889–95.
11. Chang LY, Lin TY, Huang YC, Tsao KC, Shin SR, Kuo ML, et al. Comparison of enterovirus 71 and coxsackievirus A16 clinical illnesses during the Taiwan enterovirus epidemic, 1998. Pediatr Infect Dis J. 1999;18:1092–6.
12. Chang LY, Huang LM, Gau SS, Wu YY, Hsia SH, Fan TY, et al. Neurodevelopment and cognition in children after enterovirus 71 infection. N Engl J Med. 2007;356:1226–34.

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Seasonal Cholera from Multiple Small Outbreaks, Rural Bangladesh

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Clinical and environmental *Vibrio cholerae* organisms collected from February 2004 through April 2005 were systematically isolated from 2 rural Bangladeshi locales. Their genetic relatedness was evaluated at 5 loci that contained a variable number of tandem repeats (VNTR). The observed minimal overlap in VNTR patterns between the 2 communities was consistent with sequential, small outbreaks from local sources.

Cholera is a major cause of illness in the developing world. The World Health Organization reported in 2006 that 236,896 cases of cholera occurred in 52 countries, a 79% increase over 2005 (*1*). Although major advances in the understanding of the molecular basis of *Vibrio cholerae* pathogenicity have been made, including defining the environmental reservoirs for the microorganism (*2*–*4*), we do not fully understand the cause of seasonal epidemics in cholera-endemic areas nor the factors that drive epidemics. Specifically, whether these seasonal epidemics arise from a single clonal strain or reflect superimposition of multiple small outbreaks is not clear.

The Study

From February 2004 through April 2005, we systematically collected clinical and environmental *V. cholerae* from Bakerganj and Mathbaria, 2 small communities 50 miles apart in the southern part of coastal Bangladesh. Samples were collected on 3 consecutive days every 2 weeks throughout the year. Clinical isolates were collected from ≈20% of all patients who had symptoms of cholera when seen at the local clinics. Environmental isolates were cultured from water, sediment, and plankton samples taken at 6 sites (ponds or river sites) in each of the 2 communities. The same sites were used throughout the 15-month study, and the same method was applied at all sites and across all time points. Isolation was performed by standard culture methods, and *V. cholerae* was identified by a combination of biochemical (*5*), molecular, and serologic techniques (*6*). All samples were collected according to protocols approved by Institutional Review Boards at Johns Hopkins University, University of Maryland, and the International Centre for Diarrheal Disease Research, Bangladesh.

For multilocus sequence typing (MLST) and variable number of tandem repeat (VNTR) determinations, each locus was PCR amplified by using standard conditions and appropriate primers from the literature (*7*) (see online Technical Appendix, available from www.cdc.gov/EID/content/14/5/831-Techapp.pdf). The resulting fragments were sequenced by using Big Dye Kit (Applied Biosystems, Foster City, CA, USA). Trace files were generated by using an ABI 3730xl automatic sequencer and read using either 1) the Phred (*8*,*9*), Phrap (www.washington.edu), or Consed (*10*) package or 2) Sequencher (AGCT, Gene Codes Corporation, Ann Arbor, MI, USA).

A total of 391 environmental and clinical isolates of *V. cholerae* were collected and identified from February 2004 through April 2005. Of these, 267 environmental isolates were identified as belonging to non-O1 and non-O139 serogroups and did not carry the gene for cholera toxin (*ctx*). Analysis of these 267 by MLST (using the 7 loci identified previously [*7*]) yielded a genetic background that was distinct from that of the clinical/epidemic strains. The other 68 (20%) of 335 environmental *V. cholerae* isolates shared a genetic background identical or nearly identical to clinical/epidemic *V. cholerae.* These 68 and all 56 clinical isolates collected (all of which were related by MLST) were further analyzed by examining 5 VNTR loci.

Sequence typing was based on 5 polymorphic VNTR loci. These loci were identified with the program Tandem Repeat Finder (*11*). Four of the 5 loci had hexameric repeats in coding regions. The loci were identified by those genes in which they occur: VC0147vntr, VC0436–7vntr (intergenic), VC1650vntr, VC0171vntr, and VCA0283vntr. Alleles were distinguished by the number of tandem repeats as determined by Tandem Repeat Finder (*11*) (online Technical Appendix). Sequences from 1 locus with identical numbers of repeats were assigned to the identical allele. The alleles at the 5 loci were ordered to generate a sequence type (ST), for example, 3,5,2,2,8. Each locus was polymorphic with 7, 6, 6, 20, and 16 alleles, respectively. Thirty-six STs were observed. The various STs were defined as related if they were identical at 4 of the 5 loci. When we defined a VNTR genetic group as differing by a single locus variant from another member of the group, 3 large VNTR genetic groups were identified and 5 VNTR genetic groups composed of only 2 isolates and 7 unrelated strains. These 7 singletons differed from all other STs at 2 or more loci.

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There was statistically significant agreement between serogroup and VNTR genetic group. For *V. cholerae* O139, all STs were 4,1,1,x,x (online Technical Appendix). Thus, the isolates were considered to be related because x,x = 1,1; 2,1; or 2,8, i.e., a change in a single locus serially connected all isolates. Summing the number of isolates of a sequence type, we found that the 23 ctx^+ O139 strains formed a VNTR genetic group. A second group comprised 75 ctx^+ O1 Inaba isolates. Finally, 18 ctx^+ O1 Ogawa clustered into 3 additional VNTR genetic groups. There were 10 exceptions, i.e., 3 non-O1, non-O139 ctx^+ isolates were in groups; 3 ctx^- O139, 2 ctx^- O1 Inaba, 1 ctx^-, and 1 ctx^+ O1 Ogawa were not.

We found that Bakerganj and Mathbaria yielded distinct *V. cholerae* populations; only 2 (ST 3,5,2,2,7 and 1,1,3,9,8) of 36 STs identified were found at both locations (Table 1; online Technical Appendix). There was substantial divergence in STs among strains isolated from patients, compared with strains from the environment in Mathbaria; only 1 (ST 3,5,2,2,7) of 16 STs were found in both patient and environmental isolates. Similarly, in Bakerganj, only 2 (ST 3,5,2,2,6 and 3,5,2,1,5) of 24 STs were found in both clinical and environmental isolates.

Clinical or environmental isolates from a given period were more likely to have a common ST (online Technical Appendix). For example, at Mathbaria, 49 of the 53 isolates with an ST identical to that of another isolate were found in the same or neighboring month. Similarly, at Bakerganj, 33 of 36 isolates with identical STs were found in the same or neighboring month.

Variation in the VNTR loci appeared to be greater among clinical isolates than among environmental isolates. A total of 29 STs occurred in clinical isolates, whereas only 12 occurred in environmental isolates (Table 1). When we controlled for location and month of collection (Table 2), the total number of STs among environmental isolates (7 ST/35 isolates) was less than that among clinical isolates (16 ST/32 isolates) ($\chi^2 = 4.4$, df 1, $p = 0.036$). Common STs were found among environmental isolates, despite the isolates coming from samples from different ponds and distinct subsamples (e.g., water, phytoplankton, zooplankton).

Table 1. Number of *Vibrio cholerae* sequence types in distinct serotypes and sample types in Bakerganj and Mathbaria, Bangladesh, 2004–2005

Serotype	Source	Bakerganj	Mathbaria
O1 Inaba	Clinic	10	7
	Environment	7	1
O1 Ogawa	Clinic	6	6
	Environment	1	0
O139	Environment	0	3

Conclusions

Our data do not support the concept of seasonal cholera epidemics occurring by movement of a single clonal wave across the countryside. They are consistent, instead, with the natural occurrence of *V. cholerae* year-round in the aquatic environment of each site, with each site having its own, distinct grouping of strains (*12*,*13*). The limited overlap between STs in environmental and clinical isolates is an enigma that remains to be resolved. However, the extensive variation in VNTR STs in this short time frame and small geographic area suggests that VNTR STs can be useful in assessing genetic relatedness of isolates during outbreaks/epidemics. The strong temporal clustering of the variation arising in the VNTR STs of clinical isolates is consistent with the hypothesis that clinical cases reflect the occurrence of multiple small outbreaks.

Our data are drawn from rural Bangladesh; however, cholera is a global disease. Its epidemiology may well differ in sub-Saharan Africa, the Americas, or other parts of Asia, or in the mega-cities that are increasingly the hallmark of the developing world. These variations emphasize the need for application of similar techniques in these diverse settings.

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Table 2. Sequence type (ST) variations among *Vibrio cholerae* O1 Inaba isolates from environmental and clinical sources by month of collection, Bangladesh, 2004–2005

Location	Date	Source	No. ponds	No. isolates	No. STs	Variation*
Mathbaria	2004 Dec	Environment	4	12	1	0.08
Bakerganj	2004 Sep	Environment	5	16	4	0.25
Bakerganj	2005 Apr	Environment	4	7	2	0.29
Bakerganj	2004 Oct	Clinic		9	4	0.44
Mathbaria	2004 May	Clinic		11	5	0.45
Mathbaria	2004 Apr	Clinic		8	4	0.50
Bakerganj	2004 Dec	Clinic		4	3	0.75

*Variation, no. STs/no. isolates.

References

1. World Health Organization. Cholera 2005. Wkly Epidemiol Rec. 2006;81:297–308.
2. Huq A, Small EB, West PA, Huq MI, Rahman R, Colwell RR. Ecological relationships between *Vibrio cholerae* and planktonic crustacean copepods. Appl Environ Microbiol. 1983;45:275–83.
3. Brayton PR, Tamplin ML, Huq A, Colwell RR. Enumeration of *Vibrio cholerae* O1 in Bangladesh waters by fluorescent-antibody direct viable count. Appl Environ Microbiol. 1987;53:2862–5.
4. Franco AA, Fix AD, Prada A, Paredes E, Palomino JC, Wright AC, et al. Cholera in Lima, Peru, correlates with prior isolation of *Vibrio cholerae* from the environment. Am J Epidemiol. 1997;146: 1067–75.
5. Tison DL, Kelly MT. *Vibrio* species of medical importance. Diagn Microbiol Infect Dis. 1984;2:263–76.
6. Alam M, Sultana M, Nair GB, Sack RB, Sack DA, Siddique AK, et al. Toxigenic *Vibrio cholerae* in the aquatic environment of Mathbaria, Bangladesh. Appl Environ Microbiol. 2006;72:2849–55.
7. Garg P, Aydanian A, Smith D. J Glenn M Jr, Nair GB, Stine OC. Molecular epidemiology of O139 *Vibrio cholerae*: mutation, lateral gene transfer, and founder flush. Emerg Infect Dis. 2003;9:810–4.
8. Ewing B, Green P. Base-calling of automated sequencer traces using phred. II. Error probabilities. Genome Res. 1998;8:186–94.
9. Ewing B, Hillier L, Wendl MC, Green P. Base-calling of automated sequencer traces using phred. I. Accuracy assessment. Genome Res. 1998;8:175–85.
10. Gordon D. Viewing and editing assembled sequences using Consed. In: Baxevanis A, Davison D, editors. Current protocols in bioinformatics. New York: John Wiley & Co; 2004. p. 11.2.1–11.2.43.
11. Benson G. Tandem repeats finder: a program to analyze DNA sequences. Nucleic Acids Res. 1999;27:573–80.
12. Colwell RR. Global climate and infectious disease: the cholera paradigm. Science. 1996;274:2025–31.
13. Huq A, Sack RB, Nizam A, Longini IM, Nair GB, Ali A, et al. Critical factors influencing the occurrence of *Vibrio cholerae* in the environment of Bangladesh. Appl Environ Microbiol. 2005;71: 4645–54.

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New Saffold Cardioviruses in 3 Children, Canada

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In Canada, cardiovirus isolates related to Saffold virus were detected in nasopharyngeal aspirates from 3 children with respiratory symptoms. Polyprotein sequence of the Can112051-06 isolate had 91.2% aa identity with Saffold virus; however, EF and CD loops of the viral surface varied substantially.

The family *Picornaviridae* contains 9 genera: *Enterovirus, Hepatovirus, Rhinovirus, Kobuvirus,* and *Parechovirus* infect humans, whereas *Aphtovirus, Erbovirus, Teschovirus,* and *Cardiovirus* are animal pathogens (*1*). The genus *Cardiovirus* is divided into 2 species: Theiler viruses and the encephalomyocarditis viruses (EMCVs) (*2–5*). Although rats and mice are the natural hosts for EMCVs, these cardioviruses have been found to infect many animal species including pigs, rodents, elephants, macaques, and humans (*6–9*). Recently, a new cardiovirus provisionally named Saffold virus (SAF-V) was isolated from a stool sample of an 8-month-old girl with fever (*10*). This virus is believed to constitute a novel cardiovirus species and is more genetically related to Theiler-like virus than to other known cardioviruses (*10*). We report the identification and characterization of 3 SAF-V isolates recovered from children with respiratory symptoms.

The Patients

The first patient was a 23-month-old girl who was referred on March 6, 2006, to a tertiary hospital for bilateral otitis media that had not responded to amoxicillin or later to cefprozil. She also had cough, rhinorrhea, and fever of 39°C. Her 5-month-old brother had similar clinical signs. Blood cultures were negative, as were antigen detection tests for influenza A and B viruses, the respiratory syncytial virus, and adenoviruses. After 24 hours, the girl was discharged with a diagnosis of bilateral acute otitis media secondary to a viral infection. A nasopharyngeal aspirate collected at the time of admission was inoculated onto different continuous cell lines including human lung adenocarcinoma (A-549); human rhabdosarcoma (RD); transformed human kidney (293); human colon adenocarcinoma (HT-29); human laryngeal carcinoma (Hep-2); human foreskin fibroblast; mink lung; and Vero, MDCK, and rhesus monkey kidney (LLC-MK2) cells. Cultures were incubated for 3 weeks at 37°C in 5% CO_2. A viral isolate (Can112051-06) with cytopathic effects (round cells) suggestive of a picornavirus was observed only in LLC-MK2 cells after 6 days of incubation (Figure 1). An immunofluorescent assay that used the Pan-Enterovirus Blend kit (Light Diagnostics, Levingston, UK) gave a moderate fluorescent signal. Nucleic acid extracts from Can112051-06 were further analyzed with a multiplex real-time reverse transcription–PCR (RT-PCR) assay for common respiratory viruses (influenza A and B viruses,

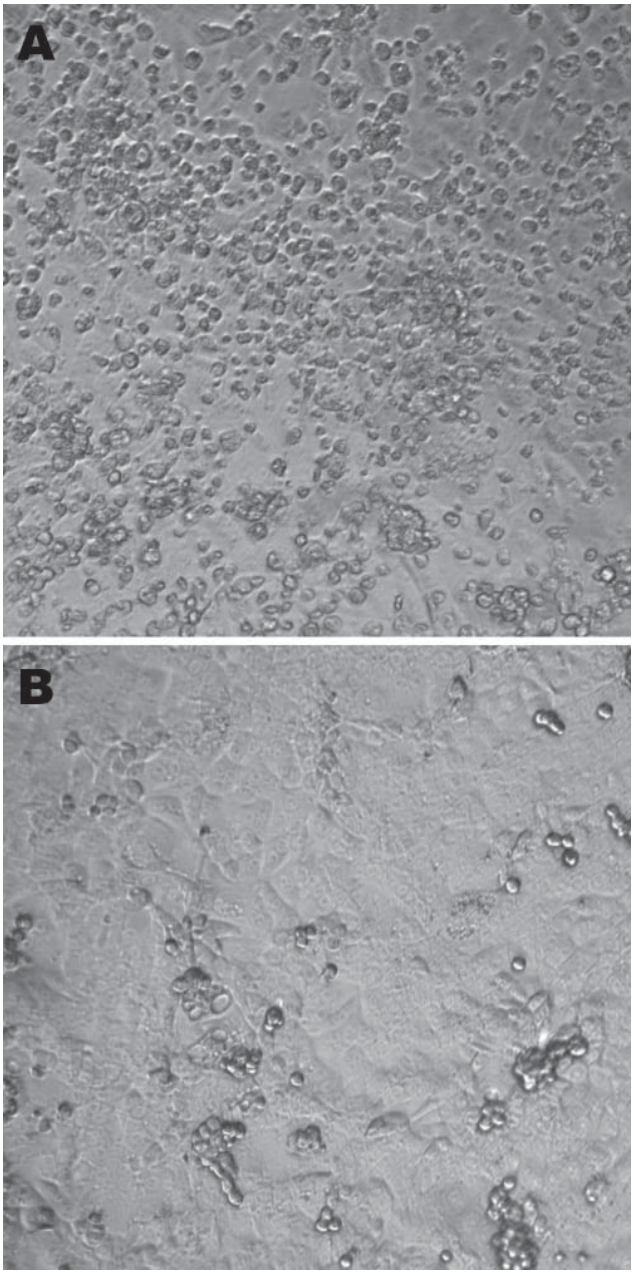

Figure 1. A) Cytopathic effects (round cells) observed 6 days after infection of rhesus monkey kidney (LLC-MK2) cells (second passage) with the Can112051-06 Saffold virus–like cardiovirus strain. B) Uninfected LLC-MK2 cells. Magnification ×10.

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human respiratory syncytial virus, and human metapneumovirus) (*11*) as well as RT-PCR assays for enteroviruses and parechoviruses (*12*); results were negative.

The supernatant from LLC-MK2–infected cells was treated with DNase and divided into 2 aliquots for DNA and RNA extractions by using the QIAamp Blood Mini Kit and QIAamp Viral RNA extraction kits (QIAGEN, Mississauga, Ontario, Canada), respectively. Nucleic acids were then used in the sequence-independent single-primer amplification method as described (*13*). Amplicons of 800–1,200 bp obtained from RNA samples were cloned and sequenced.

Sequence determination of cloned amplicons followed by tBLASTx analysis showed similarity of Can112051-06 sequences with the SAF-V VP4 and 2C sequences (data not shown). Subsequent PCR amplifications and sequencing reactions that used primers selected from our clones and the complete SAF-V genome sequence (GenBank accession no. EF165067) enabled us to determine the complete polyprotein encoding region of the Can112051-06 isolate (GenBank accession no. AM922293). This region was 6,879 nt long compared with 6,888 nt for the SAF-V polyprotein sequence; nucleotide identity between the 2 strains was 82.5%. The Can112051-06 polyprotein comprised 2,293 aa compared with 2,296 aa for the SAF-V polyprotein; amino acid identity between the 2 strains was 91.2%. Deletions of 1 aa in the VP2 and 2 in the VP1 proteins were found in Can112051-06 with regard to the prototype SAF-V strain. As expected, the Can112051-06 and SAF-V polyproteins contained 11 putative cleavage sites. The 8 aa flanking these sites were conserved; 6 sites were identical in the 2 strains, whereas the remaining sites had 1- or 2-aa differences (Table 1). The resulting 12 proteins of Can112051-06 and SAF-V had 76.1%–100% aa identities (Table 2). The highest difference level was seen in the L peptide. In addition to the L peptide, some cardioviruses, in particular Theiler's murine encephalomyelitis virus strains that are associated with persistent infections, contain an alternate open reading frame (ORF), the so-called L* (*14*). As for the prototype SAF-V strain, the Can112051-06 putative L* ORF is unlikely to encode a protein because it has an ACG (instead of ATG) start codon (data not shown). In addition, contrasting with the SAF-V L*, which contained 57 aa, the Can112051-06 L* sequence contained only 34 aa. Comparison of the L* sequence of Can112051-06 with the first 34 aa of the SAF-V L* sequence showed 60.6% identity (data not shown). Four small loops are exposed on the virion surface of cardioviruses; 2 are part of the VP2 EF loop structure, and 2 are part of the VP1 CD loop structure. The EF loop structure of Can112051-06, which contained 55 aa (residues 274-328 of the polyprotein), had 61.8% aa identity with that of SAF-V (Figure 2, panel A). Similarly, the CD loop structure of Can112051-06, which contained 40 aa (residues 712-751 of the polyprotein), had 67.5% aa identity with the SAF-V counterpart (Figure 2, panel B).

Table 1. Cleavage sites of Can112051-06 and prototype Saffold virus cardiovirus polyproteins*

Cleavage site	Can112051-06	Saffold virus
L / VP4	MEPQ / GNSN	MEPQ / GNSN
VP4 / VP2	PLLM / DQNT	PLLM / DQNT
VP2 / VP3	LEDQ / SPIP	LEAD / SPIP
VP3 / VP1	YTPH / GVDN	YTPQ / GVDN
VP1 / V2A	LELQ / NPIS	LELQ / DPIS
2A / 2B	FQLQ / GGVL	FQLQ / GGVL
2B / 2C	LQQQ / SPVR	LQQQ / SPIR
2C / 3A	LVAQ / SPGN	LVAQ / SPGN
3A / 3B	EGEQ / AAYS	EGEQ / AAYS
3B / 3C	LDVQ / GGGK	LDVQ / GGGK
3C / 3D	LIPQ / GAIV	LTPQ / GAIV

*Can112051-06 GenBank accession no. AM922293; Saffold virus GenBank accession no. EF165067.

Other respiratory samples with picornavirus-like cytopathic effects on LLC-MK2 cells and weakly immunofluorescent signals according to the Pan-Enterovirus Blend Kit were screened for cardiovirus SAF-V by using a specific RT-PCR assay targeting a 2A–2C encoding region (1,407 nt, 469 aa). With use of this strategy, 2 more cases were noted in September 2006: 1 in a 19-month-old child hospitalized for suspected bacteremia and a cold and 1 in a 4-year-old child hospitalized for right lung pneumonia and otitis media. The 2A-2C aa sequences of these additional isolates were identical and shared 96.6% and 97.2% aa identities with the corresponding regions of Can112051-06 and the prototype SAF-V, respectively.

Conclusions

Our findings suggest a pathogenic role for SAF-V-like viruses in humans. Although the polyprotein sequences of the Can112051-06 strain and the original US strain were related, the EF and CD loop structures varied substantially (61.8% and 67.5% aa identities, respectively). For com-

Table 2. Amino acid identities between Can112051-06 and prototype Saffold virus proteins*

Protein	% Identity
L	76.1
VP4	97.2
VP2	83.9
VP3	85.2
VP1	76.7
2A	95.8
2B	97.6
2C	96.6
3A	100
3B	95.0
3C	96.8
3D	97.0

*Can112051-06 GenBank accession no. AM922293; Saffold virus GenBank accession no. EF165067.

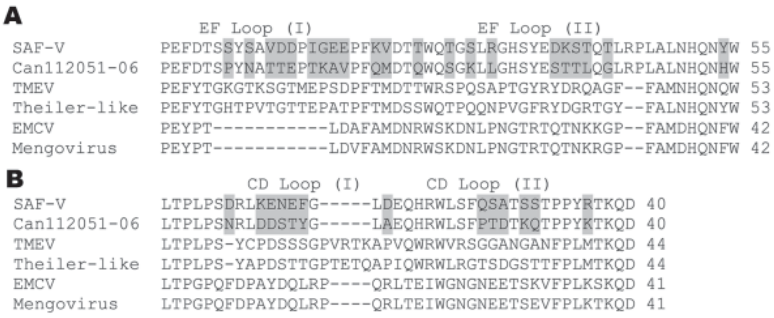

Figure 2. Comparison of amino acid sequences of the A) EF loop structure (part of the VP2 protein) and B) the CD loop structure (part of the VP1 protein) between Can112051-06 and other cardioviruses including Saffold virus (SAF-V), Theiler's murine encephalomyelitis virus (TMEV), Theiler-like virus, encephalomyocarditis virus (EMCV), and Mengovirus. Amino acid differences between Can112051-06 and SAF-V are shaded.

parison, the EF and CD loop structure sequences of EMCV and Mengovirus (2 members of the EMCV species) have 95.2% and 95.1% aa identities, respectively. The difference between time of isolation of SAF-V (1981) and the Can112051-06 strain (2006) is unlikely to be responsible for such a high level of sequence variation. We previously showed that the amino acid sequences of the VP0-VP1 capsid region of Canadian human parechovirus 1 strains isolated from 1985 through 2004 had 89.2% to 97.5% identities (*12*). Because the EF and CD loop structures are exposed on the viral surface of cardioviruses and thus constitute an important site for recognition by neutralizing antibodies (*15*), Can112051-06 and the original SAF-V might represent different serotypes, although further serologic studies are needed to confirm this hypothesis. The implication of the weak immunofluorescent signal seen in cardiovirus-infected cells stained with an enterovirus antibody is uncertain because of the considerable difference between the capsid proteins of cardioviruses and enteroviruses, which constitute 2 separate picornavirus genera.

In contrast to the initial recovery of this virus from a stool sample (*10*), our 3 strains were recovered from nasopharyngeal aspirate samples of children with fever and some other respiratory signs. The cardioviruses were the only pathogens identified in these samples. Whether SAF-V and the related Canadian strains described in this study should be classified as a new human *Cardiovirus* species or as a new clade within the *Theilovirus* species remains to be determined.

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References

1. King AMQ, Brown F, Christian P, Hovi T, Hyypia T, Knowles NJ, et al. Family *Picornaviridae*. In: Van Regenmortel MHV, Fauquet CM, Bishop DHL, Carstens EB, Estes MK, Lemon SM, et al., editors. Virus taxonomy. San Diego: Academic Press; 2000. p. 657–78.
2. Ohsawa K, Watanabe Y, Miyata H, Sato H. Genetic analysis of a Theiler-like virus isolated from rats. Comp Med. 2003;53:191–6.
3. Lipton HL, Friedman A, Sethi P, Crowther JR. Characterization of Vilyuisk virus as a picornavirus. J Med Virol. 1983;12:195–203.
4. Craighead JE, Huber SA, Haynes MK. Diverse patterns of immune and non-immune-mediated disease in EMC M-variant-infected mice. J Autoimmun. 1990;3:27–9.
5. Martin LR, Neal ZC, McBride MS, Pamenberg AC. Mengovirus and encephalomyocarditis virus poly(C) tract lengths can affect virus growth in murine cell culture. J Virol. 2000;74:3074–81.
6. Knowles NJ, Dickinson ND, Wilsden G, Carra E, Brocchi E, De Simone F. Molecular analysis of encephalomyocarditis viruses isolated from pigs and rodents in Italy. Virus Res. 1998;57:53–62.
7. Grobler DG, Raath JP, Braak LE, Keet DF, Gerdes GH, Barnard BJ, et al. An outbreak of encephalomyocarditis-virus infection in free-ranging African elephants in the Kruger National Park. Onderstepoort J Vet Res. 1995;62:97–108.
8. Emerson CL, Wagner JL. Antibody responses to two encephalomyocarditis virus vaccines in rhesus macaques (*Macaca mulatta*). J Med Primatol. 1996;25:42–5.
9. Kirkland PD, Gleeson AB, Hawkes RA, Naim HM, Broughton CR. Human infection with encephalomyocarditis virus in New South Wales. Med J Aust. 1989;151:176–8.
10. Jones MS, Lukashov VV, Ganac RD, Schnurr DP. Discovery of a novel human picornavirus in a stool sample from a pediatric patient presenting with fever of unknown origin. J Clin Microbiol. 2007;45:2144–50.
11. Boivin G, De Serres G, Cote S, Gilca R, Abed Y, Rochette L, et al. Human metapneumovirus infections in hospitalized children. Emerg Infect Dis. 2003;9:634–40.
12. Abed Y, Boivin G. Human parechovirus infections in Canada. Emerg Infect Dis. 2006;12:969–75.
13. Allander T, Tammi MT, Eriksson M, Bjerkner A, Tiveljung-Lindell A. Cloning of a human parvovirus by molecular screening of respiratory tract samples. Proc Natl Acad Sci U S A. 2005;102:12891–6.
14. Michiels T, Jarousse N, Brahic M. Analysis of the leader and capsid coding regions of persistent and neurovirulent strains of Theiler's virus. Virology. 1995;214:550–8.
15. Jnaoui K, Minet M, Michiels T. Mutations that affect the tropism of DA and GDVII strains of Theiler's virus in vitro influence sialic acid binding and pathogenicity. J Virol. 2002;76:8138–47.

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Bacteremia Caused by Group G Streptococci, Taiwan

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A retrospective observational study in Taiwan, 1998–2004, identified 92 patients with group G streptococcal bacteremia; 86 had *Streptococcus dysgalactiae* subspecies *equisimilis*. The most common diagnosis was cellulitis (48 cases), followed by primary bacteremia (34 cases). Infection recurred in 9 patients. Mortality rate was low (3.3%); resistance to quinupristin-dalfopristin was high.

Group G streptococci (GGS) are part of the normal microbial flora of the gastrointestinal tract, vagina, and skin and cause a variety of infections (*1*). Major underlying illnesses in patients with GGS bacteremia are malignancy, cardiovascular disease, diabetes mellitus, bone and joint diseases, and cirrhosis (*1*,*2*). Reported mortality rates for patients with GGS bacteremia also vary, ranging from 5% to 30% (*1*–*3*). Recent studies of β-hemolytic streptococci isolates carrying Lancefield group G antigen showed that they consist of *Streptococcus dysgalactiae* subspecies *equisimilis*, *S. anginosus*, and *S. canis* (*2*,*4*–*6*). To supplement the limited clinical information about bacteremia caused by GGS strains identified to the species level (*2*–*4*), we conducted a retrospective observational study.

The Study

We included all patients with GGS-positive blood cultures who had been treated from April 1998 through August 2004 at National Taiwan University Hospital, a 2,000-bed teaching hospital in northern Taiwan. We recorded demographic parameters, underlying illness, clinical diagnosis, and outcome for each patient. Clinical diagnosis was based on the attending physician's judgment and examination results. Recurrence of bacteremia was defined as repeated positive blood culture after complete treatment (at least 14 days) of previous bacteremia.

Differentiation of GGS was based on colony size, hemolytic reaction, Voges-Proskauer reaction, and β-glucuronidase activity. All β-hemolytic streptococci, whether large or small colonies, were tested for Lancefield group by using an agglutination kit (Streptex; Murex Biotech Ltd., Dartford, UK). PCR to differentiate between *S. anginosus* and *S. dysgalactiae* subsp. *equisimilis* was performed for all GGS isolates as described (*7*). For identification of *S. canis*, a probable isolate was identified by a negative β-glucuronidase result and further confirmed with the 16sRNA method as described (*8*). Susceptibilities of these isolates were tested by using the broth microdilution method as defined by the Clinical and Laboratory Standards Institute (formerly National Committee for Clinical Laboratory Standards) (*9*).

To determine the similarity of isolates in cases of recurrence, we used pulsed-field gel electrophoresis (PFGE) as described (*10*). The *emm* typing of isolates in cases of recurrence were also determined as described (*11*). The first 160 bases sequenced by emmseq2 that had ≥95% identity were defined as having the same genotype (*11*).

During the study period, 106 episodes of GGS bacteremia in 92 patients had been recorded; 56 episodes occurred during the first half of the study period (before June 2001) and 50 episodes during the second half. The causative agent was *S. dysgalactiae* subsp. *equisimilis* for 99 episodes, *S. anginosus* for 5, and *S. canis* for 2. Bacteremia recurred for 9 patients (1 had 4 episodes, and 3 had 3 episodes); bacteremia was nosocomial for 7 patients and polymicrobial for 5. The clinical characteristics of the patients are summarized in Table 1. All 3 patients who died had a diagnosis of the primary bacteremia caused by S**.** *dysgalactiae* subsp. *equisimilis*.

Among the 9 patients with recurrent bacteremia, the causative agent was *S. dysgalactiae* subsp. *equisimilis* for 8 and *S. canis* for 1. PFGE performed with all 13 available isolates from recurrent cases showed that 10 were identical to that of the initial episode, including 1 in a patient with recurrence of *S. canis* bacteremia. Sequence typing showed *emm* type stG485 for 4 patients. The clinical characteristics of the patients and *emm* typing results are shown in Table 2; PFGE results are shown in the Figure. The underlying diseases of patients with recurrent episodes included genital cancer (4 [44.4%] patients) and history of cellulitis (6 [66.7%]), each of which was significantly correlated with the likelihood of recurrence ($p<0.01$ for each). Further analysis showed that a previous history of cellulitis was significantly correlated with female sex ($p = 0.01$), genital cancer ($p<0.01$), tissue edema ($p = 0.02$), heart disease ($p = 0.04$), and post–coronary artery bypass graft ($p = 0.03$).

Bacteremia caused by β-hemolytic *S. anginosus* with group G antigen was identified for 5 patients, none of whom had cellulitis, compared with 48 (55.8%) of the 86 patients with *S. dysgalactiae* subsp. *equisimilis* who did have cellulitis ($p = 0.03$). Polymicrobial bacteremia and nosocomial bacteremia were found in a higher percentage of patients with *S. anginosus* (60% and 40.0%, respectively) than of patients with *S. dysgalactiae* subsp. *equisimilis* bacteremia

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Table 1. Clinical characteristic of 92 patients with group G streptococcal bacteremia, April 1998–August 2004, Taiwan

Characteristic	No. (%) patients
Age, y	
<10	1 (1.1)
10–50	12 (13.0)
51–75	68 (73.9)
>75	11 (12.0)
Median (range)	72 (10–93)
Sex	
Male	58 (63.0)
Female	34 (37.0)
Underlying diseases	
Malignancy	35 (38.0)
Genital	10 (10.9)
Head and neck	8 (8.7)
Gastrointestinal	6 (6.5)
Hematologic	3 (3.3)
Tissue edema	25 (27.2)
Heart disease	20 (21.7)
Post–coronary artery bypass graft	6 (6.5)
Diabetes mellitus	16 (17.4)
Central nervous system disease	15 (16.3)
Liver cirrhosis	9 (9.8)
Chronic renal disease	8 (8.7)
Chronic lung disease	6 (6.5)
Bone disease	5 (5.4)
Deep venous thrombosis	2 (2.2)
Type of infection	
Cellulitis	48 (52.1)*
Primary bacteremia	34 (36.9)
Deep-seated abscess	4 (4.2)†
Neutropenia and fever	3 (3.3)
Septic arthritis	2 (2.2)
Urinary tract infection	1 (1.1)
Infective endocarditis	1 (1.1)
Pneumonia	1 (1.1)
Initial findings	
Fever	86 (93.5)
Leukocytosis (>10,000 cells/μL)	34 (37.0)
Septic shock	4 (5.4)
Outcome	
Death	3 (3.3)
Recurrence of bacteremia	9 (9.8)

*Includes 2 patients who also had septic arthritis.
†Includes 2 patients with psoas muscle abscess, 1 with epidural abscess, and 1 with deep neck infection.

(4.7% and 5.8%, respectively); $p<0.01$ and $p = 0.02$, respectively. The 1 patient with *S. canis* bacteremia was a 33-year-old man with no history of dog bite. He had alcohol-associated liver cirrhosis of Child C (severe) classification and leg edema. He had 2 episodes of *S. canis* bacteremia 1 month apart. Echocardiogram results showed no evidence of valvular vegetation. For the first episode, the patient received a 14-day course of cefotaxime.

Antimicrobial drug–susceptibility testing showed decreased susceptibility to only macrolides (susceptibity rates: azithromycin 67.4%, clarithromycin 73.9%), clindamycin (87.0%), and quinupristin-dalfopristin (33.7%) (online Appendix Table, available from www.cdc.gov/EID/content/14/5/837-appT.htm). No clinical factor correlated with macrolide resistance. All isolates of recurrent bacteremia were susceptible to macrolides.

Conclusions

We documented 5 cases of primary bacteremia caused by β-hemolytic group G *S. anginosus* and unintentionally documented recurrence of *S. canis* bacteremia. *S. canis* bacteremia in humans was first clearly described in 1997 (*12*).

Our finding of 5 β-hemolytic *S. anginosus* isolates and 1 *S. canis* isolate in patients with GGS bacteremia in this study differs from findings of previous studies (*2*,*3*). Factors that may have contributed to this discrepancy include serotype determination and PCR method. Serotype determination was performed for all β-hemolytic streptococci isolated in our hospital, whether colonies were large or small, which might have led to the detection of more streptococcal isolates with G antigen. The PCR method developed in our hospital and used in this study could effectively differentiate *S. anginosus* from *S. dysgalactiae* subsp. *equisimilis* (*7*).

Information about clinical infection with *S. milleri* with group G antigen is limited (*4*). In a previous study of GGS bacteremia, Cohen-Poradosu et al. reported that 6 of 84 patients had recurrence of bacteremia (*3*). We found recurrence in 9 of the 92 patients. Risk factors were similar to those previously reported for non–group A streptococcal cellulitis (*13*). PFGE of these isolates showed that a high percentage of recurrence was caused by identical strains. Although Cohen-Poradosu et al. reported that *emm* type stG840 was the most common strain (*3*), we found *emm* type stG485 to be most common.

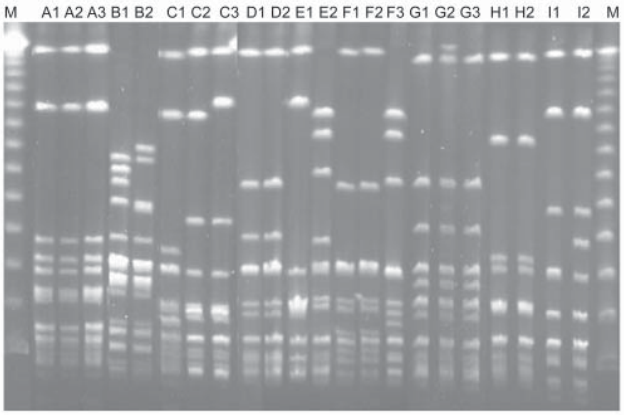

Figure. Pulsed-field gel electrophoresis profiles of all isolates from patients with recurrent group G streptococcal bacteremia. Isolates B1 and B2, *Streptococcus canis;* other isolates, *S. dysgalactiae* subsp. *equisimilis* (see designation of the isolates in Table 2). Lane M, molecular mass marker

Table 2. Summary of characteristics of patients with recurrence of group G streptococcus bacteremia, April 1998–August 2004, Taiwan*

Patient no.	Age, y/ sex	Isolate	Date of isolation	Underlying disease	Clinical diagnosis	*emm* type	PFGE pattern
1	67/F	A1	2001 May 28	Coronary heart disease, post–coronary artery bypass graft	Cellulitis	stG166b	–
		A2	2002 Jul 18		Cellulitis	stG166b	Identical
		A3	2003 Oct 14		Cellulitis	stG166b	Identical
2	33/M	B1†	2002 Nov 13	Alcoholic liver cirrhosis, child C	Primary bacteremia	STL1929.1	–
		B2†	2002 Oct 15		Primary bacteremia	STL1929.1	Identical
3	47/F	C1	1998 May 15	Vulvar cancer after surgery and radiotherapy	Cellulitis	stG166b	–
		C2	2002 Jan 18		Cellulitis	stG6.1	Related
		C3	2002 Dec 19		Cellulitis	stG6.1	Identical
4	49/M	D1	2000 May 24	Nasopharyngeal carcinoma after chemotherapy and radiotherapy	Cellulitis	stG485	–
		D2	2000 Aug 9		Cellulitis	stG485	Identical
5	28/M	E1	1998 Dec 26	von Willebrand disease, type I	Cellulitis	stG485	–
		E2	1999 Aug 28		Cellulitis	stG840	Different
6	72/F	F1	1998 Aug 24	Cervical cancer after surgery and radiotherapy, diabetes mellitus	Cellulitis	stG485	–
		F2	1998 Oct 23		Cellulitis	stG485	Identical
		F3	1999 Dec 3		Cellulitis	stG840	Different
7	55/F	G1	1999 Oct 9	Cervical cancer after surgery and radiotherapy	Cellulitis	stG485	–
		G2	2000 Apr 18		Cellulitis	stG485	Identical
		G3	2001 Sep 24		Cellulitis	stG485	Identical
		NA	2000 Jul 19		Cellulitis	NA	NA
8	46/M	H1	2001 Aug 21	Acute myeloid leukemia (M4)	Primary bacteremia	stGLP 1.0	–
		H2	2001 Sep 6		Primary bacteremia	stGLP 1.0	Identical
9	80/F	I1	2003 May 5	Cervical cancer with lung metastasis and obstructive uropathy	Primary bacteremia	stG245.0	–
		I2	2003 Nov 17		Primary bacteremia	stG245.0	Identical

*PFGE, pulsed-field gel electrophoresis; NA, not available.
†*Streptoccus canis*.

For years in Taiwan, macrolide resistance of streptococci has been a major health problem (*14*,*15*). A previous study found erythromycin resistance in 23.5% of GGS strains (*14*). Although we did not test for erythromycin resistance, we found some resistance even to new macrolides. Since restriction of macrolide use in Taiwan, a linear relationship has been noted between the decline in erythromycin use and the decline in erythromycin resistance in *S. pyogenes* (*15*). Our study, however, found no decline in macrolide resistance from first half of the study period (27.1%) to the second half (37.0%).

In summary, in our study, infection with *S. dysgalactiae* subsp. *equisimilis* was the most common cause of GGS bacteremia. Infection recurred for ≈10%. The mortality rate for patients with GGS bacteremia was relatively low (<10%), but resistance to quinupristin-dalfopristin was extremely high.

Dr Liao is an infectious diseases specialist in the Department of Internal Medicine, Far-Eastern Memorial Hospital. His major research interests are clinical and epidemiologic studies and pathogenesis of gram-positive bacterial infections, particularly streptococcal and methicillin-resistant *Staphylococcus aureus* infections.

References

1. Auckenthaler R, Hermans PE, Washington JA II. Group G streptococcal bacteremia: clinical study and review of the literature. Rev Infect Dis. 1983;5:196–204.
2. Woo PC, Fung AM, Lau SK, Wong SS, Yuen KY. Group G beta-hemolytic streptococcal bacteremia characterized by 16S ribosomal RNA gene sequencing. J Clin Microbiol. 2001;39:3147–55.
3. Cohen-Poradosu R, Jaffe J, Lavi D, Grisariu-Greenzaid S, Nir-Paz R, Valinsky L, et al. Group G streptococcal bacteremia in Jerusalem. Emerg Infect Dis. 2004;10:1455–60.
4. Facklam R. What happened to the streptococci: overview of taxonomic and nomenclature changes. Clin Microbiol Rev. 2002;15: 613–30.
5. Vandamme P, Pot B, Falsen E, Kersters K, Devriese LA. Taxonomic study of Lancefield streptococcal groups C, G, and L (*Streptococcus dysgalactiae*) and proposal of *S. dysgalactiae* subsp. *equisimilis* subsp. nov. Int J Syst Bacteriol. 1996;46:774–81.
6. Lawrence J, Yajko DM, Hadley WK. Incidence and characterization of beta-hemolytic *Streptococcus milleri* and differentiation from *S. pyogenes* (group A), *S. equisimilis* (group C), and large-colony group G streptococci. J Clin Microbiol. 1985;22:772–7.
7. Liu LC, Tsai JC, Hsueh PR, Teng LJ. Rapid differentiation between members of the *anginosus* group and *Streptococcus dysgalactiae* subsp. *equisimilis* within beta-hemolytic group C and G streptococci by PCR. J Clin Microbiol. 2006;44:1836–8.
8. Whatmore AM, Engler KH, Gudmundsdottir G, Efstratiou A. Identification of isolates of *Streptococcus canis* infecting humans. J Clin Microbiol. 2001;39:4196–9.
9. National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A6. 5th ed. Wayne (PA): The Committee, 2004.

10. Hsueh PR, Teng LJ, Lee LN, Yang PC, Ho SW, Luh KT. Dissemination of high-level penicillin–, extended-spectrum cephalosporin–, and erythromycin–resistant *Streptococcus pneumoniae* clones in Taiwan. J Clin Microbiol. 1999;37:221–4.
11. Facklam R, Beall B, Efstratiou A, Fischetti V, Johnson D, Kaplan E, et al. *emm* typing and validation of provisional M types for group A streptococci. Emerg Infect Dis. 1999;5:247–53.
12. Bert F, Lambert-Zechovsky N. Septicemia caused by *Streptococcus canis* in a human. J Clin Microbiol. 1997;35:777–9.
13. Baddour LM, Bisno AL. Non-group A beta-hemolytic streptococcal cellulitis. Association with venous and lymphatic compromise. Am J Med. 1985;79:155–9.
14. Wu JJ, Lin KY, Hsueh PR, Liu JW, Pan HI, Sheu SM. High incidence of erythromycin-resistant streptococci in Taiwan. Antimicrob Agents Chemother. 1997;41:844–6.
15. Hsueh PR, Shyr JM, Wu JJ. Changes in macrolide resistance among respiratory pathogens after decreased erythromycin consumption in Taiwan. Clin Microbiol Infect. 2006;12:296–8.

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Unique *Cryptosporidium* Population in HIV-Infected Persons, Jamaica

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A cryptosporidiosis survey showed the presence of *Cryptosporidium hominis*, *C. parvum, C. canis*, and *C. felis* in 25, 7, 1, and 1 HIV-positive persons from Jamaica, respectively; 1 person had both *C. hominis* and *C. felis*. Multilocus sequence typing indicated the presence of a homogeneous but geographically distinct *C. hominis* population in Jamaica.

Cryptosporidiosis is endemic to most tropical countries including Jamaica (*1*). Some evidence suggests that clinical manifestations of cryptosporidiosis may vary according to *Cryptosporidium* species and subtypes (*2*,*3*). Differentiating the species and subtypes with an aim of understanding the transmission dynamics of the parasites in disease-endemic areas requires the use of high resolution molecular tools (*4*). So far, few studies have reported on the molecular epidemiology of cryptosporidiosis in the Caribbean region, and only 2 small-scale studies on *Cryptosporidium* spp. in HIV-infected persons in Haiti have been conducted (*5*,*6*). In this preliminary study, genotyping and multilocus sequence typing (MLST) techniques were used to investigate the transmission of cryptosporidiosis among HIV-infected patients in Jamaica.

The Study

Stool specimens were obtained from HIV-infected adults in Kingston, Jamaica, as part of routine parasitologic diagnosis. All patient identifiers were removed before specimen acquisition. A total of 35 *Cryptosporidium*-positive stool specimens were collected from May 2003 through July 2007. Only 1 specimen per patient was included in the study. Specimens were collected from multiple hospitals in Kingston; some of the patients were from outlying areas who came to Kingston for medical care. Specimens were stored in 2.5% potassium dichromate at 4°C before analysis. Thereafter, DNA was extracted by using the FastDNA Spin Kit for Soil (Qbiogene Inc., Carlsbad, CA, USA); *Cryptosporidium* spp. were identified on the basis of PCR–restriction fragment length polymorphism analysis of the small subunit ribosomal RNA gene as described (*7*). Identification of *C. hominis* and *C. parvum* subtypes was based on sequence polymorphism at the 60-kDa glycoprotein gene (GP60) locus by using the nomenclature described (*8*). Nineteen of the 25 *C. hominis*–positive specimens and all 7 *C. parvum*–positive specimens were analyzed by MLST. The typing targeted 5 additional loci, including the 47-kDa protein (CP47 microsatellite), a serine repeat antigen (MSC6–7 minisatellite), a hypothetical retinitis pigmentosa GTPase regulator (RPGR minisatellite), and a hydroxyproline-rich glycoprotein (DZHRGP minisatellite and microsatellite) in chromosome 6 and the 70-kDa heat shock protein (HSP70 minisatellite) in chromosome 2. Nomenclature and classification of subtypes at these 5 loci have been described (*9*).

Genotype analysis of the 35 *Cryptosporidium*–positive specimens showed that 25 had *C. hominis*, 7 had *C. parvum*, 1 had *C. canis*, 1 had *C. felis,* and 1 had both *C. hominis* and *C. felis*. Initial subtyping of *C. hominis* and *C. parvum* used sequence analysis of the GP60 gene. Most (22) *C. hominis* specimens had subtype IbA10G2; 3 had subtype IeA12G3T3. All 7 *C. parvum* specimens belonged to subtype IIcA5G3d, which was identical to *C. parvum* identified in children in South Africa (GenBank accession no. AF440636).

Results of the MLST analysis of the additional 5 loci showed limited gene diversity in *C. hominis*. At the CP47 locus, all 19 specimens analyzed belonged to subtype IA39G22. Most of the other loci were also monomorphic, and subtypes at these loci were identified by amplicon sizes. All specimens were 590 bp at the DZHRGP locus, 358 bp at the RPGR locus, and 1,095 bp at the HSP70 locus. At the MSC6–7 locus, 18 of the 19 *C. hominis* specimens analyzed were 494 bp; 1 was 509 bp.

Diversity was more pronounced in the MLST analysis of *C. parvum,* although all specimens belonged to IIcA5G3d subtype at the GP60 locus. There were 3 subtypes at the DZHRGP locus. Subtype 1 was 496 bp and was found in 4 specimens. Subtype 2 was also 496 bp but had 1 single nucleotide polymorphism at position 314; this subtype was seen in 2 specimens. Subtype 3 was 499 bp and was found in 1 specimen. The HSP70 locus had 2 subtypes, differentiated by the presence of a single nucleotide polymorphism (a change of C to T at position 172), with 6 of the 7 specimens in 1 subtype. All *C. parvum* specimens were monomorphic at the CP47, MSC6–7, and RPGR loci. Results of the *C. hominis* and *C. parvum* subtyping at the 5 loci are shown in the Table.

To measure the degree of heterogeneity of *C. hominis* and *C. parvum*, an MLST analysis was conducted to

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Table. Subtypes of *Cryptosporidium hominis* and *C. parvum* at 6 loci identified in specimens from Jamaica*

GP60	CP47	MSC6-7	DZHR-GP	RPGR	HSP70
Cryptosporidium hominis, n = 25 (19 used in MLST)					
IbA10G2 (22)	IA39G22 (19)	494 bp (18)	590 bp (19)	358 bp (19)	1095 bp (19)
IeA12G3T3 (3)		509 bp (1)			
C. parvum, n = 7 (7 used in MLST)					
IIcA5G3d (7)	IIA24G11C1 (7)	461 (7)	496a bp (4)	391 bp (7)	1059a bp (6)
			496b bp (2)		1059b bp (1)
			499 bp (1)		

*Numbers in parentheses show the frequency of subtypes at each locus. MLST, multilocus sequence typing.

compare MLST types from Jamaica with those from other geographic areas of similar GP60 subtype families Ib, Ie and IIc (Kenya, Peru, India, and USA). All sequences for each specimen at the 5 loci were concatenated and multilocus subtypes generated. The analysis showed *C. hominis* multilocus subtypes from Jamaica clustered in 1 group, regardless of their GP60 subtype designation. Specimens of the same GP60 subtypes from other regions clustered in clades separate from the Jamaica specimens, forming largely distinct monophyletic groups defined by geographic origin. Specimens from Kenya and India clustered in 1 clade because of extensive human migration between the 2 countries. Although all *C. parvum* specimens from Jamaica were identified as IIcA5G3d, 4 MLST types were present, highlighting the extent of genetic diversity of *C. parvum* in this study. The relationship of the multilocus subtypes based on the 5 loci inferred by neighbor-joining analysis is shown in the Figure.

Conclusions

Results of our study show that anthroponotic transmission is important in the epidemiology of cryptosporidiosis in Jamaica. This conclusion is based on our finding of *C. hominis* and anthroponotic *C. parvum* (the IIc subtype family) in 33 (94%) of 35 specimens analyzed. Because all *C. parvum*–positive specimens belonged to the anthroponotic subtype family IIc and because 1 of the *C. felis*–infected patients had concurrent infection with *C. hominis*, it is possible that some of the *C. canis* and *C. felis* infections seen in this study were transmitted through anthroponotic pathways.

Results of the GP60 subtyping showed only 2 *C. hominis* subtype families (Ib and Ie); most belonged to the IbA10G2 subtype. Although the sample size is small, homogeneity within *C. hominis* at the GP60 locus is unusual, as common subtype families such as Ia and Id are usually equally abundant in most developing countries (*2*,*10*,*11*). Only some industrialized nations, such as Portugal and the United Kingdom, are known to have limited heterogeneity in *C. hominis* infections in humans (*12*,*13*). The GP60 subtype IbA10G2 identified here is identical to that previously reported in the United States, the United Kingdom, Portugal, South Africa, and Peru. Another Ib subtype, IbA9G3, which was commonly reported in India, Malawi, and Australia (*4*,*8*,*10*–*12*), was not seen in the specimens from Jamaica. Likewise, most Ie infections in humans are caused by IeA11G3T3, but our study identified a less common subtype, IeA12G3T3, which was also found in specimens from New Orleans (USA) and Australia (*4*). The limited num-

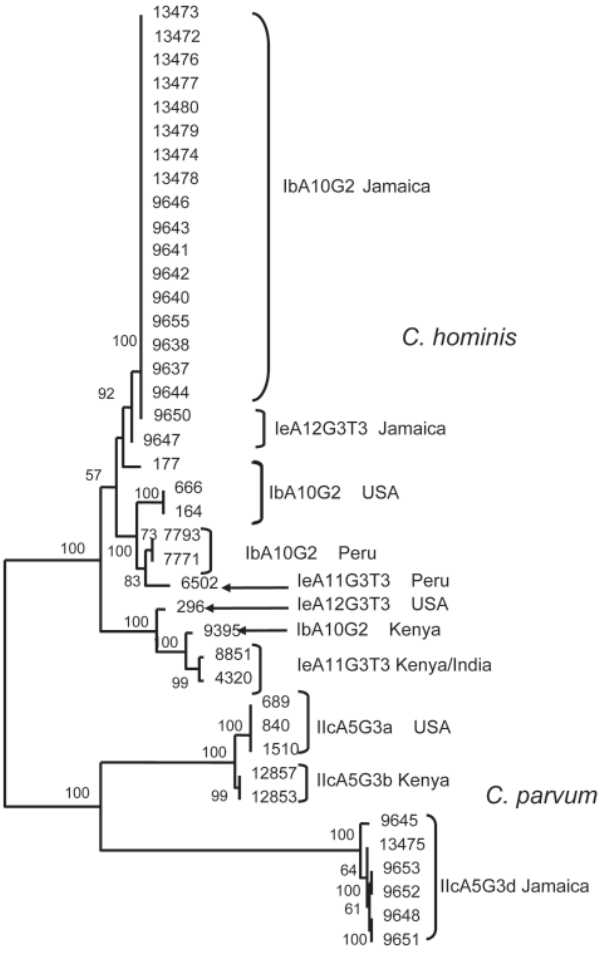

Figure. Relationships among *Cryptosporidium hominis* and *C. parvum* multilocus sequence subtypes at 5 genetic loci. Parasite population from Jamaica was compared with that from other regions by neighbor-joining analysis of concatenated sequence of 5 genetic loci, by using GP60 subtype identification in specimen selection. The Kimura 2-parameter model was used in the distance calculations. The sequences reported in this paper are available in the GenBank database under accession nos. EU141710–EU141727.

ber of GP60 subtypes would suggest an epidemic mode of transmission. The extended period of specimen collections and the identification of other *Cryptosporidium* species do not indicate that a cryptosporidiosis outbreak occurred during this long study period.

All *C. hominis* specimens from Jamaica, irrespective of the GP60 subtype (IbA10G2 or IeA12G3T3), belonged mostly to 1 MLST group distinct from other subtypes from other geographic regions. These results suggest that a unique *C. hominis* parasite population is being transmitted in Jamaica. Heterogeneity was higher in the *C. parvum* population despite the smaller number of specimens that we analyzed. As with *C. hominis*, a unique population of *C. parvum* in Jamaica also seems to exist. These results further show the distinct population of *Cryptosporidium* spp. that can arise because of geographic segregation. When one considers that all *C. parvum* specimens belonged to only 1 GP60 subtype, whether the high diversity seen with the MLST is due to recent expansion of an ancestral type in the region is not clear. A study of population genetics of *Cryptosporidium* spp. from humans in Haiti showed limited multilocus subtypes in both *C. hominis* and *C. parvum*, which was interpreted as an indication for an epidemic clonal population for both species (*6*).

The occurrence of unique multilocus subtypes in both *C. hominis* and *C. parvum* populations in Jamaica that can be differentiated from other geographic regions, including the nearby United States, is notable and requires further investigations. The distinction is important in mapping the transmission of the parasite, especially where tracking infection pathways is necessary, such as in investigations of outbreaks and traveler's diarrhea. The fact that *C. hominis* from different areas may have an identical GP60 subtype but a different MLST subtype is also noteworthy, as some *C. hominis* subtypes, such as IbA10G2, are commonly associated with water-borne outbreaks in industrialized countries. The clinical manifestations of the GP60 subtypes in relation to the distinct MLST from different geographic areas needs to be studied further. This would clarify how geographic segregation in *Cryptosporidium* MLST subtypes relates to GP60 subtypes that have various pathogenicities.

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Dr Gatei is a postdoctoral research scientist at the Centers for Disease Control and Prevention. Her research interests primarily concern molecular epidemiology of enteric pathogens, especially *Cryptosporidium.*

References

1. Lindo JF, Levy VA, Baum MK, Palmer CJ. Epidemiology of giardiasis and cryptosporidiosis in Jamaica. Am J Trop Med Hyg. 1998;59:717–21.
2. Cama VA, Ross JM, Crawford S, Kawai V, Chavez-Valdez R, Vargas D, et al. Differences in clinical manifestations among *Cryptosporidium* species and subtypes in HIV-infected persons. J Infect Dis. 2007;196:684–91.
3. Matos O, Alves M, Xiao L, Cama V, Antunes F. *Cryptosporidium felis* and *C. meleagridis* in persons with HIV, Portugal. Emerg Infect Dis. 2004;10:2256–7.
4. Chalmers RM, Ferguson C, Caccio S, Gasser RB, Abs ELOYG, Heijnen L, et al. Direct comparison of selected methods for genetic categorisation of *Cryptosporidium parvum* and *Cryptosporidium hominis* species. Int J Parasitol. 2005;35:397–410.
5. Raccurt CP, Brasseur P, Verdier RI, Li X, Eyma E, Stockman CP, et al. Human cryptosporidiosis and *Cryptosporidium* spp. in Haiti [in French]. Trop Med Int Health. 2006;11:929–34.
6. Ngouanesavanh T, Guyot K, Certad G, Le Fichoux Y, Chartier C, Verdier RI, et al. *Cryptosporidium* population genetics: evidence of clonality in isolates from France and Haiti. J Eukaryot Microbiol. 2006;53:S33–6.
7. Jiang J, Alderisio KA, Singh A, Xiao L. Development of procedures for direct extraction of *Cryptosporidium* DNA from water concentrates and for relief of PCR inhibitors. Appl Environ Microbiol. 2005;71:1135–41.
8. Sulaiman IM, Hira PR, Zhou L, Al-Ali FM, Al-Shelahi FA, Shweiki HM, et al. Unique endemicity of cryptosporidiosis in children in Kuwait. J Clin Microbiol. 2005;43:2805–9.
9. Gatei W, Hart CA, Gilman RH, Das P, Cama V, Xiao L. Development of a multilocus sequence typing tool for *Cryptosporidium hominis.* J Eukaryot Microbiol. 2006;53:S43–8.
10. Gatei W, Das P, Dutta P, Sen A, Cama V, Lal AA, et al. Multilocus sequence typing and genetic structure of *Cryptosporidium hominis* from children in Kolkata, India. Infect Genet Evol. 2007;7:197–205.
11. Peng MM, Meshnick SR, Cunliffe NA, Thindwa BD, Hart CA, Broadhead RL, et al. Molecular epidemiology of cryptosporidiosis in children in Malawi. J Eukaryot Microbiol. 2003;50:557–9.
12. Hunter PR, Hadfield SJ, Wilkinson D, Lake IR, Harrison FC, Chalmers RM. Subtypes of *Cryptosporidium parvum* in humans and disease risk. Emerg Infect Dis. 2007;13:82–8.
13. Alves M, Xiao L, Sulaiman I, Lal AA, Matos O, Antunes F. Subgenotype analysis of *Cryptosporidium* isolates from humans, cattle, and zoo ruminants in Portugal. J Clin Microbiol. 2003;41:2744–7.

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Human Astrovirus Gastroenteritis in Children, Madagascar, 2004–2005

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We report data regarding the molecular epidemiology of human astrovirus (HAstV) infections among children in Madagascar. In a 13-month study, 5 HAstV isolates were detected in fecal samples from 237 children (2.1%) by reverse transcription–PCR. Phylogenetic analysis showed the cocirculation of usual and unusual HAstVs.

Human astroviruses (HAstVs) belong to the *Astroviridae* family, which is divided into 2 genera: *Mamastrovirus* (mammalian viruses) and *Avastrovirus* (avian viruses) (*1*). They are single-stranded positive-sense RNA viruses with a characteristic 5- or 6-pointed star, which has an electron-dense center when viewed by negative-stain electron microscopy. Their genome is 6.8–7.3 kb in length and includes 3 overlapping open reading frames (ORFs) (*2*). ORF1a and ORF1b encode nonstructural proteins (serine protease and RNA polymerase, respectively). ORF2 encodes the capsid precursor. There are 8 serotypes of HAstVs; type 1 is the most prevalent worldwide (*3*).

Molecular assays such as reverse transcription–PCR (RT-PCR) and molecular typing methods have advanced our understanding of HAstV epidemiology. Today, astroviruses, along with rotaviruses and caliciviruses, are considered important viral agents of acute pediatric gastroenteritis (*2*). These viruses have been associated with endemic diarrheal episodes and outbreaks of gastroenteritis in industrialized (*4*,*5*) and nonindustrialized countries (*6*,*7*). Although Madagascar is a society of considerable diversity, no studies have yet been reported on the prevalence and molecular epidemiology of HAstV among children with acute gastroenteritis in the country.

The Study

From May 2004 through May 2005, a study of acute gastroenteritis in children (newborn to 16 years) was undertaken by Institut Pasteur, Antananarivo, Madagascar. This study was approved by the Ethical Review Board of Institute Pasteur, Madagascar, and the National Ethical Committee of Madagascar. It was funded internally by the University of Liverpool. Antananarivo is the capital city of Madagascar with a population of ≈4 million persons. Fecal samples were collected from children with acute dehydrating gastroenteritis who had been brought to the rehydration clinics and hospitals of Antananarivo for treatment; samples were stored at –80°C until they were analyzed at the University of Liverpool, United Kingdom.

All samples were screened by RT-PCR for astrovirus and norovirus and by ELISA with subsequent genotyping for rotavirus, as described (*8*–*11*). In brief, viral RNA was extracted from 150 μL of 10%–20% fecal suspensions in phosphate-buffered saline by using a guanidine and silica method (*12*) and reverse transcribed by using random hexamers (Sigma-Genosys, Dorset, UK). Primers Mon244/245 and Mon269/270 were then used to amplify a 413-bp and a 449-bp fragment of ORF2, respectively (*8*). If no amplicon was obtained, primers Mon 340/348 were used to amplify a 289-bp fragment of ORF1a. Amplification products were purified by using Minispin columns (Amersham, Buckinghamshire, UK) and sequenced by Cogenics Lark Technologies (Hope End, Essex, UK).

Phylogenetic relationships were examined by using the ClustalW multiple alignment program (EMBL, Heidelberg, Germany). Phylogenetic trees were constructed according to the neighbor-joining method with ClustalX (version 1.83); the alignment file obtained by analysis with ClustalW bootstrap values on a scale from 1 to 1,000 was also calculated. Unrooted phylograms of HAstV isolates from the present report and reference strains were plotted in the PHYLIP format output by using the TreeView software version 3.0 (http://taxonomy.zoology.gla.ac.uk/rod/treeview.html). Assignment of HAstV to genotype was done according to the scheme proposed by Belliot et al. (*9*). The nucleotide sequences of the Malagasy strains have been deposited at the GenBank database under accession nos. EF490425–EF490429 and EF519312.

During this 13-month study, 237 children (142 boys and 95 girls) were screened for HAstV infection. Overall, 85% of the children were <3 years, 77% were <2 years, 43% were <1 year, and 3% were newborns. The median age of the study population was 20 months (range 1 day–16 years); for children with astrovirus infection, the median age was 10 months (5–21 months). No infections were recorded among newborns.

No RT-PCR–confirmed isolate was detected by electron microscopy. No HAstVs were detected by using primers Mon269/270. One positive sample was found with primers Mon244/245. This sample and an additional 4 strains produced amplicons with primers Mon340/348.

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[1]Deceased.

Table. Detection of human astrovirus in fecal specimens from children of Antananarivo, Madagascar, 2004–2005*

Sample no.	ID no. (GenBank accession no.)	Sample date	Patient age, mo/sex	Norovirus PCR	Rotavirus PCR	Astrovirus PCR 340/348	Astrovirus PCR 244/245	Astrovirus PCR 269/270	Astrovirus Sequence typing ORF1a	Astrovirus Sequence typing ORF2
1	DT1004 (EF490429)	2004 Jun	12/M	Neg	G2P[4]	Pos	Neg	Neg	Type 1	–
2	DG6013 (EF490425)	2004 Jun	10/F	Neg	Neg	Pos	Neg	Neg	Type 3	–
3	DR0034 (EF490427)	2004 Nov	10/M	Neg	Neg	Pos	Neg	Neg	Type 8	–
4	DR0038 (EF490428, EF519312)	2004 Nov	5/M	Neg	G2P[4]	Pos	Pos	Neg	Type 8	Type 2
5	DR0075 (EF490426)	2005 Feb	21/M	Neg	Neg	Pos	Neg	Neg	Type 3	–

*ID, identification; ORF, open reading frame; Neg, negative; Pos, positive; –, not amplified.

Each isolate was typed successfully by nucleotide sequencing of either partial ORF1a or ORF2 products obtained by using primers Mon340/348 (n = 5) and Mon244/245 (n = 1), respectively (Table).

In total, 5 HAstVs were detected in 237 children (2.1%). HAstVs were detected throughout the year. Two co-infections with G2P[4] rotavirus were confirmed by RT-PCR. No co-infection with norovirus was found (Table). Sequence analysis of the ORF1a region showed that isolates from Madagascar clustered within the genogroup A strains as described by Belliot et al. (9). In particular, 1 strain (DT1004_Madagascar2004_EF490429) grouped with genotype 1, a total of 2 strains (DG6013_Madagascar2004_EF490425 and DR0075_Madagascar2005_EF490426) grouped with genotype 3, and notably, 2 strains (DR0034_Madagascar2004_EF490427 and

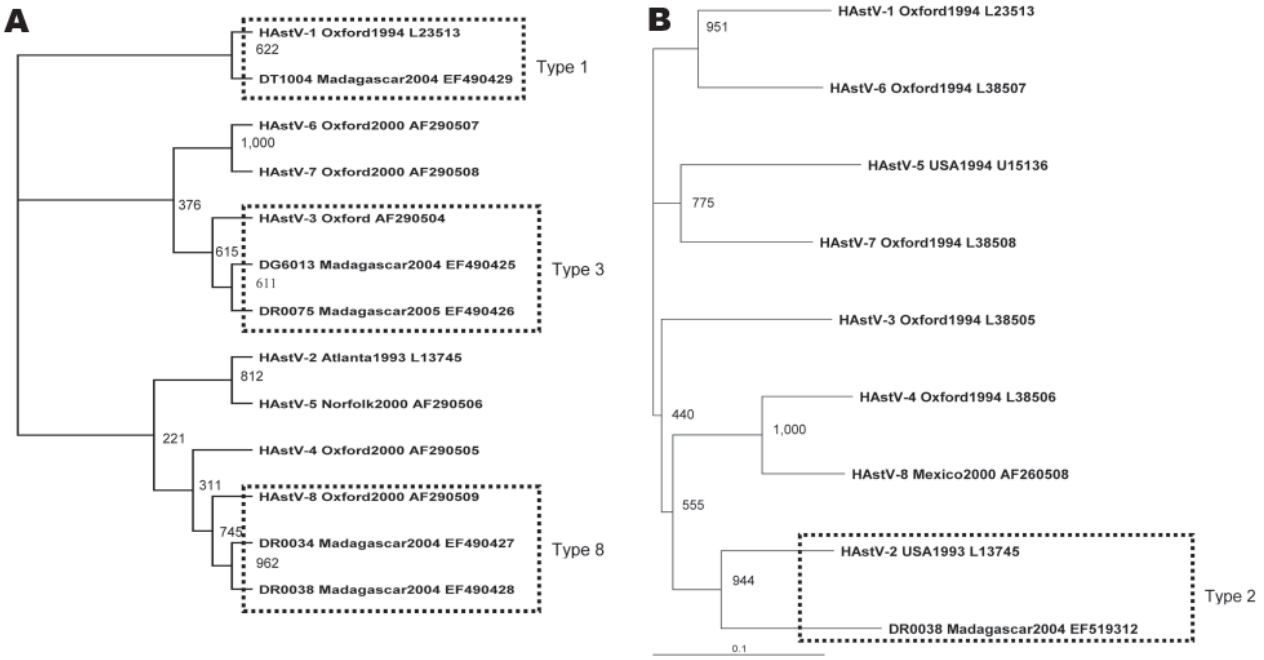

Figure. Phylogenetic tree of human astrovirus (HAstV) based on the 289-base region of the open reading frame (ORF) 1a gene (A) and the 413-base region of the ORF2 gene (B). We included 6 novel sequences designated according to isolate code_place/year_GenBank accession no. A) DG6013_Madagascar2004_EF490425, DR0075_Madagascar2005_EF490426, DR0034_Madagascar2004_EF490427, DR0038_Madagascar2004_EF490428, DT1004_Madagascar2004_EF490429; B) DR0038_Madagascar2004_EF519312. We also included 16 sequences of reference astrovirus strains obtained from GenBank, designated according to HAstV genotype_place/year_GenBank accession no.: A) HAstV-1_Oxford1994_ L23513, HAstV-2_Atlanta1993_L13745, HAstV-3_Oxford_AF290504, HAstV-4_Oxford2000_AF290505, HAstV-5_Norfolk2000_AF290506, HAstV-6_Oxford2000_AF290507, HAstV-7_Oxford2000_AF290508, HAstV-8_Oxford2000_AF290509; B) HAstV-1_Oxford1994_L23513, HAstV-2_USA1993_ L13745, HAstV-3_Oxford1994_L38505, HAstV-4_Oxford1994_L38506, HAstV-5_USA1994_U15136, HAstV-6_Oxford1994_L38507, HAstV-7_Oxford1994_L38508, HAstV-8_Mexico2000_AF260508. Bootstrap values based on 1,000 generated trees are displayed at the nodes.

DR0038_Madagascar2004_EF490428) grouped with the emerging genotype 8. Phylogenetic analysis of the ORF2 region showed that the 1 amplifiable isolate from Madagascar (DR0038_Madagascar2004_EF519312), which was clustered as genotype 8 at the ORF1a region, could now be classified as genotype 2 (Figure).

Conclusions

This study systematically examined the role of HAstV in acute gastroenteritis in Madagascar. In a 13-month study, we detected HAstV by RT-PCR in 2.1% of children with gastroenteritis in Antananarivo. This finding establishes HAstV as the third most commonly detected enteric virus in this population, after rotavirus (38%) and norovirus (6%) (*11*). These findings agree with those of previous studies that reported astrovirus infection rates of 1.5% to as high as 26% (*13*,*14*).

The median age of children with HAstV infection was 10 months (range 5–21 months), equivalent to that of the rotavirus-infected group (median age 10 months; range 1 day to 48 months) but lower than that of the norovirus-infected group (median age 18 months, range 3 to 51 months) (*11*). HAstVs were detected in June and November 2004 and February 2005 (2 isolates during each of June and November 2004 and 1 isolate in February 2005). However, because of the small number of isolates detected, we could not determine whether the pattern of HAstV infections in Madagascar was seasonal.

Compared with electron microscopy, new molecular detection and typing methods have greatly enhanced our ability to study the endemic circulation of enteric viruses. Our data indicate that HAstVs are important agents of acute gastroenteritis among children <24 months in Madagascar and that simultaneous circulation of multiple astrovirus genotypes is not rare. Although HAstV-1 has been reported to be the most prevalent type detected worldwide, the presence of HAstV-8 among our isolates indicates the continued worldwide emergence of this unusual astrovirus strain. In addition, phylogenetic analysis of the serine protease and capsid regions of our HAstV strains provided contradictory genotyping results; RNA recombinations may have contributed to significant genomic rearrangements (*15*). Further analysis is needed to confirm our preliminary findings and to investigate the importance of HAstV infections among children in Madagascar.

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References

1. Matsui SM, Greenberg HB. Astroviruses. In: Fields BN, Knipe DM, Howley PM, editors. Virology. 4th ed. Philadelphia: Lippincott-Raven Press; 2001. p. 875–93.
2. Walter JE, Mitchell DK. Astrovirus infection in children. Curr Opin Infect Dis. 2003;16:247–53.
3. Guix S, Bosch A, Pinto RM. Human astrovirus diagnosis and typing: current and future prospects. Lett Appl Microbiol. 2005;41:103–5.
4. Schnagl RD, Belfrage K, Farrington R, Hutchinson K, Lewis V, Erlich J, et al. Incidence of human astrovirus in central Australia (1995 to 1998) and comparison of deduced serotypes detected from 1981 to 1998. J Clin Microbiol. 2002;40:4114–20.
5. Sakamoto T, Negishi H, Wang QH, Akihara S, Kim B, Nishimura S, et al. Molecular epidemiology of astroviruses in Japan from 1995 to 1998 by reverse transcription-polymerase chain reaction with serotype-specific primers (1 to 8). J Med Virol. 2000;61:326–31.
6. Bhattacharya R, Sahoo GC, Nayak MK, Ghosh S, Dutta P, Bhattacharya MK, et al. Molecular epidemiology of human astrovirus infections in Kolkata, India. Infect Genet Evol. 2006;6:425–35.
7. Cunliffe NA, Dove W, Gondwe JS, Thindwa BD, Greensill J, Holmes JL, et al. Detection and characterization of human astroviruses in children with acute gastroenteritis in Malawi. J Med Virol. 2002;67:563–6.
8. Noel JS, Lee TW, Kurtz JB, Glass RI, Monroe SS. Typing of human astroviruses from clinical isolates by enzyme immunoassay and nucleotide sequencing. J Clin Microbiol. 1995;33:797–801.
9. Belliot G, Laveran H, Monroe SS. Detection and genetic differentiation of human astroviruses: phylogenetic grouping varies by coding region. Arch Virol. 1997;142:1323–34.
10. Cunliffe NA, Dove W, Bunn JE, Ben Ramadan M, Nyangao JW, Riveron RL, et al. Expanding global distribution of rotavirus serotype G9: detection in Libya, Kenya, and Cuba. Emerg Infect Dis. 2001;7:890–2.
11. Papaventsis DC, Dove W, Cunliffe NA, Nakagomi O, Combe P, Grosjean P, et al. Human norovirus infection among children with acute gastroenteritis in Antananarivo, Madagascar, 2004–2005. Emerg Infect Dis. 2007;13:908–11.
12. Boom R, Sol CJ, Salimans MM, Jansen CL, Wertheim-Van Dillen PM, Van Der Noordaa J. Rapid and simple method for purification of nucleic acids. J Clin Microbiol. 1990;28:495–503.
13. Kang YH, Park YK, Ahn JB, Yeun JD, Jee YM. Identification of human astrovirus infections from stool samples with diarrhea in Korea. Arch Virol. 2002;147:1821–7.
14. Maldonado Y, Cantwell M, Old M, Hill D, Sanchez ML, Logan L, et al. Population-based prevalence of symptomatic and asymptomatic astrovirus infection in rural Mayan infants. J Infect Dis. 1998;178:334–9.
15. Walter JE, Briggs J, Guerrero ML, Matson DO, Pickering LK, Ruiz-Palacios G, et al. Molecular characterization of a novel recombinant strain of human astrovirus associated with gastroenteritis in children. Arch Virol. 2001;146:2357–67.

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The Mystery of Increased Hospitalizations of Elderly Patients

Martin I. Meltzer*

This issue of Emerging Infectious Diseases contains 2 articles that report increases in England in the incidence of hospitalizations for specific diseases, one on pneumonias (*1*) and the other on community-acquired staphylococcal diseases (*2*). Both articles report increases in disease among those >65 years of age.

Trotter et al. found a 20%–39% increase in pneumonia-related hospitalizations in persons >65 years from April 1997 through March 2005 (*1*). These researchers found that the older a person, the greater the risk for pneumonia-related hospitalization. Those older patients with coexisting conditions, as measured by a severity-of-illness scale (the Charlson Comorbidity Index), were also more likely to be hospitalized than those with no such recorded conditions. What is surprising, however, is that although the percentage of patients with moderate or severe coexisting conditions increased over time, the percentage who died within 30 days of admission decreased slightly. It is hard to think of an advance in medical science (e.g., a new "wonder drug") within the period studied that would explain the stability of a 30-day mortality rate when hospitalizations among the frail elderly are increasing.

Hayward et al. (*2*) measured a 6-fold increase in hospitalizations for staphylococcal pneumonia and an ≈4-fold increase in hospitalizations for abscesses or cellulitis for patients ≥65 years of age from 1989–90 through 2003–04, (*2*). While these increases may be real and actual, they should be placed in some context by moving the focus from relative increases to actual numbers. As reported by Hayward et al., comparing 1989–90 to 2003–04, the age-adjusted rate of admission for abscesses, carbuncles, furuncles and cellulitis (all 4 combined) increased from 500 to 1,488 per million general population. For staphylococcal pneumonia, the rates increased from 2 to 12 per million (*2*). The rate for pneumonia (primary diagnosis) rose from 1,480 (1997–98) to 1,980 per million in 2004–05 (*1*).

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These rates are not unexpected. In the United States in 2000, the rate of hospitalizations for staphylococcal diseases was ≈1,060 per million general population (*3*,*4*). Among those >65 years of age in 2002, the rate of hospitalizations for pneumonia (primary diagnosis) was equivalent to 2,100 per million all-ages general population (17,000/million ≥65 years) (*5*). (In the United States in 2005, there were 116,200 hospital discharges per million persons [*3*]. In 2000, a diagnosis of *S. aureus* infection was made in 9.13 of every 1,000 hospital discharges, or 0.913% [*4*].)

The rates and increases measured in England may seem large, but during the fiscal year 2004–05 the British National Health System recorded ≈12 million hospital "admission episodes," including 6.8 million operations in England (*6*), a rate of ≈240,000 admissions per million population. Thus, while hospital staffs are very likely to see cases of staphylococcal disease and pneumonia, they will also see a much larger combined volume of patients with other diseases and conditions.

Being very busy, hospital staff may not actually notice the increases reported by Hayward et al. and Trotter et al. Hidden or not, the reported results are real, and the incidence of hospitalizations for certain diseases has clearly risen. However, as both sets of authors carefully point out, we do not know the cause of such increases. Both studies used the same dataset: the United Kingdom's National Health Services hospital admissions database (Hospital Episodes Statistics). This is an administrative dataset, primarily designed to track data to allow administrators, politicians, and the public to gauge how well the healthcare system is responding to demand. For example, mean time waited until admitted to hospital is a key measure of the system's ability to meet demand (in 2004–05, the mean wait time was 84 days [*7*]).

Such administrative databases do not, typically, contain the type of data that allow detailed analyses of epidemiologic risk factors. Both Hayward's and Trotter's articles contain extensive discussion of factors that could influence the measured increases in rates of hospitalizations.

Until we understand what caused these increases, designing interventions to target the root causes of such increases will be challenging. Although the increases should cause concern, and although the phrase "more research is needed" is often hackneyed and self-serving, in this situation it is warranted.

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References

1. Trotter CL, Stuart JM, George R, Miller E. Increasing hospital admissions for pneumonia, England. Emerg Infect Dis. 2008;14:727–33.
2. Hayward A, Petersen I, Livermore DM, Duckworth G, Islam A, Knott F, et al. Increasing hospitalizations and general practice prescriptions for community-onset staphylococcal disease, England. Emerg Infect Dis. 2008;14:720–6.
3. National Center for Health Statistics. Health United States, 2007. Hyattsville (MD): The Center; 2007. p. 341.
4. Kuehnert MJ, Hill HA, Kupronis BA, Tokars JI, Solomon SL, Jernigan DB. Methicillin-resistant *Staphylococcus aureus* hospitalizations, United States. Emerg Infect Dis. 2005;11:868–72.
5. Fry AM, Shay DK, Holman RC, Curns AT, Anderson LJ. Trends in hospitalizations for pneumonia among persons aged 65 years or older in the United States, 1988–2002. JAMA. 2005;294:2712–9.
6. The Information Centre. Hospital Episode Statistics Online. Headline figures table: 2004–05 [cited 2008 Feb 17]. Available from http://www.hesonline.nhs.uk/Ease/servlet/ContentServer?siteID=1937&categoryID=193
7. The Information Centre. Hospital Episode Statistics Online. A decade in view [cited 2008 Feb 17]. Available from http://www.hesonline.nhs.uk/Ease/servlet/ContentServer?siteID=1937&categoryID=451

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Extensively Drug-Resistant Tuberculosis, Taiwan

To the Editor: In Taiwan, the incidence of tuberculosis (TB) was 74.1/100,000 population in 2004 and 72.7/100,000 in 2005; the mortality rate was 4.2/100,000 in 2004 and 4.3/100,000 in 2005 (*1*). Because of these high incidences and the increasing effects of multidrug-resistant TB (MDR TB), i.e., resistant to at least both isoniazid (INH) and rifampin (RIF), the laboratory-based Taiwan Surveillance of Drug Resistance in TB (TSDRTB) program was established in 2003 (*2*). Surveillance demonstrated that combined drug resistance rates were 11.3% (2004) and 10.1% (2005) for INH; 7.5% (2004) and 6.2% (2005) for RIF; 4.3% (2004) and 2.1% (2005) for ethambutol (EMB); 10.6% (2004) and 9.8% (2005) for streptomycin (SM); 20.4% (2004) and 18.1% (2005) for any first-line drug; and 5.3% (2004) and 4.0% (2005) for multidrug resistance. These resistance rates are higher than those reported by the third TB global drug resistance surveillance. Global surveillance reported median prevalence of combined drug resistance was 6.6% for INH, 2.2% for RIF, 1.3% for EMB, 6.1% for SM, 10.4% for any drug, and 1.7% for multidrug resistance. TB (*3*).

Extensively drug-resistant TB (XDR TB) was initially defined as an MDR isolate that was resistant to at least 3 of the 6 main classes of second-line drugs: aminoglycosides, polypeptides, fluoroquinolones, thioamides, cycloserine, and para-aminosalicylic acid (*4*). In October 2006, the World Health Organization (WHO) redefined XDR TB as an isolate "resistant to at least INH and RIF (i.e., MDR TB) plus resistant to at least 1 of the fluoroquinolones and 1 of the following 3 injectable drugs: capreomycin, kanamycin, and amikacin" (*5*). Clearly, XDR TB is a global threat and the demands on XDR TB surveillance systems are urgent.

Because no guidelines for drug susceptibility testing of second-line drugs existed in Taiwan before 2007, clinical mycobacteriology laboratories performed drug susceptibility testing of second-line drugs using the agar proportion method by clinicians' request only. Critical concentrations of second-line drugs for drug susceptibility testing were 2 μg/mL for ofloxacin, 6 μg/mL for kanamycin, 10 μg/mL for ethionamide, and 8 μg/mL for para-aminosalicylate. Of the 215 MDR isolates, 92 (42.8%), 35 (16.3%), 34 (15.8%), and 56 (26.0%) were resistant to fluoroquinolone, kanamycin, ethionamide, and para-aminosalicylate, respectively. Of the 116 MDR isolates tested for susceptibility to second-line drugs in 2004, 10.3% (12/116) were XDR TB; of the 99 MDR isolates tested in 2005, 10.1% (10/99) were XDR TB.

With their broad spectrum antimicrobial activity, fluoroquinolones are widely used for the treatment of bacterial respiratory infections in Taiwan. In addition, fluoroquinolones are the preferred oral agents for treating drug-resistant TB that is known or presumed to be sensitive to this class of drugs, or when first-line agents cannot be used because of intolerance (*6*). In contrast to injectable agents that have a higher incidence of renal and hearing impairment after long-term use, fluoroquinolones have high oral bioavailability, convenient dosing intervals, and a lower incidence of side effects (*7*). Therefore, despite TB treatment recommendations, some clinicians prescribe fluoroquinolones instead of injectable agents. A previous study by Yu et al. (*8*) showed that fluoroquinolone-resistant *Mycobacterium tuberculosis* isolates were rare among patients not previously exposed to fluoroquinolones; however, the increased rate of resistance to fluoroquinolones was observed among patients with MDR TB because of inadequate treatment regimens or poor compliance (*8*,*9*).

In this study, 215 MDR isolates were tested; among these, 42.8% (92/215) were fluoroquinolone-resistant, a much higher percentage than the 10.2% (22/215) that fulfilled the definition of XDR TB. Because the adequate use of fluoroquinolones for TB per WHO and national guidelines (either for intolerance or drug resistance) is important, the use of fluoroquinolones is strictly regulated by the National Health Insurance program. Since 2007, clinicians in Taiwan have been required to apply for these and second-line drugs through the Taiwan Centers for Disease Control (CDC) and to accept professional supervision in their administration. Furthermore, these drugs can only be given under the direct observed treatment program.

An outbreak in rural South Africa highlighted the risk of XDR TB for persons co-infected with HIV (*10*). The current Taiwan TB and HIV Register shows that <1% of TB patients are co-infected with HIV and documents no XDR TB patients who are co-infected with HIV. However, because persons co-infected with HIV and *M. tuberculosis* have the highest rates of progression to active disease, continued monitoring of co-infected patients is essential for control of TB.

The initial purpose of the TSDRTB program was to survey drug resistance of first-line anti-TB drugs in Taiwan. Therefore, our study data are limited; the rate of XDR TB among MDR TB may be an underestimate because we did not have adequate representative cases and methods. The present surveillance system does clearly show the emergence of XDR TB cases in Taiwan, which highlights the need to reinforce diagnosis and treatment strategies recommended by the National TB Control Program. In addition to the established TSDRTB program, Taiwan CDC started an enhanced population-based surveillance of MDR TB/XDR TB in 2007.

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References

1. Centers for Disease Control, Taiwan. Statistics of Communicable Diseases and Surveillance Report, Republic of China, 2004 and 2005 [cited 2008 Mar 18]. Available from http://www.cdc.gov.tw/public/Data/8324174197.pdf
2. Jou R, Chuang PC, Wu YS, Yan JJ, Luh KT. Drug-resistant *Mycobacterium tuberculosis,* Taiwan. Emerg Infect Dis. 2006;12:871–2.
3. World Health Organization. Anti-tuberculosis drug resistance in the world report no. 3. Geneva: The Organization. 2004 [cited 2008 Mar 18]. Available from http://www.who.int/tb/publications/who_htm_tb_2004_343/en/index.html
4. Centers for Disease Control and Prevention. Emergence of *Mycobacterium tuberculosis* with extensive resistance to second-line drugs—worldwide, 2000–2004. MMWR. 2006;55:301–5.
5. World Health Organization. Extensively drug-resistant tuberculosis (XDR-TB): recommendations for prevention and control. Wkly Epidemiol Rec. 2006; 81:430–2.
6. American Thoracic Society, Centers for Disease Control and Prevention, Infectious Diseases Society of America. Treatment of tuberculosis. Am J Respir Crit Care Med. 2003;167:603–62.
7. Ginsburg AS, Hooper N, Parrish N, Dooley KE, Dorman SE, Booth J, et al. Fluoroquinolone resistance in patients with newly diagnosed tuberculosis. Clin Infect Dis. 2003;37:1448–52.
8. Yu MC, Suo J, Lin TP, Luh KT. In vitro activity of ofloxacin against *Mycobacterium tuberculosis.* J Formos Med Assoc. 1997;96:13–6.
9. Huang TS, Kunin CM, Lee SS, Chen YS, Tu HZ, Liu YC. Trends in fluoroquinolone resistance of *Mycobacterium tuberculosis* complex in a Taiwanese medical center: 1995–2003. J Antimicrob Chemother. 2005;56:1058–62.
10. Gandhi NR, Moll A, Sturm AW, Pawinski R, Govender T, Lalloo U, et al. Extensively drug-resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa. Lancet. 2006;368:1575–80.

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Hantavirus Outbreak, Germany, 2007

To the Editor: Hantavirus disease (for review see [*1*]) has been reportable in Germany since 2001, according to the Federal Infection Protection Act. In this country, Puumala virus (PUUV) causes most clinical hantavirus cases, although Dobrava-Belgrade virus and Tula virus also circulate (*1*). From 2001 through 2006, an average of ≈220 cases were reported per year (incidence 0.267/100,000) with a maximum of 448 cases in 2005. In contrast, 1,687 cases were reported in 2007 (*2*). Whereas in 2005 the highest incidence of infection was in metropolitan areas (*3*), the current outbreak is focused in the rural areas in southern and western Germany. Clinical case-patients exhibit key characteristics of hantavirus disease (nephropathia epidemica): acute high fever; pain in the back, head, and/or abdomen; proteinuria; rise of serum creatinine; thrombocytopenia; and renal failure (*1*). The outbreak provided considerable numbers of clinical samples from the viremic phase and thus has enabled a molecular epidemiologic analysis of the circulating virus.

At the National Consultation Laboratory for Hantavirus Infections (Berlin), we received early-phase serum specimens from the outbreak regions for confirmation assays. In enzyme immunoassays and Western blot tests (*4*), 80 samples from patients during the early clinical phase were positive for PUUV-specific immunoglobulin (Ig) M antibodies. All IgM data were accompanied by simultaneous or subsequent detection of PUUV-specific IgG. The samples were screened for hantavirus RNA by reverse transcription–PCR (RT-PCR) (*5*). Of the 80 early-phase serum samples, 42 (53%) were RT-PCR positive. For a subset of 14 of the 42 samples, a 557-nt segment of the nucleocapsid (S) gene underwent nucleotide sequence analysis as described previously (*6*).

The Figure, panel A, shows a map of Germany with the residences of those patients from whom virus sequences were amplified (marked by letter H in front of the specimen number). In the phylogenetic analysis, despite a substantial evolutionary distance to PUUV strains from other parts of Europe, the virus sequences unambiguously grouped within the PUUV species (Figure, panel B). The few previously known human PUUV sequences from individual clinical case-patients in Germany, "Berkel" from Munsterland (*7*) and "Heidelberg" from a region located between Swabian Jura and Spessart Forest (*8*), as well as human-derived strains from a small 2004 outbreak in the Bavarian Forest (*6*), were included in this analysis. The results showed a clustering of the new viral sequences strictly according to residential areas of the patients, forming the following 4 clades: Swabian Jura (SJ), Spessart Forest (SF), Munsterland (ML), and Bavarian Forest (BF). Two different single sequences, Karlsruhe (from a region in northwestern Swabian Jura) and Essen (in southern Munsterland), represent 2 putative additional lineages.

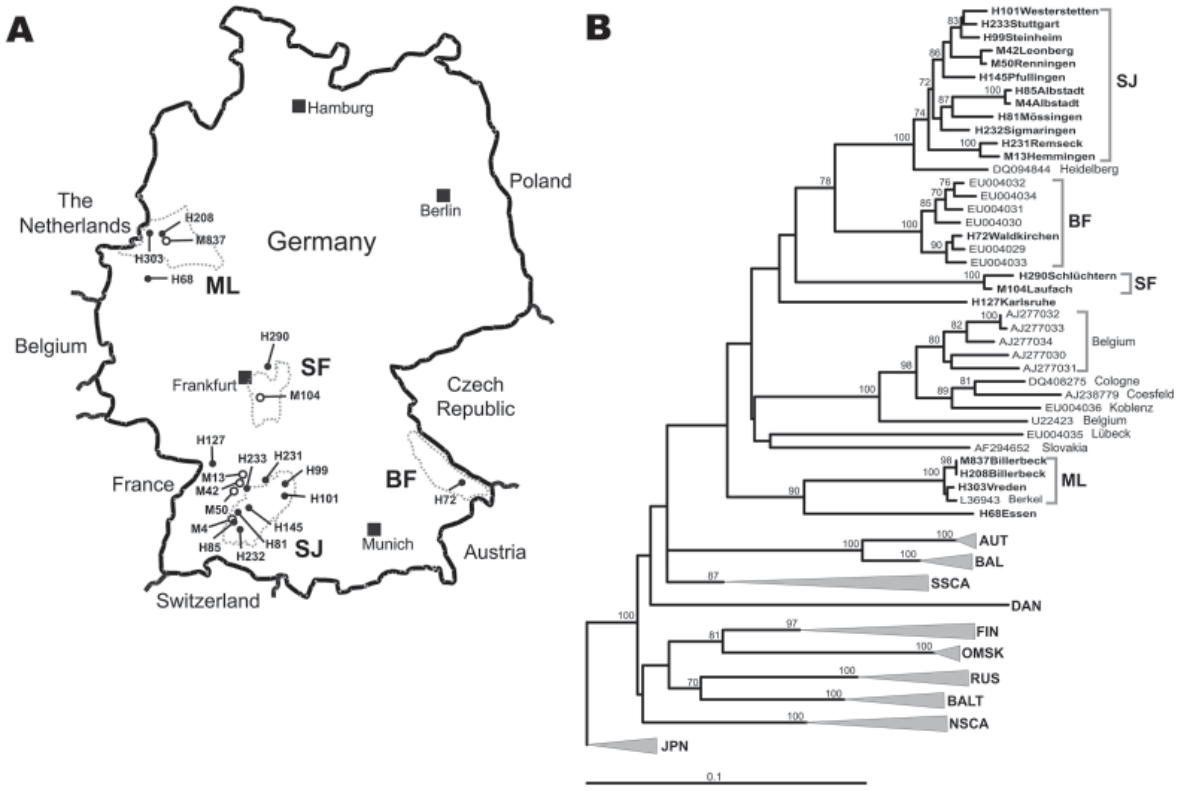

Figure. A) Map of Germany showing origins of viral sequences from the 2007 outbreak. H, sequences of human origin; M, sequences of rodent origin (*Myodes glareolus*). Dotted circles mark the outbreak regions characterized by particular virus sequence clusters; SJ, Swabian Jura; BF, Bavarian Forest; SF, Spessart Forest; ML, Munsterland. B) Neighbor-joining phylogenetic tree (TN93 evolutionary model) of European Puumala virus (PUUV) strains based on partial sequences of the S segment (557 nt, position 385–941). Bootstrap values ≥70%, calculated from 10,000 replicates, are shown at the tree branches. PUUV-like sequences from Japan (JPN) were used as an outgroup. Sequences taken from GenBank are indicated by their accession numbers. New sequences from this study are given in **boldface.** Accession numbers of new sequences are H101, EU266757; H233, EU266758; H99, EU266759; M42, EU085563; M50, EU085565 ; H145, EU266760; H85, EU266761; M4, EU266762; H81, EU266763; H232, EU266764; H231, EU266765; M13, EU085558; H72, EU266766; H290, EU266767; M104, EU246963; H127, EU266768; M837, EU266769; H208, EU266770; H303, EU266771; H68, EU266772. For clarity, previously characterized PUUV clades from other parts of Europe are shown in simplified form. However, the complete dataset of PUUV sequences as presented by Schilling et al. (*6*) was used to calculate the tree. Previously defined lineages are indicated by abbreviated names: AUT, Austrian; BAL, Balkan; BALT, Baltic; DAN, Danish; FIN, Finnish; NSCA, North Scandinavian; OMSK, Russian from Omsk region; RUS, Russian; SSCA, South Scandinavian. Scale bar indicates an evolutionary distance of 0.1 substitutions per position.

Most sequences in this study were obtained from Swabian Jura, the region with the highest illness rate of the outbreak (incidence 32.9/100,000). The Swabian Jura was previously identified as a hantavirus-endemic area characterized by higher seroprevalence rates in the population compared with the rest of Germany (*9*). Sequence alignments within this clade showed a nucleotide sequence diversity of up to 5.5%. Within the BF clade, the diversity is up to 4%. However, between the 4 phylogenetic clades mentioned above (SJ, SF, ML, and BF), a sequence variability of 12%–18% was found.

The natural reservoir of PUUV is the bank vole, *Myodes glareolus*; the virus is transmitted to humans by the aerosolized excreta of these rodents (*1*). Sequence comparisons showed a tight correlation between human- and rodent-derived PUUV sequences obtained from the same regional provenance (nucleotide identity >98%) and high variability of sequences originating from different geographic regions (nucleotide identity ≈85%). Neighbor-joining analyses confirmed the direct clustering of human- and rodent-derived sequences in the different phylogenetic clades (Figure, panel B).

In this study we focused on the analysis of a 557-nt S-segment region. For more detailed studies, analysis of the complete S and M sequences of the virus strains will be necessary. Nevertheless, our results demonstrate a high variability among the German PUUV strains but a strong clustering of viral sequences of human and rodent origin in the same geographic region. The diversity of the PUUV clusters suggests their separate evolutionary history in

the different regions of Germany. In contrast, within these particular geographic areas, only slight sequence differences were found in longitudinal analysis over several years. This conclusion is supported by the novel human Waldkirchen sequence (H72), which is almost identical to the BF strains from 2004 (*6*,*10*) and the similarity of newly derived human sequences from Munsterland (H208, H303) to the Berkel strain from 1994 (*7*). The molecular characterization of the viral sequences of patient and rodent origin from the outbreak areas demonstrates that PUUV is the causative agent of the current outbreak.

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References

1. Kruger DH, Ulrich R, Lundkvist A. Hantavirus infections and their prevention. Microbes Infect. 2001;3:1129–44.
2. Robert Koch Institute, Berlin, Germany. Epidemiologisches Bulletin. 2008;(13) [cited 2008 Mar 28]. Available from www.rki.de
3. Abu Sin M, Stark K, van Treek U, Dieckmann H, Uphoff H, Hautmann W, et al. Risk factors for hantavirus infection in Germany, 2005. Emerg Infect Dis. 2007;13:1364–6.
4. Meisel H, Wolbert A, Razanskiene A, Marg A, Kazaks A, Sasnauskas K, et al. Development of novel immunoglobulin G (IgG), IgA, and IgM enzyme immunoassays based on recombinant Puumala and Dobrava hantavirus nucleocapsid proteins. Clin Vaccine Immunol. 2006;13:1349–57.
5. Kramski M, Meisel H, Klempa B, Kruger DH, Pauli G, Nitsche A. Detection and typing of human pathogenic hantaviruses by real-time reverse transcription-PCR and pyrosequencing. Clin Chem. 2007;53:1899–905.
6. Schilling S, Emmerich P, Klempa B, Auste B, Schnaith E, Schmitz H, et al. Hantavirus disease outbreak in Germany: limitations of routine serological diagnostics and clustering of virus sequences of human and rodent origin. J Clin Microbiol. 2007;45:3008–14.
7. Pilaski J, Feldmann H, Morzunov S, Rollin PE, Ruo SL, Lauer B, et al. Genetic identification of a new Puumala virus strain causing severe hemorrhagic fever with renal syndrome in Germany. J Infect Dis. 1994;170:1456–62.
8. Bahr U, Zeier M, Muranyi W. Characterization of a new Puumala virus genotype associated with hemorrhagic fever with renal syndrome. Virus Genes. 2006;33:229–34.
9. Zoller L, Faulde M, Meisel H, Ruh B, Kimmig P, Schelling U, et al. Seroprevalence of hantavirus antibodies in Germany as determined by a novel recombinant enzyme immunoassay. Eur J Clin Microbiol Infect Dis. 1995;14:305–13.
10. Essbauer S, Schmidt J, Conraths FJ, Friedrich R, Koch J, Hautmann W, et al. A new Puumala hantavirus subtype in rodents associated with an outbreak of Nephropathia epidemica in South-East Germany in 2004. Epidemiol Infect. 2006;134:1333–44.

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Chikungunya Virus in *Aedes albopictus*, Italy

To the Editor: Chikungunya virus (CHIKV) infection is a self-limiting illness characterized by fever, headache, weakness, rash, and arthralgia. Some patients show prolonged weakness or arthralgia lasting several months. In 2006, several Indian Ocean states and India experienced outbreaks of CHIKV infection, where the vector was postulated to be *Aedes albopictus* in at least some areas (*1*,*2*).

Starting from mid July 2007, in 2 villages in Ravenna Province in Italy, Castiglione di Ravenna (≈1,700 inhabitants) and Castiglione di Cervia (≈2,000 inhabitants), several residents sought treatment at local hospitals and health centers for high fever and arthralgia, joint and muscular pain, severe headaches, body aches, and in some cases, rash. Since the beginning of August 2007, an increasing number of febrile syndromes associated with arthralgia have been recorded among the residents of the area. By the end of August, the number of sick persons had increased to ≈150 (*3*). At the beginning of September, the disease was confirmed as chikungunya fever by the Superior Institute of Health (*4*).

On August 21 and 22, 2007, an entomologic investigation was carried out in the area. *Ae. albopictus* (215 females and 57 males), *Culex pipiens* (369 females and 15 males), and a few specimens of *Ae. caspius* (5 females) and *Anopheles* spp. (2 females) were collected by using 3 light traps without CO_2 (Centers for Disease Control and Prevention [CDC], Atlanta, GA, USA) and 8 CO_2-baited traps (similar to the CDC light trap) activated once overnight. Collections were obtained by using 2 small-handled aspirators per day of sampling. Collected mosquitoes were divided by species and pooled as described in the Table.

Table. Pooled mosquito samples analyzed for chikungunya virus, Italy

Pool	No. mosquitoes	Sex	Species	Site of collection	PCR results
1	125	F	*Aedes albopictus*	Castiglione Ravenna	Positive
2	90	F	*Ae. albopictus*	Castiglione Cervia	Positive
3	214	F	*Culex pipiens*	Castiglione Ravenna	Negative
4	155	F	*Cx. pipiens*	Castiglione Cervia	Negative
5	5	F	*Ae. caspius*	Castiglione Ravenna and Castiglione Cervia	Negative
6	2	F	*Anopheles* spp.	Castiglione Ravenna and Castiglione Cervia	Negative
7	57	M	*Ae. albopictus*	Castiglione Ravenna and Castiglione Cervia	Negative
8	15	M	*Cx. pipiens*	Castiglione Ravenna and Castiglione Cervia	Negative

Each pool was analyzed for CHIKV or nucleic acid (viral isolation and CHIKV-specific reverse transcription–PCR [RT-PCR]). Total RNA was extracted from supernatant of mosquitoes homogenized in minimal essential medium by using TRIzol LS (Invitrogen, Carlsbad, CA USA), and cDNA was synthesized by using SuperScript II (Invitrogen) and random primers. Two PCR protocols were used on the same samples: a nested RT-PCR (*5*) and a real-time PCR (*6*). Positive results were obtained from samples 1 and 2 (Table) with both PCR protocols. No positive control was available to the authors at the time of the first PCR-positive detection of virus from mosquitoes; therefore, laboratory contamination can be excluded. Moreover, from the same PCR-positive samples of *Ae. albopictus* (Table), viral isolation was achieved.

The 172-bp PCR fragment obtained after the second round of the nested RT-PCR (*5*) was located in the E2 gene between nt positions 9486 and 9660 according to nucleotide sequence of the S27 strain genome (GenBank accession no. AF369024). The sequence of this fragment was obtained by using a BigDye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA) and compared with the available CHIKV sequences, including Indian isolates obtained during 2005–2006 (IND-06), the earlier Indian isolates (1963 and 1973), the isolate from Yawat in 2000, Reunion isolates during 2005–2006 (RU), the Senegal strain (1983), and the S27 and Ross isolates (1952). The sequence of the isolate from Italy is clustered into the Central/East African genotype and showed highest nucleotide identity (99.4%–100.0%) with isolates from IND-06 and 100% identity at the amino acid level with isolates from IND-06 and from RU. These results must be considered carefully because the short-sequenced fragment does not enable confirmation of the epidemiologic origin of the isolate from Italy. The sequence of the whole genome of CHIKV isolates from mosquitoes in Italy is an ongoing process; when completed, results will be available in GenBank.

The remainder of insect samples, which included a few male samples of *Ae. albopictus*, generated PCR-negative results with both protocols tested. Male mosquitoes were tested to detect evidence of transovarian transmission, but the small number of mosquitoes tested suggests possible vertical transmission of the virus in the Italian outbreak. Chikungunya fever in Italy has been reported recently by Beltrame et al. (*7*) but those cases involved 9 patients who were infected while traveling in regions where CHIKV was endemic.

In spite of the large diffusion of *Ae. albopictus* in Italy recorded since 1990 and broadly distributed all over the country (*8*), this outbreak of Chikungunya fever is evidence of an active endogenous circulation of the virus and could represent a possible introduction of this disease in Italy. No prediction can be made about spread and persistence of the virus in Italy because the vector is now present in all areas of the country and recent winters have been characterized by mild temperatures. Most likely, because transovarial infection has not been demonstrated for CHIKV, spread of infection will remain limited. However, as this report documents, Italy is at risk for infection with arboviruses, such as dengue virus and West Nile virus, which have serious effects on public health.

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References

1. Bodenmann P, Genton B. Chikungunya: an epidemic in real time. Lancet. 2006; 368:258.
2. Schuffenecker I, Iteman I, Michault A, Murri S, Frangeul L, Vaney MC, et al. Genome microevolution of chikungunya viruses causing the Indian Ocean outbreak. PLoS Med. 2006;3:e263.
3. Pavan WO. Chikununya - Italy (Emilia Romagna): suspected. ProMed. 2007 Aug 31 [cited 2008 Mar 17]. Available from http://www.promedmail.org, archive no. 20070901.2877.

4. Pavan WO. Chikununya - Italy (Emilia Romagna): confirmed. ProMed. 2007 Sep 3 [cited 2008 Mar 17]. Available from http://www.promedmail.org, archive no. 20070903.2899.
5. Pfeffer M, Linssen B, Parke MD, Kinney RM. Specific detection of chikungunya virus using a RT-PCR/nested PCR combination. J Vet Med B Infect Dis Vet Public Health. 2002;49:49–54.
6. Pastorino B, Bessaud M, Grandadam M, Murri S, Tolou HJ, Peyrefitte CN. Development of a TaqMan RT-PCR assay without RNA extraction step for the detection and quantification of African chikungunya viruses. J Virol Methods. 2005;124:65–71.
7. Beltrame A, Angheben A, Bisoffi Z, Monteiro G, Marocco S, Calleri G, et al. Imported chikungunya infection, Italy [letter]. Emerg Infect Dis [serial on the Internet]. 2007 Aug [cited 2008 Mar 17]. Available from http://www.cdc.gov/EID/content/13/8/1264.htm
8. Romi R. *Aedes albopictus* in Italy: an underestimated health problem. Ann Ist Super Sanita. 2001;37:241–7.

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Persistent Human Metapneumovirus Infection in Immunocompromised Child

To the Editor: Respiratory viral infections can be associated with a wide range of clinical manifestations from self-limiting upper respiratory tract diseases to pneumonia (*1*). However, in general, respiratory viral infections are more likely to progress to more severe diseases in immmunocompromised patients. Human metapneumovirus (hMPV) has been reported in most parts of the world as a cause of acute respiratory tract infections in persons of all age groups (*2*). Fatal hMPV infections have been reported in immunocompromised patients, including a 17-month-old girl who had acute lymphoblastic leukemia (*3*) and a 33-year-old woman who had received a hematopoietic stem cell transplant (HSCT) (*4*). In adult HSCT recipients, fatal pneumonia (*5*) and persistent hMPV infection without respiratory symptoms have been described (*6*). In addition, adult lung transplant recipients have been able to clear hMPV infection despite high levels of immunosuppression (*7*). We report a case of persistent hMPV infection in a child with severe combined immunodeficiency disorder (SCID) who shed hMPV during an 11-month period.

The child, a girl who was born in January 2002, received an allogeneic haploidentical stem cell transplant from her father in May 2002 after her diagnosis of SCID. Infection with influenza A virus (H3N2) was diagnosed on April 2005 and progressed to a chronic pneumonitis of the lingula. She received successive courses of anti-influenza agents (amantadine, oseltamivir, and zanamivir) for 1 year during which time several positive influenza cultures were obtained (*8*). Four years after the transplant, she was still lymphopenic (800×10^9/L, mostly T cells) and had chronic graft-versus-host disease, which had been treated with steroids (prednisone 2.5 mg twice a day for many months). She also had a mild chronic cough but did not need supplemental oxygen while she was receiving nebulized zanamivir (10–20 mg twice a day). Her 2 nasopharyngeal aspirate (NPA) specimens from June and July 2006 were negative for influenza virus. However, positive cultures for hMPV were obtained from NPA and bronchoalveolar lavage specimens collected on July 2006. After receiving this result, we performed retrospective and prospective molecular detection studies for hMPV for this patient. HMPV was detected by reverse transcription–PCR for the F and G genes (*9*) in 6 and 7 NPA samples, respectively, collected during an 11-month period from November 4, 2005, through October 4, 2006. These samples were obtained for surveillance of influenza infection in this child with persistent cough.

Amplified hMPV G sequences were aligned by using the Clustal W program (www.molecularevolution.org/cdc/software/clustalw). A phylo genetic tree was constructed with MEGA 3.1 software (www.megasoftware.net) by using the neighbor-joining algorithm with Kimura-2 parameters. Sequence analysis of the hMPV G gene showed that all strains belonged to the B2 genotype, which clustered with hMPV Can98–75 and NL1/94 reference strains (Figure, panel A). Amplified hMPV G gene sequences of the 6 samples collected in 2006 were identical, but they had 96.7% and 92.8% nucleotide and amino acid identities, respectively, with the initial strain from November 2005, which clearly indicates 2 viral strains (Figure, panel B). Similar results were obtained with the F gene (data not shown). Inoculation of the respiratory samples on a panel of 10 cell lines as previously described (*10*) showed that only 2 of 7 NPA samples were positive for hMPV by culture; 2 of the 5 remaining samples were positive for influenza A, which may have masked the cytopathic effects of hMPV on rhesus monkey kidney (LLC-MK2) cells.

Persistent hMPV infection in asymptomatic adult HSCT recipients has been described (*6*). In that study, hMPV was isolated from 2 patients in 2 consecutive samples collected 12–56 days apart. However, virus evolution was not adequately investigated because it was based on sequence analysis of a 150-bp fragment from the highly conserved nucleoprotein gene (*6*). Unlike in previous reports (*6*,*7*), characterization of hMPV strains in our study was performed by sequence

analysis of a 633-bp fragment from the most variable hMPV G gene. Our findings showed 2 distinct hMPV variants of the same genotype (B2). These variants might represent a viral drift after immune pressure but most likely was the result of 2 different infections in the immunocompromised child. The latter hypothesis is suggested by the considerable amino acid variability (15 aa differences in the 211-aa region of the G protein) between strains collected on November, 4, 2005, and January, 20, 2006, compared with identical sequences for the strains recovered over the next 10 months. Debiaggi et al. (*6*) previously suggested that persistent hMPV infection in HSCT patients was attributable to their inability to clear the virus because of impaired immune response. By contrast, adult lung transplant recipients were found to be able to achieve hMPV clearance despite their severe immunosuppression status (*7*). Because both fatal and mild or asymptomatic hMPV infections have been reported in immunocompromised hosts, additional studies are needed to determine whether such differing outcomes are due to viral, host, or environmental factors. In conclusion, this case of persistent hMPV infection associated with relatively mild respiratory symptoms in an immunocompromised child suggests that the host's immune response may play a key role in disease pathogenesis.

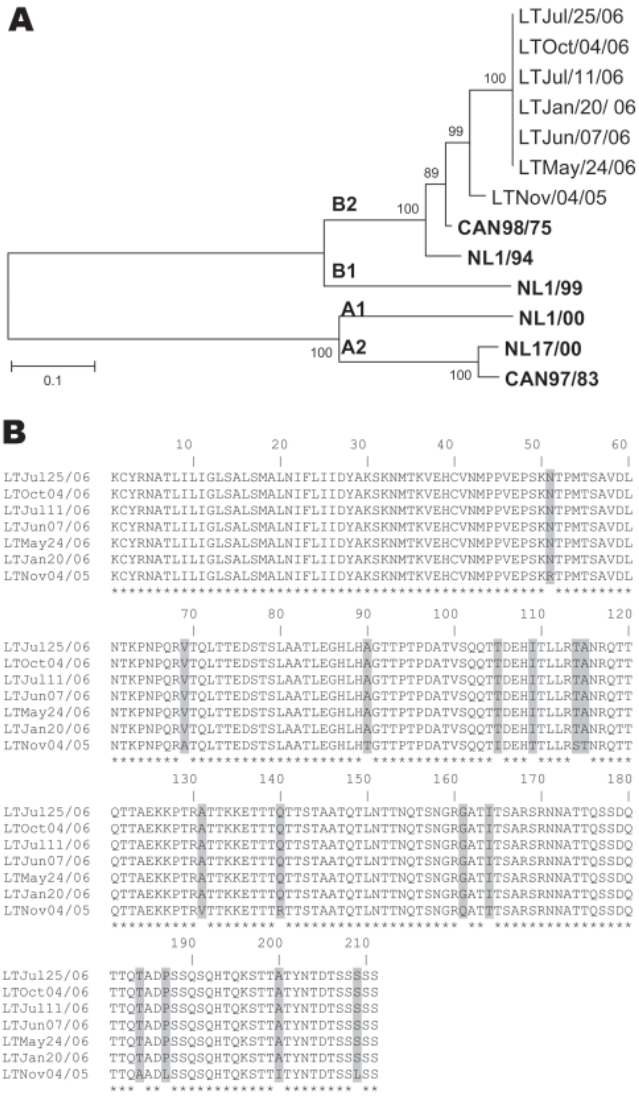

Figure. A) Phylogenetic analysis of human metapneumovirus (hMPV) strains isolated during an 11-month period based on nucleotide sequences of the G gene. Multiple nucleotide sequence alignments were performed by using the ClustalW program (www.molecularevolution.org/cdc/software/clustalw); a phylogenetic tree was constructed with MEGA 3.1 software (www.megasoftware.net) by using the neighbor-joining algorithm with Kimura-2 parameters. The analysis included the following hMPV reference strains: Can98/75 (GenBank accession no. AY485245), NL1/94 (AY304362), NL1/99 (AY304361), NL1/00 (AF371337), NL17/00 (AY304360), and Can97/83 (AY485253). Scale bar indicates 1 substitution for every 10 nucleic acid residues. **Boldface** indicates reference isolates. B) Comparison of the partial amino acid sequences (residues 26–236) of the G protein of hMPV isolates recovered during an 11-month period from an immunocompromised child. Asterisks denote identical residues; shaded boxes highlight different amino acids between the hMPV variant of November 4, 2005, and the subsequent variants from January 20, 2006, to October 4, 2006.

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References

1. Abed Y, Boivin G. Treatment of respiratory viral infections. Antiviral Res. 2006;70:1–16.
2. Hamelin ME, Abed Y, Boivin G. Human metapneumovirus: a new player among respiratory viruses. Clin Infect Dis. 2004;38:983–90.
3. Pelletier G, Déry P, Abed Y, Boivin G. Respiratory tract reinfections by the new human *Metapneumovirus* in an immunocompromised child. Emerg Infect Dis. 2002;8:976–8.
4. Cane PA, van den Hoogen BG, Chakrabarti S, Fegan CD, Osterhaus AD. Human metapneumovirus in a hematopoietic stem cell transplant recipient with fatal lower respiratory tract disease. Bone Marrow Transplant. 2003;31:309–10.
5. Englund JA, Boeckh M, Kuypers J, Nichols WG, Hackman RC, Morrow RA, et al. Fatal human metapneumovirus infection in stem-cell transplant recipients. Ann Intern Med. 2006;144:344–9.

6. Debiaggi M, Canducci F, Sampaolo M, Marinozzi MC, Parea M, Terulla C, et al. Persistent symptomless human metapneumovirus infection in hematopoietic stem cell transplant recipients. J Infect Dis. 2006;194:474–8.
7. Larcher C, Geltner C, Fischer H, Nachbaur D, Muller LC, Huemer HP. Human metapneumovirus infection in lung transplant recipients: clinical presentation and epidemiology. J Heart Lung Transplant. 2005;24:1891–901.
8. Baz M, Abed Y, McDonald J, Boivin G. Characterization of multidrug-resistant influenza A/H3N2 viruses shed during one year by an immunocompromised child. Clin Infect Dis. 2006;43:1555–61.
9. Ludewick HP, Abed Y, Van Niekerk N, Boivin G, Klugman KP, Madhi SA. Human metapneumovirus genetic variability, South Africa. Emerg Infect Dis. 2005;11:1074–8.
10. Boivin G, Abed Y, Pelletier G, Ruel L, Moison D, Côté S, et al. Virological features and clinical manifestations associated with human metapneumovirus: a new paramyxovirus responsible for acute respiratory-tract infections in all age groups. J Infect Dis. 2002;186:1330–4.

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Leptospirosis in Taiwan, 2001–2006

To the Editor: Leptospirosis is a zoonotic disease that has now been identified as an emerging infectious disease (*1*,*2*). It is caused by pathogenic spirochetes of the genus *Leptospira*. The natural hosts for *Leptospira* spp. come from a variety of species (*2*–*4*), of which the rodent is the most important reservoir (*4*,*5*). The incubation period range for leptospirosis is usually 5–14 days, with a range of 2–30 days (*4*). Leptospirosis is a disease of humid tropical and subtropical countries. According to the World Health Organization (*4*), probable leptospirosis incidence ranges from ≈0.1–1 case/100,000 population/year in temperate climates to 10–100 cases/100,000 population/year in humid tropical climates. Leptospirosis epidemics are often related to heavy rainfall and flooding (*1*,*6*,*7*). Because of its climate, Taiwan may be at high risk for leptospirosis. We therefore investigated human leptospirosis in Taiwan and the relationship between leptospirosis incidence and rainfall pattern.

Taiwan is a medium-sized archipelago in East Asia; the Tropic of Cancer runs through its center. The northern part of Taiwan is subtropical; the southern part is tropical. Taiwan lies in the path of many tropical storms and typhoons that bring extremely heavy rainfall usually during July–September. The annual "plum rain" season in May and June also brings a lot of precipitation. Because of its tropical and subtropical marine climate, Taiwan enjoys rich agricultural productivity throughout the year, which is favorable for rodent infestations (*8*,*9*).

In Taiwan, reported cases of leptospirosis have been investigated by the Centers for Disease Control since 2001. Leptospirosis should be suspected in patients who have fever; headache; myalgia; abdominal pain; prostration; conjunctival suffusion; meningeal irritation and aseptic meningitis; anuria, oliguria, or proteinuria; jaundice; acute renal insufficiency; or gastrointestinal or lung hemorrhage. Patients with suspected leptospirosis are reported by physicians to Taiwan's Centers for Disease Control through the Notifiable Disease Surveillance System, after which local health bureaus collect urine and blood samples for confirmation by serologic testing. Urine and blood samples from patients with clinically suspected leptospirosis are inoculated into Ellinghausen-McCullough-Johnson-Harris culture medium plus 5-fluorouracil and incubated at 30°C for 8–12 weeks. Cultures are examined by dark-field microscopy every week. Alternatively, latex agglutination assay may be used for rapid serologic diagnosis of serum from patients with clinically suspected leptospirosis (*10*). Samples with positive latex agglutination assay results should be confirmed by microscopic agglutination test (MAT). An antibody titer ≥100 as determined by MAT is regarded as a probable case of leptospirosis. The local health bureau again collects patients' serum during the convalescent phase of illness for confirmation by MAT. A laboratory-confirmed case is defined as the isolation of leptospires from urine and blood or a 4-fold increase in antibody titer between acute- and convalescent-phase samples.

During 2001–2006, of 7,733 suspected human cases of leptospirosis, 291 cases were confirmed. The major serotype identified was *L. santarosai* serovar Shermani. The mean annual incidence was 0.21 cases/100,000 population. The laboratory-confirmed cases were observed in Taiwan, mostly in male patients (83.5%) (Figure, panel A). Cases occurred in all age groups but were more common (90%) in those 25–74 years of age. Age-specific incidence was highest for persons 55–64 years of age; mean annual incidence was 0.57/100,000 population.

Rainfall data from the Central Weather Bureau of the Republic of China showed typically high rainfall (252–433 mm/month) in Taiwan during May–September. Heavy rains were followed by an increase in laboratory-confirmed cases of leptospirosis (Figure, panel B); June–October accounted for 60% of cases, with a higher incidence of 0.022–0.028 cases/100,000 population. In October–December, monthly rainfall was below average (201.1 mm/month), but leptospirosis incidence was above average (0.018/100,000). Specifically, 25 (74%) of leptospirosis cases in October, 10 (42%) in November, and 4 (19%) in December were likely as-

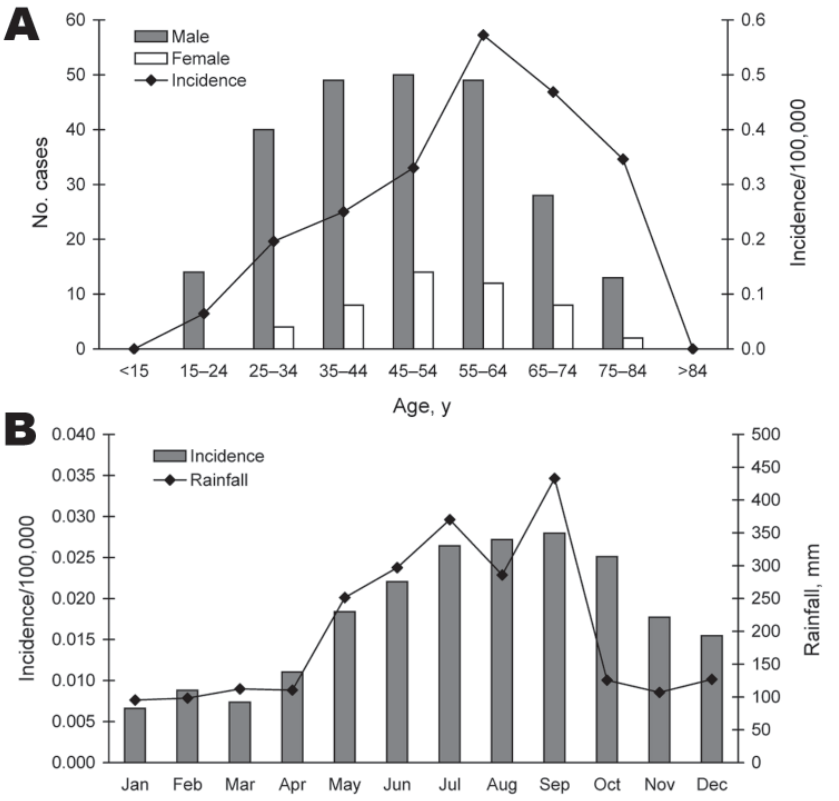

Figure. Leptospirosis cases (N = 291) in Taiwan, 2001–2006. A) Distribution by age and sex. B) Relationship between rainfall and leptospirosis incidence. Data represent averages for each month during the 6 years.

sociated with several days of heavy rainfall from typhoons. Therefore, the typhoons may be the reason for high incidence in October–December.

The annual incidence of leptospirosis in Taiwan is relatively lower than that in other countries with tropical or subtropical climates. Our study does not conclusively document the reason for lower incidence, although it does suggest an association between amount of rainfall and incidence. An understanding of the relationship between leptospirosis incidence and rainfall is indispensable for implementing appropriate preventive measures.

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References

1. Levett PN. Leptospirosis. Clin Microbiol Rev. 2001;14:296–326.
2. Vinetz JM. Leptospirosis. Curr Opin Infect Dis. 2001;14:527–38.
3. Bharti AR, Nally JE, Ricaldi JN, Matthias MA, Diaz MM, Lovett MA, et al. Leptospirosis: a zoonotic disease of global importance. Lancet Infect Dis. 2003;3: 757–71.
4. World Health Organization. Human leptospirosis: guidance for diagnosis surveillance and control. Geneva: The Organization; 2003. p. 109.
5. Michel V, Branger C, Andre-Fontaine G. Epidemiology of leptospirosis. Rev Cubana Med Trop. 2002;54:7–10.
6. Trevejo RT, Rigau-Perez JG, Ashford DA, McClure EM, Jarquín-González C, Amador JJ, et al. Epidemic leptospirosis associated with pulmonary hemorrhage—Nicaragua, 1995. J Infect Dis. 1998;178:1457–63.
7. Narita M, Fujitani S, Haake DA, Paterson DL. Leptospirosis after recreational exposure to water in the Yaeyama Islands, Japan. Am J Trop Med Hyg. 2005;73:652–6.
8. Ku TY, Lin CC. Abundance and distribution of field rodents in Taiwan. Plant Prot Bull. 1980;22:397–420.
9. Lu KH. 2004. Strategy of field rodent control in Taiwan. In: Proceedings of seminar on management of plant health. Taiwan. p. 259–76.
10. Smits HL, van der Hoorn MA, Goris MG, Gussenhoven GC, Yersin C, Sasaki DM, et al. Simple latex agglutination assay for rapid serodiagnosis of human leptospirosis. J Clin Microbiol. 2000;38:1272–5.

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Leishmania (Leishmania) amazonenis Infection, Suriname

To the Editor: A 17-year-old man was seen at the Dermatology Service in Paramaribo (Suriname) with a skin condition that he had had since he was 5 years of age. The condition consisted of multiple cutaneous ulcerations, nodules, and fibrotic plaques disseminated on his face, limbs, and trunk, and subcutaneous nodules on lymph-draining tracts on his hands, arms, and legs (online Appendix Figure, panel A, available from www.cdc.gov/EID/content/14/5/857-appG.htm). He had lived his entire life in an inland village, located at Brokopondo Lake (central-eastern Suriname); he had never traveled outside the country. The diagnosis of cutaneous leishmaniasis (CL, a parasitic disease caused by the protozoa *Leishmania*) was presumed. The patient received pentamidine therapy in 1997, 1998, and 2005, but without sustained clinical effect. Rapid screening tests for HIV were negative (Determine [Abbott Laboratories, Tokyo,

Japan] and Unigold [Trinity Biotech, Co. Wicklow, Ireland]). In 2006, the diagnosis of CL was confirmed with histopathology, culture, and PCR. The parasite was identified by a PCR restriction fragment length polymorphism method on the small subunit–internal transcribed spacer genes (*1*) and by multilocus enzyme electrophoresis at the National Reference Center of *Leishmania* (Montpellier, France).

After promising results were obtained with miltefosine in a patient with anergic diffuse cutaneous leishmaniasis (ADCL) in Venezuela (*2*), the patient received 150 mg/day oral miltefosine (Impavido, Zentaris, Germany) for 98 days and the lesional parasite load was quantified with quantitative nucleic acid sequence-based amplification (*3*). Skin biopsy specimens were collected from 1 target lesion before treatment; during treatment at day 14, day 28, day 42 (all in duplicate); and at day 70 (single biopsy).

The strain causing infection (MHOM/SR/2006/SP100) was identified as *Leishmania (Leishmania) amazonensis,* and the enzymatic profile was equal to *L. (L.) amazonensis* zymodeme MON-41. Histopathology showed large macrophages containing abundant *Leishmania* amastigotes and few lymphocytes and plasma cells without granuloma formation. A considerable clinical improvement was observed during the first 2 months of therapy. The lesions slowly decreased in size and duration. At day 70, all ulcerative lesions were re-epithelialized, without signs of infiltration or lymphangitis (online Appendix Figure, panel B). At start of treatment, parasite counts of 360,000 and 310,000 parasites per biopsy were detected; these counts decreased to 0 parasites/biopsy at day 70. Histopathologic studies at day 70 showed no *Leishmania* bodies, a dense lymphocytic and plasma cellular infiltrate, and fibrosis. Apart from mild elevation of creatine and urea during treatment, no subjective or adverse side effects were reported.

L. (L.) amazonensis causes CL and 2 very serious manifestations of CL, disseminated cutaneous leishmaniasis (DCL) and ADCL (*4*). Both forms are histopathologically characterized by heavily parasitized macrophages and an absence of cell-mediated immune responses in therapy-naive patients (*4*). ADCL is resistant to any form of therapy, and cell-mediated immune responses never seem to occur. In contrast, the cell-mediated immune response in DCL can eventually arise upon therapy response, even in patients with previous therapy failures (*4*). The therapy response in DCL patients is histopathologically characterized by the appearance of a lymphocytic and plasma cellular infiltrate. The diagnosis of DCL is plausible in our patient based on the histopathologic findings before, during, and after therapy; the clinical picture (erythematous infiltrated plaques, lymphadenitis, and lymphangitis), and the favorable therapy response. He was last seen 7 months after end of therapy, at which time new lesions had not developed.

In general, *L. (L.) amazonensis* infection is rare in humans (*5*). In French Guiana, bordering the eastern side of Suriname, few patients (≈1.9%) are reported to be infected with this species (*5*). However, the sandfly vector of *L. (L.) amazonensis*, *Lutzomyia flaviscutellata,* was detected earlier in Suriname (*6*), which may indicate transmission of *L. (L.) amazonensis* infection to humans by means of the bite of this sandfly in Suriname. Our patient had no history of transfusion or intravenous drug use.

Many gold diggers from the northern part of Brazil work and travel in Suriname and are familiar with CL. In the Brazilian State Pará, a region bordering Suriname in the South, the infection rate with *L. (L.) amazonensis* is high (34.8%) (*7*). It is thus conceivable that infected gold diggers from that area have introduced *L. (L.) amazonensis* into Suriname. Our patient used to live in a village where many Brazilian gold diggers worked around the time that his skin lesions developed. Migration of laborers is associated with an increased risk for CL infection (*8*). The zymodeme MON-41 is widespread in Central America and the northern part of South America, and has been reported in Venezuela, Brazil, Panama, French Guiana, and Colombia (F. Pratlong and J.P. Dedet, Montpellier International Cryobank of *Leishmania*, pers. comm., 2007). Therefore, speculations on the exact origin of the infection need to be made cautiously.

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References

1. Rotureau B, Ravel C, Couppié P, Pratlong F, Nacher M, Dedet JP, et al. Use of PCR-restriction fragment length polymorphism analysis to identify the main New World *Leishmania* species and analyze their taxonomic properties and polymorphism by application of the assay to clinical samples. J Clin Microbiol. 2006;44:459–67.
2. Zerpa O, Blanco B, Kannee C, Ulrich M, Sindermann H, Engel J, et al. Treatment of diffuse cutaneous leishmaniasis with miltefosine: a case report. Int J Dermatol. 2006;45:751–3.

3. van der Meide WF, Schoone GJ, Faber WR, Zeegelaar JE, de Vries HJ, Ozbel Y, et al. Quantitative nucleic acid sequence-based assay as a new molecular tool for the detection and quantification of *Leishmania* parasites in skin biopsies. J Clin Microbiol. 2005;43:5560–6.
4. Silveira FT, Lainson R, Corbett CE. Further observations on clinical, histopathological, and immunological features of borderline disseminated cutaneous leishmaniasis caused by *Leishmania (Leishmania) amazonensis.* Mem Inst Oswaldo Cruz. 2005;100:525–34.
5. Rotureau B, Ravel C, Nacher M, Couppié P, Curtet I, Dedet JP, et al. Molecular epidemiology of *Leishmania (Viannia) guyanensis* in French Guiana. J Clin Microbiol. 2006;44:468–73.
6. Burgos A, Hudson J. Annotated list of the phlebotominae (diptera) of Suriname. Mem Inst Oswaldo Cruz. 1994;89:171–8.
7. Silveira FT, Lainson R, Shaw JJ, De Souza AA, Ishikawa EA, Braga RR. Cutaneous leishmaniasis due to *Leishmania (Leishmania) amazonensis* in Amazonian Brazil, and the significance of a negative Montenegro skin-test in human infections. Trans R Soc Trop Med Hyg. 1991;85:735–8.
8. Rotureau B, Joubert M, Clyti E, Djossou F, Carme B. Leishmaniasis among gold miners, French Guiana. Emerg Infect Dis. 2006;12:1169–70.

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Household Transmission of Carbapenemase-producing *Klebsiella pneumoniae*

To the Editor: Since its first description in 2001, carbapenemase-producing *Klebsiella pneumoniae* has become a frequent nosocomial pathogen in the eastern United States (*1*). This bacterium was introduced into Israel in 2005 and is endemic now in several hospitals in the country (*2*). We recently documented transmission of this organism within a household, the source being a debilitated patient who returned home after a long hospitalization.

A 73-year-old man had a urologic procedure (transurethral resection of the bladder neck) in a community hospital in early October 2007. He was initially evaluated on September 23, 2007, at an outpatient clinic where a routine urine sample was obtained for culture. Carbapenemase-producing *K. pneumoniae* was cultured. Identification and susceptibility testing of the isolate were completed by using the VITEK 2 system (bioMérieux, Marcy l'Etoile, France). *K. pneumoniae* carbapenemase was confirmed by using the modified Hodge test (*3*). Two repeat urine cultures grew the same organism; however, a stool culture was negative for carbapenemase-producing *K. pneumoniae*.

The medical history of the patient included hypertension and carcinoma of the prostate gland that was treated with high-intensity focused ultrasound in May 2007, followed by transurethral resection of prostate in June 2007. The 2 procedures were performed in 2 different private hospitals, and each required a 24-hour hospitalization. No carbapenemase-producing *K. pneumoniae* was documented in these hospitals. Two months before detection of carbapenemase-producing *K. pneumoniae*, the patient received a 1-week course of oral amoxicillin-clavulanate for presumed urinary tract infection, although urine culture obtained on July 29, 2007 was sterile. A repeat urine culture 2 weeks later (August 13, 2007) remained sterile.

Because the circumstances of strain acquisition and patient characteristics were not typical for epidemiology of carbapenemase-producing *K. pneumoniae* (*3*), he was further questioned about possible contacts of relevance. The patient disclosed that his wife, who had amyotrophic lateral sclerosis that required mechanical ventilation, had been hospitalized in a tertiary hospital in the Tel Aviv area for 9 weeks until July 19, 2007. After discharge, she has been staying at home where she was cared for by her son, sister, and nurses; the patient stated that he had limited contact with his wife (he did not participate in her care). The infection control unit of the tertiary hospital was contacted, and the name of the wife was identified in the hospital registry. Carbapenemase-producing *K. pneumoniae* was isolated from her urine on June 8, 2007.

Despite limited contact, the patient probably acquired carbapenemase-producing *K. pneumoniae* from his wife, who was a documented carrier of this organism. Because his early urine cultures (taken after his wife was discharged from hospital) were sterile, we can assume that the transmission of the organism occurred at their home. We cannot rule out that the strain was transferred by an intermediary, such as the couple's son. It is unlikely that the organism was acquired at the private hospitals from which no case of carbapenemase-producing *K. pneumoniae* was reported (in Israel reporting carbapenemase-producing *K. pneumoniae* isolates to health authorities is mandatory). Also, the patient had 2 negative urine cultures.

Carbapenemase-producing *K. pneumoniae* is a recent addition to the pool of multidrug-resistant nosocomial pathogens. Most publications on this organism have focused on issues of structural and molecular epidemiology. Little is known regarding clinical characteristics and importance of infection with this organism. Until now, the strain has been recovered only from hospitalized patients with a longer hospital stay, those given multiple antimicrobial drug courses, and those mechanically ventilated (*3*,*4*). The strain can colonize the urinary,

intestinal, and respiratory tracts, as well as wounds; bloodstream infection is associated with higher death rates than infection at other sites (*4*). Hand carriage is probably the biggest factor in transmission of extended-spectrum β-lactamase producers, and there is little evidence to suggest that carriers of carbapenemase-producing *K. pneumoniae* would be different. Environmental contamination plays a limited role in transmission of the organism (*3*). Caregivers should be aware that multidrug-resistant organisms of nosocomial origin can be transmitted in the community (*5*). Acquisition of such strains is probably of negligible importance in an otherwise healthy person. However, consequences may be different if the recipient of the strain is a debilitated patient.

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References

1. Desphande LM, Jones RN, Fritsche TR, Sader HS. Occurrence and characterization of carbapenemase-producing *Enterobacteriacea*: report from the SENTRY Antimicrobial Surveillance Program (2000–2004). Microb Drug Resist. 2006;12:223–30.
2. Leavitt A, Navon-Venezia S, Chmelnitsky I, Schwaber MJ, Carmeli Y. Emergence of KPC-2 and KPC-3 in carbapenem-resistant *Klebsiella pneumoniae* strains in an Israeli hospital. Antimicrob Agents Chemother. 2007;51:3026–9.
3. Patel JB, Srinivasan A. Carbapenem resistance in *Enterobacteriaceae*. Presented at the 107th American Society for Microbiology General Meeting; 2007 May 21–25; Toronto, Ontario, Canada.
4. Agmon O. Shwartz O, Gotesman T, Dan M. A year with KPC at an urban hospital in Israel. Presented at the 8th Congress of the International Federation of Infection Control; 2007 Oct 18–21; Budapest, Hungary.
5. Calbo E, Romaní V, Xercavins M, Gómez L, Vidal CG, Quintana S, et al. Risk factors for community-onset urinary tract infections due to *Escherichia coli* harbouring extended-spectrum beta-lactamases. J Antimicrob Chemother. 2006;57:780–3.

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Alternatives to Ciprofloxacin Use for Enteric Fever, United Kingdom

To the Editor: In cases of typhoid and paratyphoid fever, it is often necessary to commence treatment before the results of laboratory sensitivity tests are available. It is therefore important to be aware of optional drug therapies available because some organisms may be resistant to key antimicrobial drugs. For typhoid and paratyphoid, ciprofloxacin has become the first-line drug of choice since the widespread emergence and spread of strains resistant to chloramphenicol, ampicillin, and trimethoprim (*1*).

The Laboratory of Enteric Pathogens (LEP) of the Health Protection Agency of England and Wales is the reference center for *Salmonella enterica* serovars Typhi and Paratyphi A for the United Kingdom; as such, this laboratory receives isolates from all cases of infection. Isolates are screened by breakpoint for resistance to antimicrobial drugs at the following levels: chloramphenicol, 8 mg/L; ampicillin, 8 mg/L; trimethoprim, 2 mg/L; ciprofloxacin, 0.125 mg/L (decreased susceptibility); and 1.0 mg/L (high-level resistance), ceftriaxone, 1 mg/L, and cefotaxime, 1 mg/L. The levels for testing for resistance to chloramphenicol, ampicillin, trimethoprim, ceftriaxone, and cefotaxime correspond to internationally accepted therapeutic levels for these antimicrobial agents. In contrast, the levels for ciprofloxacin (0.125 and 1.0 mg/L) have been chosen after observations of treatment failures at levels when used at below the expected recommended serum concentrations (*2*,*3*). Since 2005, a proportion of isolates exhibiting decreased susceptibility and high-level resistance to ciprofloxacin have been tested for resistance to azithromycin by Etest (AB Biodisk, Solna, Sweden), using drug-sensitive strains of *S.* Typhi and *S.* Paratyphi A as controls.

From January 2001 through December 2006, LEP reported 1,215 cases of *S.* Typhi infection and 1,274 cases of *S.* Paratyphi A infection. Of these, ≈60% (1,493) reported recent travel abroad; India and Pakistan were the most frequently visited countries (*4*). Other cases were associated with persons who had a history of such travel, but the numbers involved were difficult to document accurately because of underreporting of foreign travel and other communication problems.

For *S.* Typhi, the occurrence of isolates resistant to ciprofloxacin at 0.125 mg/L increased from 60 (35%) of 170 in 2001 to 169 (70%) of 240 cases in 2006, with 4.8 (2%) of isolates in 2006 resistant at 1.0 mg/L (Table). The corresponding figures for *S.* Paratyphi A were 58 (25%) of 232 cases in 2001, rising to 84% in 2004, with an incidence of 73% in 2006; 9% of these were resistant to ciprofloxacin at 1.0 mg/L (Table). Moreover, in 2006, 56 isolates of *S.* Typhi (23% of total) exhibited resistance to chloramphenicol, ampicillin, and trimethoprim, 54 (96%) were also resistant to ciprofloxacin at 0.125 mg/L. When tested for resistance to ceftriaxone and cefotaxime, none of the isolates (either *S.* Typhi or *S.* Paratyphi A) were resistant at 1.0 mg/L.

Although the levels of resistance to ciprofloxacin were for the most part below that regarded as therapeutic (MIC 0.25–1.0 mg/L), at least 21 treatment failures have been documented since 2005. These findings demonstrate that the efficacy of ciprofloxacin for first-line treatment of

Table. Incidence of resistance/decreased susceptibility to key antimicrobial agents in isolates of *Salmonella enterica* serovars Typhi and Paratyphi A, United Kingdom, 2001–2006*

Year	% *S.* Typhi resistant to						% *S.* Paratyphi A resistant to					
	No. studied	C	A	Tm	Cp_L	Cp_H	No. studied	C	A	Tm	Cp_L	Cp_H
2001	170	24	23	23	35	0	232	28	27	27	23	2
2002	150	18	17	17	35	1	149	10	9	10	39	3
2003	218	20	20	21	43	1	177	17	18	17	65	12
2004	215	23	23	24	47	2	221	5	5	5	70	14
2005	222	29	29	29	62	2	217	7	7	7	60	12
2006	240	23	24	24	68	2	278	2	3	2	64	9

*C, chloramphenicol; A, ampicillin, Tm, trimethoprim, Cp_L, ciprofloxacin MIC 0.25–1.0 mg/L; Cp_H, ciprofloxacin MIC >1.0 mg/L. No isolates exhibited resistance to ceftriaxone or cefotaxime; of 50 *S.* Typhi and 40 *S.* Paratyphi A isolated in 2005 and 2006, the MIC to azithromycin by E test (AB Biodisk, Solna, Sweden) was not greater than 8 mg/L for *S.* Typhi and 12 mg/L for *S.* Paratyphi A, which corresponds to those of drug-sensitive controls of the respective serotypes.

enteric fever in the United Kingdom has been seriously jeopardized. In cases of treatment failures, commonly used alternative antimicrobial agents have included third-generation cephalosporins such as ceftriaxone. The macrolide antimicrobial azithromycin is also being increasingly used, particularly for patients with hypersensitivity to penicillins (*5*). With this in mind, 50 *S.* Typhi and 40 S. Paratyphi A strains isolated from January 2005 through December 2006, which exhibited resistance to ciprofloxacin at 0.125 mg/L, were tested for resistance to azithromycin by Etest. Results indicated that none of the isolates of *S.* Typhi exhibited MICs >8 mg/L, which corresponded to the MIC to azithromycin of a drug-sensitive control strain of *S.* Typhi (range 4–8 mg/L, MIC_{90} 6 mg/L). For *S.* Paratyphi A, none of the isolates exhibited MICs >12 mg/L, corresponding to that of a drug-sensitive control strain of this serovar (range 6–12 mg/L, MIC_{90} 10 mg/L). Although there are no definitive data on resistance levels for azithromycin in relation to treatment of typhoid and paratyphoid, these findings suggest that resistance to this antimicrobial agent in terms of treatment efficacy has not yet been jeopardized.

These results indicate that the availability of effective antimicrobial agents for the treatment of typhoid and paratyphoid infection is becoming increasingly limited for patients in the United Kingdom. Nevertheless, despite the dramatic upsurge in the occurrence of strains with decreased susceptibility, ciprofloxacin still remains the drug of choice for many physicians. It is reassuring that in cases of treatment failure, third-generation cephalosporins such as ceftriaxone and macrolide antimicrobial agents such as azithromycin appear to be viable alternatives.

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References

1. Threlfall EJ, Ward LR. Decreased susceptibility to ciprofloxacin in *Salmonella enterica* serotype Typhi, United Kingdom. Emerg Infect Dis. 2001;7:448–50.
2. Rowe B, Ward LR, Threlfall EJ. Ciprofloxacin and typhoid fever. Lancet. 1992;339:740.
3. Aarestrup FM, Wiuff C, Mølbak K, Threlfall EJ. Is it time to change the break points for fluoroquinolones for *Salmonella* spp.? Antimicrob Agents Chemother. 2003;47:827–9.
4. Health Protection Agency. Foreign travel-associated illness in England, Wales and Northern Ireland–2007 report. London: Health Protection Agency; 2007.
5. Threlfall EJ, Day M, De Pinna E, Lewis H, Lawrence J. Drug-resistant enteric fever in the UK. Lancet. 2006;367:1576.

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Usutu Virus Sequences in *Culex pipiens* (Diptera: *Culicidae*), Spain

To the Editor: *Usutu virus* (USUV) is an arbovirus and a member of the mosquito-borne cluster within the *Flavivirus* genus. USUV belongs to the *Japanese encephalitis virus* antigenic group, which is closely related to pathogens such as *West Nile virus* (WNV) (*1*).

USUV has been isolated from a human in the Central African Republic and from several mosquito species from tropical and subtropical Africa (*2*). In late summer 2001, USUV emerged in central Europe and caused deaths in several species of resident birds in Austria (*3*). However, monitoring of USUV in dead birds from 2003 through 2005 showed that the absolute numbers of USUV–associated bird deaths declined, although USUV detection persisted in bird tissues (*4*). This decrease in USUV-associated bird deaths was attributed to herd immunity in the bird population (*5*). In the summer of 2005, USUV was detected in a blackbird in Hungary. The complete genomic sequence of the Hungarian USUV strain shared 99.9% identity with the strain circulating in Austria since 2001 (*6*). On the other hand, neutralizing antibodies against USUV have been detected in sera of resident and migrant birds

in the United Kingdom without causing an obvious reduction in the bird population (*7*).

From May through October 2006, monitoring of flaviviruses in mosquitoes was performed in the northeast region of Spain (Catalonia). This monitoring was implemented in the 3 main wetlands of the region: Aigüamolls de la Empordà (Girona Province) near France, where WNV was detected in dead horses in 2000 (*8*); Delta del Llobregat (Barcelona Province); and Delta de l'Ebre (Tarragona Province). Mosquitoes were collected by mosquito control services in these areas. Female mosquitoes were classified and grouped in pools according to date, species, and localization. During this period, 436 pools belonging to 9 mosquito species were collected. The most abundant species was *Culex pipiens* (n = 168).

Viral RNA was recovered from mosquito pools by homogenization and viral RNA extraction with QIAamp Viral RNA Mini Kit (QIAGEN, Valencia, CA, USA), and then generic reverse transcription (RT)-nested PCR was used to identify flaviviruses (*9*). This procedure was used to amplify a specific fragment of the NS5 gene within the flavivirus genome. The 143-bp amplification product was detected by electrophoresis and purified by using QIAquick PCR Purification Kit (QIAGEN). Sequencing reactions were performed with ABI Prism BigDye Terminator Cycle Sequencing v.3.1 Ready Reaction (Applied Biosystems, Foster City, CA, USA), and analyzed by using an ABI PRISM model 3730 automated sequencer (Applied Biosystems). Assembly of the consensus sequences and translation into amino acid sequences was performed with Larsergene DNASTAR group of programs (DNASTAR Inc., Madison, WI, USA). Comparisons with published sequences of known flaviviruses were performed by searches with FASTA program in EMBL database (available from www.ebi.ac.uk/embl/) to identify the detected agent and to study the level of homology.

One pool of *Cx. pipiens* captured in the middle of August 2006 from Delta del Llobregat, in a typical Mediterranean climate that contained 3 female mosquitoes, was positive for flaviviruses. That positive pool was obtained from the center of the village of Viladecans, where different common migratory and sedentary birds such as *Passer domesticus*, *Hirundo rustica*, or *Delichon urbica* feed and nest. The Spanish USUV sequence showed 97.97% homology to USUV strain SAAR-1776 from South Africa; it showed 94.94% similarity with USUV strain Vienna 2001 from Austria and USUV strain Budgapest from Hungary, with 2-nt and 5-nt differences, respectively (Figure). All of these were synonymous mutations and thus did not result in amino acids replacements. The homology data showed that the Spanish strain belongs to USUV species and is more related to the African USUV isolates than to central European isolates.

To date, no bird deaths observed in Barcelona Province have been associated with viral encephalitis. However, this region is where the USUV-specific RT-PCR–positive samples were obtained from *Cx. pipiens* mosquitoes. One possible explanation for these findings is that Spanish USUV could be naturally avirulent for birds because the African strains of USUV appear to be in Africa. Alternatively, USUV and other related viruses such as WNV may have been circulating in Spain for many years, as a result of regular reintroduction by birds migrating from Africa. Under such circumstances, natural genetic resistance, herd immunity, and cross-protective immunity caused by related viruses likely provided at least some protection against symptomatic infections. The discovery of USUV-specific RNA, most related to the African strains of USUV, in *Cx. pipiens* in Spain extends previous evidence (*7*,*10*) that USUV and related flaviviruses such as WNV are being introduced into western Europe from Africa, presumably by migratory birds.

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References

1. Kuno G, Chang GJ, Tsuchiya KR, Karabatsos N, Cropp CB. Phylogeny of the genus *Flavivirus*. J Virol. 1998;72:73–83.
2. Adam F, Diguette J-P. Virus d'Afrique (base de donnes). Centre collaborateur OMS de référence et de recherche pour les arbovirus et les virus de fièvres hémorrhagies (CRORA); Institut Pasteur de Dakar [cited 2008 Mar 1]. Available from http://www.pasteur.fr/recherche/banques/CRORA

Figure. Comparison at nucleotide level of sequenced fragment among related Usutu virus (USUV). Dot indicates coincident nucleotide. The partial nucleotide sequence of detected Spanish USUV has been deposited in the GenBank database under accession no. AM909649. S. Africa, South Africa.

3. Weissenböck H. Kolodziejek J, Url A, Lussy H, Rebel-Bauder B, Nowotny N. Emergence of Usutu virus, an African mosquito-borne *Flavivirus* of the Japanese encephalitis virus group central Europe. Emerg Infect Dis. 2002;8:652–6.
4. Chvala S, Bakonyi T, Bukovsky C, Meister T, Brugger K, Rubel F, et al. Monitoring of *Usutu virus* activity and spread by using dead bird surveillance in Austria, 2003–2005. Vet Microbiol. 2007;122:237–45.
5. Meister T, Lussy H, Bakonyi T, Šikutová S, Rudolf I, Vogl W, et al. Serological evidence of continuing high *Usutu virus* (*Flaviviridae*) activity and establishment of herd immunity in wild birds in Austria. Vet Microbiol. 2008;127:237–48.
6. Bakonyi T, Erdélyi K, Ursu K, Ferenczi E, Csörgő T, Lussy H, et al. Emergence of *Usutu virus* in Hungary. J Clin Microbiol. 2007;45:3870–4.
7. Buckley A, Dawson A, Moss SR, Hinsley SA, Bellamy PE, Gould EA. Serological evidence of *West Nile virus*, *Usutu virus* and *Sindbis virus* infection of birds in the UK. J Gen Virol. 2003;84:2807–17.
8. Murgue B, Murri S, Zientara S, Durand B, Durand JP, Zeller H. West Nile outbreak in horses in Southern France, 2000: the return after 35 years. Emerg Infect Dis. 2001;7:692–6.
9. Sánchez-Seco MP, Rosario D, Domingo C, Hernández L, Valdés K, Guzmán MG, et al. Generic RT-nested-PCR for detection of flaviviruses using degenerated primers and internal control followed by sequencing for specific identification. J Virol Methods. 2005;126:101–9.
10. Figuerola J, Jiménez-Clavero MA, Rojo G, Gómez-Tejedor C, Soriguer R. Prevalence of West Nile virus neutralizing antibodies in colonial aquatic birds in southern Spain. Avian Pathol. 2007;36:209–12.

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Rotavirus P[4]G2 in a Vaccinated Population, Brazil

To the Editor: Gurgel et al. provide an early examination of postmarketing surveillance data from Brazil, one of the first countries to implement routine childhood immunization with Rotarix vaccine (*1*). In a community with reported vaccination coverage of 50%, the P[4]G2 strain was detected in all 21 rotavirus-positive stool samples identified during November 2006–February 2007. Although monitoring effectiveness of Rotarix against P[4]G2 strains is of interest (*2*), the small sample size, short duration of surveillance, and lack of a comparison group preclude firm assessment of an association between P[4]G2 predominance and vaccination.

Because Rotarix was introduced in Brazil in March 2006, most children >12 months old (66 [51%] of 129) in the study were ineligible for vaccination. Genotype P[4]G2 was the only strain identified even in older children, which suggests either a change in disease ecology from vaccination or the random circulation of P[4]G2 strains in the community. Ongoing hospital-based surveillance during 2006 in 3 regional countries that had not introduced rotavirus vaccine (El Salvador, Guatemala, and Honduras) showed that P[4]G2 was the predominant circulating strain (prevalence 68%–81%). Thus, as previously documented (*3*,*4*), the predominance of P[4]G2 strains after Rotarix introduction in Brazil could represent a natural shift unrelated to vaccination.

Evaluation of vaccine effectiveness against specific strains will allow full assessment of the public health impact of vaccination. Although the data are sparse in the study from Gurgel et al., a comparison of the odds of vaccination among rotavirus-positive (cases) versus rotavirus-negative (controls) children shows 80% vaccine effectiveness against P[4]G2 strains among infants <1 year of age, in accordance with recently published data from a controlled trial (*5*). To further elucidate vaccine impact, we are providing support for vaccine effectiveness studies in Nicaragua and El Salvador and conducting strain monitoring before and after licensure throughout Latin America.

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References

1. Gurgel RQ, Cuevas LE, Vieira SCF, Barros VCF, Fontes PB, Salustino ET, et al. Predominance of rotavirus P[4]G2 in a vaccinated population, Brazil. Emerg Infect Dis. 2007;13:1571–3.
2. Glass RI, Parashar UD, Bresee JS, Turcios R, Fischer TK, Widdowson MA, et al. Rotavirus vaccines: current prospects and future challenges. Lancet. 2006;368:323–32.
3. Desselberger U, Iturriza-Gomara M, Gray JJ. Rotavirus epidemiology and surveillance. Novartis Found Symp. 2001;238:125–47.
4. Santos N, Hoshino Y. Global distribution of rotavirus serotypes/genotypes and its implication for the development and implementation of an effective rotavirus vaccine. Rev Med Virol. 2005;15:29–56.
5. Vesikari T, Karvonen A, Prymula R, Schuster V, Tejedor JC, Cohen R, et al. Efficacy of human rotavirus vaccine against rotavirus gastroenteritis during the first 2 years of life in European infants: randomised, double-blind controlled study. Lancet. 2007;370:1757–63.

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To the Editor: Gurgel et al. described the predominance of P[4]G2 rotaviruses in a vaccinated population in Aracaju, northeastern Brazil (*1*). However, several limitations need to be addressed to avoid misinterpretation of data that could lead to loss of confidence in the vaccine in Brazil and other countries.

Brazil was one of the first countries in Latin America to introduce a live, oral, attenuated human rotavirus vaccine into a public-sector health program. Nevertheless, vaccine coverage levels vary considerably across regions (≈40% to >80%) and are ≈50% in some parts of northern and northeastern Brazil. Therefore, drawing conclusions about the vaccine's protection and prevailing rotavirus genotypes in a setting where coverage is still low seems premature.

Two findings require special consideration. First, although the number of patients is small, children <1 year of age showed a reduced risk for severe rotavirus diarrhea among vaccinated (7%) patients compared with nonvaccinated (26%) patients: $p<0.05$; odds ratio (OR) 0.20; exact 95% confidence interval (CI) 0.029–1.24. Second, surveillance was conducted for only 4 months, which did not allow for demonstration of a true representative pattern of strain distribution over time. The sequential changing predominance of rotavirus serotypes occurring over time has been well documented for many years (*2*).

The authors stated that the "vaccine does not afford complete protection against infection" (*1*). For those not paying close attention to data analysis, this statement could be misinterpreted to mean that the vaccine may not protect against P[4]G2. To the contrary, even with a small sample size and low vaccine coverage, additional analyses of the original data show that the live, oral, attenuated human rotavirus vaccine can protect against the 100% predominance of P[4]G2. In a large phase III trial conducted in Latin America and Finland, a nonsignificant but protective trend was observed against severe disease associated with P[4]G2 (*3*). Furthermore, in a subsequent meta-analysis, protection against P[4]G2 rotavirus gastroenteritis of any severity was 81% (95% CI 31–96) and protection against severe rotavirus gastroenteritis was 71% (95% CI 20–91) (*4*).

To reinforce the hypothesis that predominance of P[4]G2 strains in Aracaju is unrelated to vaccine use, it is worth mentioning that P[4]G2 rotaviruses appear to display an ≈10-year cyclic pattern of occurrence in Brazil (*5*). Although the data presented in the original article may cause misinterpretation about vaccine protection, the article highlights the need for well-designed postmarketing studies to assess both vaccine impact and strain surveillance, in compliance with recent World Health Organization recommendations (*6*).

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References

1. Gurgel RQ, Cuevas LE, Vieira SCF, Barros VCF, Fontes PB, Salustino EF, et al. Predominance of rotavirus P[4]G2 in a vaccinated population, Brazil. Emerg Infect Dis. 2007;13:1571–3.
2. Velázquez FR, Calva JJ, Guerrero ML, Mass D, Glass RI, Pickering LK, et al. Cohort study of rotavirus serotype patterns in symptomatic and asymptomatic infections in Mexican children. Pediatr Infect Dis J. 1993;12:54–61.
3. Ruiz-Palacios GM, Pérez-Schael I, Velázquez FR, Abate H, Breuer T, Costa-Clemens SA, et al. Safety and efficacy of an attenuated vaccine against severe rotavirus gastroenteritis. N Engl J Med. 2006;354:11–22.
4. O'Ryan M. Rotarix (RIX4414): an oral human rotavirus vaccine. Expert Rev Vaccines. 2007;6:11–9.
5. Linhares AC. Rotavirus infection in Brazil: epidemiology and challenges for its control [in Portuguese]. Cad Saude Publica. 2000;16:629-46.
6. World Health Organization. Rotavirus vaccines. Wkly Epidemiol Rec. 2007;82: 285–96.

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In Response: We acknowledge the comments by Patel et al. (*1*) and by Linhares and Velázquez (*2*) about our article that documented the presence of a single rotavirus genotype (P[4]G2) in Aracaju, northeastern Brazil, after the introduction of a human, monovalent rotavirus vaccine (*3*). Both letters emphasize that the predominance of P[4]G2 may be caused by a natural genotype variation unrelated to vaccination. We agree that our observation could be explained by natural variation of circulating rotavirus genotypes in the region, but an alternative possibility is that the introduction of the G1P[8] rotavirus vaccine into the childhood immunization schedule created conditions in which P[4]G2 strains had a selective advantage over strains with which the vaccine shares G type, P type, or both.

According to a systematic review of rotavirus genotypes reported in the 25 years preceding introduction of the vaccine in Brazil, the prevalence of P[4]G2 strains varied from 19% (1986–1995) to 12% (1996–2000) to 1% thereafter, thus not reaching the detection rate we observed in Aracaju (R.Q. Gurgel et al., unpub data). Furthermore, in the ensuing 8-month period, no genotype other than P[4]G2 had been detected in Aracaju, suggesting that our initial findings were not spurious (R.Q. Gurgel et al., unpub data). In addition, in a separate study we conducted in Recife, a city 500 km north of Aracaju, we observed a significant increase in the proportion of G2 strains

detected from 47% (21/45) during the 3-month period immediately after vaccine introduction (March 2006–May 2006) to 100% (11/11) during the same 3-month period 1 year after the vaccine introduction (March 2007–May 2007) (*4*). We believe that our findings are consistent with results of field trials that indicated that the vaccine provided relatively less protection against P[4] G2 strains than against other rotavirus strain types (*5*).

The beneficial impact of rotavirus vaccination in northeastern Brazil is reflected in the reduction of the detection rate of rotavirus among severe diarrhea cases in our study in Recife, which fell from 27% (45/166 cases) to 5.0% (11/221 cases) in the postvaccine 3-month reporting periods, respectively (*4*). Our data from Aracaju are indicative of heterotypic protection, although this is not statistically significant (*1*), against P[4]G2 strains. Further postlicensure studies in Brazil are required to document continuing effectiveness of the national vaccination program as well as to closely monitor the circulating rotavirus strain types (*6*).

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References

1. Patel MM, de Oliveira LH, Bispo AM, Gentsch J, Parashar UD. Rotavirus P[4] G2 in a vaccinated population, Brazil [letter]. Emerg Infect Dis. 2008;14:863.
2. Linhares AC, Velázquez FR. Rotavirus P[4]G2 in a vaccinated population, Brazil [letter]. Emerg Infect Dis. 2008;14:864.
3. Gurgel RQ, Cuevas LE, Vieira SCF, Barros VCF, Fontes PB, Salustino ET, et al. Predominance of rotavirus P[4]G2 in a vaccinated population, Brazil. Emerg Infect Dis. 2007;13:1571–3.
4. Nakagomi T, Cuevas LE, Gurgel RQ, Elrokhsi SH, Belkhir YA, Abugalia M, et al. Apparent extinction of non-G2 rotavirus strains from circulation in Recife, Brazil, after the introduction of rotavirus vaccine. Arch Virol. 2008;153:591–3.
5. Ruiz-Palacios GM, Perez-Schael I, Velazquez FR, Abate H, Breuer T, Clemens SC, et al. Safety and efficacy of an attenuated vaccine against severe rotavirus gastroenteritis. N Engl J Med. 2006;354:11–22.
6. Rotavirus vaccines. Wkly Epidemiol Rec. 2007;82:285–96.

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Erratum: Vol. 14, No. 4

In the article "Reassortant Avian Influenza Virus (H5N1) in Poultry, Nigeria, 2007" by I. Monne et al., the author affiliations contained errors. Isabella Monne, Tony M. Joannis, Alice Fusaro, Paola De Benedictis, Giovanni Cattoli, and Ilaria Capua are affiliated with Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Padova, Italy.

We regret any confusion this error may have caused.

Twelve Diseases That Changed Our World

Irwin W. Sherman

ASM Press, Washington, DC, USA, 2007
ISBN-10: 978-1555814662
ISBN-13: 978-1555814663
Pages: 219; Price: US $29.95

Twelve Diseases That Changed Our World offers engaging observations on a dozen diseases to serve 2 goals. The opening chapters meet the title's promise by tracing the impact of hereditary blood disorders porphyria and hemophilia on the succession of European monarchs in the 16th through 18th centuries. Also presented is a riveting account of the consequences of a potato blight in 1840s Ireland, which forced migration of millions to England and North America. Thereafter, the book turns to the topic of infectious diseases and the lessons learned from earlier responses to "unanticipated outbreaks of disease" to inform preparedness for future outbreaks. Specifically, the chapters are devoted to the study of cholera, smallpox, bubonic plaque, syphilis, tuberculosis, malaria, fever, influenza, and AIDS. These topics are familiar territory for Dr. Sherman, who recently authored The Power of Plagues, in which he also examines 7 of these infections; his command of the subject matter is evident.

Each chapter is packed with information ranging from pathogenesis and clinical manifestations to epidemiologic calculations and antimicrobial drug resistance. A limited number of references are provided in the concluding book notes, grouped by chapter and page number, which offer additional resources for readers seeking more information. Of particular interest is the book's accounting of 19th-century pioneers in epidemiology and infectious diseases. John Snow's use of early epidemiologic tools to associate cholera deaths with water from the Broad Street pump, Louis Pasteur's development of vaccines, and Robert Koch's discovery of tubercle bacillus and the cholera vibrio all get their deserved attention; Florence Nightingale's use of numerical data to demonstrate improvements in patient hygiene comes as a pleasant surprise. A concise volume written for the general reader, Twelve Diseases That Changed Our World provides an excellent foundation for the study of public health and infection control.

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Superantigens: Molecular Basis for Their Role in Human Diseases

Malak Kotb and John D. Fraser, editors

ASM Press, Washington, DC, USA, 2007
ISBN-10: 1555814247
ISBN-13: 978-1555814243
Pages: 263; Price: US $129.95

This collection of short reviews by experts in the field provides a complete overview of microbial superantigens, an unusual family of proteins that form an abnormal linkage between the major histocompatibility complex class II antigens and specific T-cell repertoire Vβ families. This linkage leads to the nonspecific activation of large numbers of regulatory T lymphocytes, producing cytokine storms that can have a variety of serious clinical consequences.

The book is organized into 5 sections with a total of 16 chapters. The first section is an overview of the breadth and scope of superantigen research, including an up-to date catalog of superantigens characterized from both bacteria and viruses, their cellular interactions, and disease associations. The next 3 chapters deal with the 3-dimensional structure, function, and diversity of superantigens, including an account of the critical involvement of zinc in the optimal binding of some of these proteins. Section 3 contains an entire chapter that describes the pathophysiology of superantigens in both acute and chronic skin disorders. Several chapters in section 4 describe in vitro and animal model systems for the study of diseases caused by superantigens, including autoimmune disease, neuropathology, toxic shock, and others.

The final 4 chapters in section 5 detail various therapeutic approaches for superantigen-mediated diseases. These approaches include conventional antibiotics, antagonistic peptides, intravenous immunoglobulin, antibodies directed to T-cell costimulatory receptors, and superantigen receptor mimics, in addition to existing and experimental approaches. An unnumbered section after the first chapter contains high-quality color plate illustrations, which collectively provide outstanding visual support for several chapters.

Superantigens affords a comprehensive look at the current state of knowledge regarding these interesting proteins in a relatively compact volume. The text is certainly a must-read for any scientist engaged in their study but will also prove a rewarding read for microbiologists interested in this curious interaction between mibrobes and the immune system.

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Animal Viruses: Molecular Biology

Thomas C. Mettenleiter and Francisco Sobrino

Caister Academic Press, Norfolk, UK, 2008
ISBN: 978-1904455226
Pages: 531; Price: US $300.00

In this multi-author work, Mettenleiter and Sobrino have compiled 10 chapters that describe what is currently known about the molecular biology of some of the most interesting viruses of veterinary importance, from the tiny circovirus of pigs (1,800 nt of single-stranded DNA) to the highly complex African swine fever virus (≈200,000 nt pairs of double-stranded DNA). It is fitting that the first chapter describes foot-and-mouth disease virus, which was the first animal virus to be described by Loeffler and Frosch, who worked in Griefswald-Insel Riems, where Mettenleiter is currently the president of the Friedrich-Loeffler Institut. All 10 chapters are written by experts in their respective fields. Mettenleiter is a coauthor for a chapter about herpesviruses, whereas Sobrino is a coauthor for one on foot-and-mouth disease virus. Polly Roy wrote a chapter about bluetongue virus, one of the major threats to the livestock industry worldwide, which recently emerged in Europe, perhaps because global warming has allowed the *Culicoides* vector to survive and overwinter. Another chapter is about Hendra and Nipah viruses, which are newly emerging in Southeast Asia and Australia. There are also informative chapters on arteriviruses, coronaviruses, and pestiviruses. Finally, in 1 chapter, Hans-Dieter Klenk and colleagues write about viruses of birds, including avian influenza. They discuss the molecular mechanism of pathogenesis and host range for the virus everyone fears may give rise to the next influenza pandemic.

The book would have been improved by including a chapter on paramyxoviruses, of which rinderpest virus of cattle and Newcastle disease virus of birds are 2 important examples. But, overall, this compilation is excellent and is rounded off by a scholarly and provocative epilogue about animal virology by Esteban Domingo and Marian C. Horzinek. It is almost worth buying the book for these 10 pages alone.

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AIDS Therapy, 3rd Edition

Raphael Dolin, Henry Masur, and Michael S. Saag, editors

Churchill Livingstone, New York, New York, USA, 2007
ISBN-10: 044306752X
ISBN-13: 978-04430675257
Pages: 1,204; Price US $189.00

Reviewing and summarizing the treatment of HIV disease and its complications is a daunting task. Writing a textbook incorporating the rapidly evolving treatments and management strategies is even more difficult. In this third edition of AIDS Therapy, the authors have combined the efforts of international experts to fulfill this goal. As with every textbook, references are a little outdated; few references are more recent than 2006. The addition of online access to updates will possibly alleviate this problem, although the online version still lists the Department of Health and Human Services guidelines for antiretroviral use from October 2006.

Excellent chapters cover the serologic diagnosis of HIV disease, primary care in industrialized and resource-limited countries, strategic use of antiretroviral agents, immune-based therapies, and special clinical settings. Although the management of pregnant HIV-positive patients is discussed, no individual coverage of pediatrics is provided.

The text provides comprehensive reviews of each antiretroviral agent, summarizing pharmacology, adverse reactions, and clinical uses, and extensively reviewing major trials for each agent. For some of these agents, this represents a historical review of monotherapy without practical application. For example, a full chapter is devoted to zalcitabine, an agent that was discontinued in June 2006. For antiretroviral agents, the best summary, referred to as "recommendations for use," is included in the last section of each drug chapter.

Individual chapters describe opportunistic infections and malignancies, including their diagnosis, therapy, and prevention of these diseases. Variability in the length of these chapters does not always correlate with the importance of these processes. The inclusion of multiple charts and algorithms provides a useful approach to diagnosis and management. The last major section of the text provides approaches to specific syndromes including the major problems in patient

care. These are excellent chapters and will be useful to clinicians evaluating specific syndromes. The lack of color pictures in the dermatologic and oral manifestations sections (even in the online version) is a drawback. The final chapters on drug administration and medications are useful tabulations of drug interactions, dosing, and adverse events.

This is an excellent comprehensive source book for AIDS clinicians, although it should not be considered a rapid guide to treatment options. This is a text that will be useful for understanding the basis of our current drug therapy. In contrast, the chapters discussing specific disease processes or syndromes will be extremely useful for the busy clinician looking for a single source for these conditions.

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Bedtime at Nana and Pop's House

Stan Shuman

Requires a hug and a kiss
From the two year old
In Mickey Mouse pajamas,
Climbing on my lap,
Interrupting the crime-news on T.V.,
Smack! a kiss on the left ear,
Smack! a kiss on the right,
"Eye, eye," the imp insists,
(Thank goodness for eyeglasses)
"Nose, nose," comes the next command.

I panic (what to do?!)
This adorable, cute, bright, affectionate kid,
My own grandchild,
Heading for the lips, now!
(What a strange ritual
The young parents have invented)

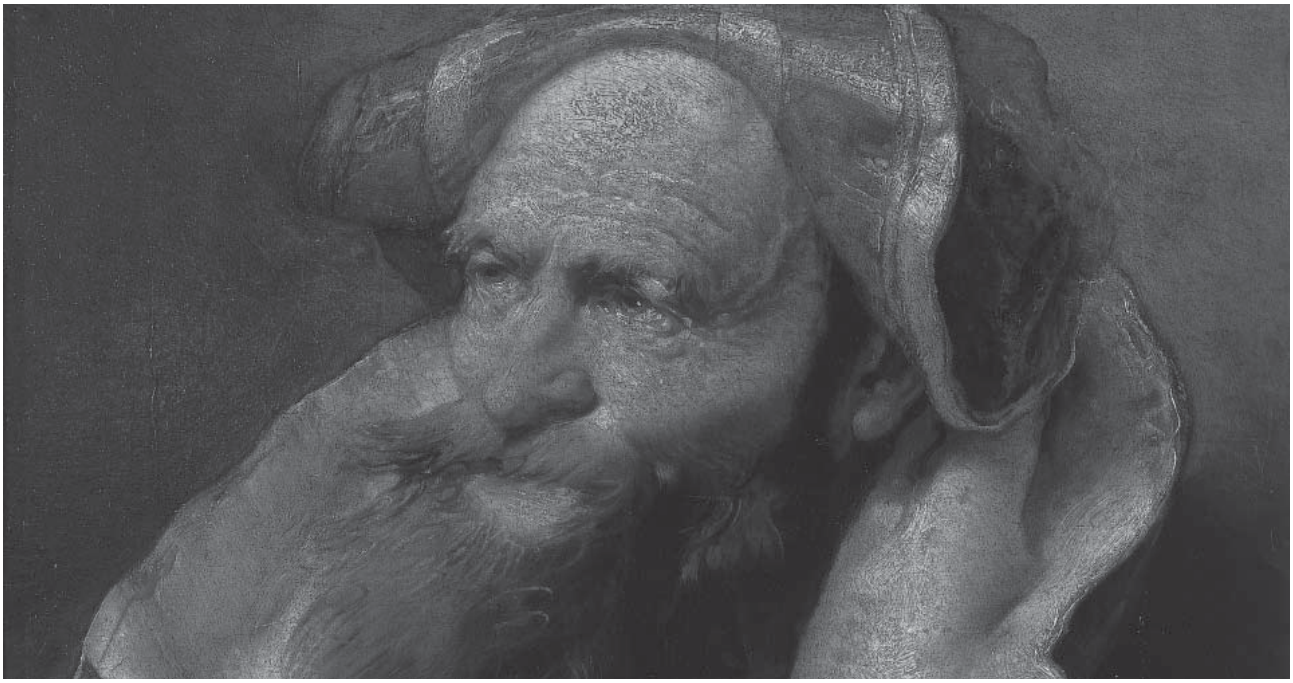
All I can think of, are GERMS:
Giardia, Hemophilus, E. coli,
Strep, Staph, and Pneumo,
A host of enterorespiratory viruses
Multiplying on this adorable child's pink
Mucous membranes, fingertips,
His droplets and aerosols a sea of microbes.

I suddenly thrust him
At arm's length, crown him
With a kiss on the curls
Of the cranium, blow
A few more long-distance
Kisses as I hand him
To his mother
(Before any more infestation can occur).

I return to the gloomy T.V.,
Wondering what the incubation
Periods are for the most likely
Forms of gastroenteritis, hepatitis,
Pink eye, U.R.I. and
Bronchopneumonia.

How fortunate the non-medical
Parents and co-grandparents,
Who hug, hug; kiss, kiss
Without worry or care!

Stan Schuman is professor emeritus at the Medical University of South Carolina, Charleston, SC, USA, and founding editor of the Journal of Agromedicine (Haworth Medical Press, New York, 1974).



Giovanni Battista (Giambattista) Tiepolo (1696–1770) Bust of an Old Man (c. 1751–1755). Oil on canvas (61 cm × 50.5 cm). National Gallery, Prague, Czech Republic

"His Lyre Is Now Attuned Only to Woe"

—Petrarch

Polyxeni Potter*

"Dreams and fables I fashion," wrote 18th-century Italian poet and librettist Pietro Metastasio, "and even while I sketch elaborate fables and dreams upon paper ... I so enter into them that I weep and am offended at ills I invented" (*1*). Metastasio might have been describing the work of his contemporary Giambattista Tiepolo, whose art blended history, mythology, legend, and scripture in a grand manner. His frescoes, which graced the palaces and princely courts of Europe, embodied the notion popular in his day that painting, like theater and opera, was staged fiction and should engage the viewer on an imaginary level (*2*).

Tiepolo was born in Venice, itself a fanciful and theatrical city of canals, lagoons, piazzas, and palaces, the center of artistic splendor, even during its decline in the 18th century. From a family of painters, draftsmen, and etchers, he was apprenticed as a youth to academic master Gregorio Lazzarini. He married Maria Cecilia, sister of painters Gianantonio and Francesco Guardi, and by age 21, he was an established painter too. He had nine children. Two followed in their father's footsteps and became his assistants; one, Domenico, became a great artist in his own right (*3*).

Tiepolo was influenced by the work of 16th-century master Paolo Veronese, particularly in his use of sumptuous often anachronistic costumes, and was often called *Veronese redivivus* (a new Veronese) (*4*). He learned eloquence and drama from Titian and Tintoretto and admired the work of his contemporary Giovanni Battista Piazzetta. His education was complex and varied, enriched by his circle of friends, patrons, collectors, and connoisseurs of the stage and its extravagance, among them cosmopolitan courtier and art critic Francesco Algarotti, who wrote a treatise on opera.

Extremely versatile, Tiepolo mastered multiple artistic forms and media (etchings, watercolors, oils) and made brilliant use of chiaroscuro—use of light and dark to create depth. He produced altarpieces as well as portraits and

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was a prodigious sketcher who infused his work with humor and irony. He is most acclaimed for his frescoes in the Palazzo Labia in Venice, the Royal Palace in Madrid, and the Würzburg Residenz in Germany.

The fresco, a style of painting on wet plaster requiring "rapid and resolute" execution, suited Tiepolo's talent and the sense of immediacy central to his work. His mastery of composition, color, and perspective enabled him to make the most of available spaces. He either ignored architectural boundaries or used them to frame or expand images and create convincing illusions in large biblical and mythologic scenes. He often collaborated with renowned architect Girolamo Mengozzi-Colonna, who designed for the theater, to increase illusionist effects by incorporating architectural features for decorative richness, affirming a centuries-old tradition of exchange between stage design and painted narrative.

"All spirit and fire" was how Tiepolo was described in his time, also the time of François Boucher, Jean-Honoré Fragonard, Jean-Antoine Watteau, and Canaletto (*5*). Though praised as "the most celebrated of the virtuosi," he remained approachable and accommodating (*5*). "Painters should aim to succeed in great works," he believed, "the kind that can please noble, rich people, for it is they who determine the fortunes of the Masters, and not other people, who cannot buy paintings of great value" (*6*).

The most representative artist of the period, Tiepolo bridged the drama and grandeur of the Baroque and the frivolity and decorativeness of Rococo. In the last years of his life, the rise of neoclassicism damaged his career but did not have a long lasting impact on his legacy.

"The mind of the painter must always be directed towards the Sublime, the Heroic, towards perfection," Tiepolo believed (*6*). He valued the world of heroes and gods and imagination over appearances. His taste for perfection also showed in his drawings of archeological artifacts: busts, statues, and decorative heads, particularly distinguished by their emphasis on exotic costumes, turbans, and antique features. His interest extended to bust-length depictions of philosophers or sages. These drawings were props for large paintings, sometimes previewed by collectors and patrons.

Bust of an Old Man, on this month's cover, likely belongs to these independent pieces within Tiepolo's work, which were later transferred into prints by his son. They were produced in the 1750s as studies but soon became valued by collectors as original works. Apart from their skillful expression of physiognomy, these portraits also displayed the artist's facility with light effects on unusual fabrics, inspired by Dutch painting (*7*).

"Help me to crease the pleats of an emerald sleeve Giambattista Tiepolo, Paolo Veronese," mused poet Derek Walcott in "Tiepolo's Hound," expressing the intensity and complexity of feeling evoked by the artist's work (*8*). Tiepolo's old man is spectacularly attired in shimmering silk and velvet tossed flamboyantly over his head and shoulders, adorned with a large pendant. His ringed hand grasps a book. His face is alert and focused, eyes glaring, mouth protruding deliberately, beard untamed.

Old age is not a frequent subject in art. This study by Tiepolo is in line with his choice of noble figures, in this case the holder of knowledge and experience as he might appear on life's stage in some elaborate production with a huge cast. Even as the old man recedes into his finery, he shows nothing of weakness, dependency, or illness. This venerable icon exemplifies verisimilitude as practiced by Tiepolo and proposed by Goethe as the object of all art, "Not to counterfeit nature but to create a harmonious whole that gave a semblance of reality" (*2*).

The sage as envisioned by Tiepolo, Rembrandt, and other Old Masters is endangered in our times. By definition immunocompromised, he and all the elderly are at increased risk for multiple health threats to their viability and prowess. In this journal issue alone, increases are noted in hospitalizations for pneumonia and community-acquired staphylococcal infections (*9*). The persistence of Osler's "old man's friend" indicates that attention has been scant to the problems of the elderly. Yet they are us. As Petrarch put it, "Men go abroad to admire the heights of mountains, the mighty waves of the sea, the broad tides of rivers, the compass of the ocean, and the circuits of the stars, yet pass over the mystery of themselves without a thought" (*10*).

References

1. Giovanni Battista Tiepolo [cited 2008 Mar 6]. Available from http://www.metmuseum.org/toah/hd/tiep/hd_tiep.htm
2. Tiepolo, theater, and the notion of theatricality [cited 2008 Mar 7]. Available from http://www.encyclopedia.com/printable.aspx?id=1G1:58926049
3. Levey M. Giambattista Tiepolo. His life and art. New Haven (CT) and London: Yale University Press; 1986.
4. Morassi A. Tiepolo. London: Phaidon; 1962.
5. History of art: Baroque and Rococo [cited 2008 Mar 7]. Available from http://www.all-art.org/history294-9.html
6. Haskell F. Patrons and painters: a study in the relations between Italian art and society in the age of the Baroque. New Haven (CT): Yale University Press; 1980.
7. National Gallery in Prague. European art from antiquity to the end of the Baroque. Prague: National Gallery in Prague; 2004.
8. Walcott D. Tiepolo's hound. New York: Farrar, Straus and Giroux; 2000.
9. Meltzer MI. The mystery of increased hospitalizations of elderly patients. Emerg Infect Dis. 2008;14:847–8.
10. Petrarch [cited 2008 Mar 7]. Available from http://humanistictexts.org/petrarch.htm

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EMERGING INFECTIOUS DISEASES

Upcoming Issue

Influenza Pandemic Preparedness in Developing Countries

Managing Potential Laboratory Exposure to Ebola Virus

Syndromic Surveillance Data for Respiratory Syndromes and Pathogen Activity

Population-attributable Risk Estimates associated with *Campylobacter* Infection, Australia

Influenza A Virus (H3N8) in Dogs with Respiratory Disease, Florida

Tuberculosis from *Mycobacterium bovis* in Binational Communities, United States

Transmission of Human Papillomavirus in Heterosexual Couples

Co-Infection of HIV with Syphilis and Hepatitis B among Sex-Trafficked Women and Girls, Nepal

Underreporting of Human Alveolar Echinococcosis, Germany

Rickettsial Seroepidemiology in Farm Workers, People's Republic of China

Lack of Evidence for Human *Neospora caninum* Infection, England

Internet-based versus Phone-based Local Outbreak Investigations

Seroprevalence of Lagos Bat Virus Antibody among Fruit Bats, West Africa

Outbreaks Caused by *Leuconostoc mesenteroides* subsp. *mesenteroides*

Fatal Human Plague and *Yersinia pestis* Persistence in Soil under Natural Conditions

Anaplasma phagocytophilum Infection among *Ixodes ricinus* Ticks, Munich, Germany

Global Distribution of a Novel Rhinovirus Genotype

Increase in Adult *Clostridium difficile*–related Hospitalizations and Case-Fatality Rate, United States

Cryptosporidium spp. and *Giardia intestinalis* in Swimming Pools, Atlanta, Georgia, United States

Complete list of articles in the June issue at http://www.cdc.gov/eid/upcoming.htm

Upcoming Infectious Disease Activities

May 5–7, 2008
Eleventh Annual Conference on Vaccine Research
Baltimore Marriott Waterfront Hotel
Baltimore, MD, USA
http://www.nfid.org/conferences/vaccine08

June 19–22, 2008
13th International Congress on Infectious Diseases
Kuala Lumpur, Malaysia
http://www.isid.org

June 24–27, 2008
ANAEROBE 2008
The 9th Biennial Congress of the Anaerobe Society of the Americas
Marriott Hotel
Long Beach, CA, USA
http://www.anaerobe.org

Announcements

To submit an announcement, send an email message to EIDEditor (eideditor@cdc.gov). In 50–150 words, describe timely events of interest to our readers. Include the date of the event, the location, the sponsoring organization(s), and a website that readers may visit or a telephone number or email address that readers may contact for more information.

Announcements may be posted on the journal Web page only, depending on the event date.

The Public Health Image Library (PHIL)

The Public Health Image Library (PHIL), Centers for Disease Control and Prevention, contains thousands of public health–related images, including high-resolution (print quality) photographs, illustrations, and videos.

PHIL collections illustrate current events and articles, supply visual content for health promotion brochures, document the effects of disease, and enhance instructional media.

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To obtain credit, you should first read the journal article. After reading the article, you should be able to answer the following, related, multiple-choice questions. To complete the questions and earn continuing medical education (CME) credit, please go to **http://www.medscape.com/cme/eid**. Credit cannot be obtained for tests completed on paper, although you may use the worksheet below to keep a record of your answers. You must be a registered user on Medscape.com. If you are not registered on Medscape.com, please click on the New Users: Free Registration link on the left hand side of the website to register. Only one answer is correct for each question. Once you successfully answer all post-test questions you will be able to view and/or print your certificate. For questions regarding the content of this activity, contact the accredited provider, CME@medscape.net. For technical assistance, contact CME@webmd.net. American Medical Association's Physician's Recognition Award (AMA PRA) credits are accepted in the US as evidence of participation in CME activities. For further information on this award, please refer to http://www.ama-assn.org/ama/pub/category/2922.html. The AMA has determined that physicians not licensed in the US who participate in this CME activity are eligible for *AMA PRA Category 1 Credits*™. Through agreements that the AMA has made with agencies in some countries, AMA PRA credit is acceptable as evidence of participation in CME activities. If you are not licensed in the US and want to obtain an AMA PRA CME credit, please complete the questions online, print the certificate and present it to your national medical association.

Article Title

Invasive Group A Streptococcal Disease in Nursing Homes, Minnesota, 1995–2006

CME Questions

1. Which one of the following is *least likely* to be a risk factor for invasive group A streptococcal (GAS) disease?
A. Diabetes mellitus
B. Cancer
C. Chronic obstructive pulmonary disease
D. Depression

2. The incidence of invasive GAS disease among persons older than 65 years compared with the general population is *best described* by which one of the following?
A. Similar
B. Two times higher
C. Three times higher
D. Four times higher

3. Which one of the following is *least likely* to be a cause of outbreaks of invasive GAS disease among nursing home residents in the United States?
A. Frequent invasive procedures
B. Resident-to-resident spread
C. Inadequate infection control
D. Chronically infected resident

4. Which one of the following *best describes* the case-fatality ratio of invasive GAS disease in nursing home residents over 65 years under the surveillance program described for Minnesota?
A. 12%
B. 20%
C. 35%
D. 50%

5. The pattern of invasive GAS disease in nursing homes is *best described* by which one of the following?
A. Sporadic
B. Chronic
C. Epidemic
D. Staff-transmitted

Activity Evaluation

1. The activity supported the learning objectives.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
2. The material was organized clearly for learning to occur.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
3. The content learned from this activity will impact my practice.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
4. The activity was presented objectively and free of commercial bias.				
Strongly Disagree				Strongly Agree
1	2	3	4	5

EMERGING INFECTIOUS DISEASES www.cdc.gov/eid

JOURNAL BACKGROUND AND GOALS

What are "emerging" infectious diseases?

Infectious diseases whose incidence in humans has increased in the past 2 decades or threatens to increase in the near future have been defined as "emerging." These diseases, which respect no national boundaries, include

- New infections resulting from changes or evolution of existing organisms.
- Known infections spreading to new geographic areas or populations.
- Previously unrecognized infections appearing in areas undergoing ecologic transformation.
- Old infections reemerging as a result of antimicrobial resistance in known agents or breakdowns in public health measures.

Why an "Emerging" Infectious Diseases journal?

The Centers for Disease Control and Prevention (CDC), the agency of the U.S. Public Health Service charged with disease prevention and health promotion, leads efforts against emerging infections, from AIDS, hantavirus pulmonary syndrome, and avian flu, to tuberculosis and West Nile virus infection. CDC's efforts encompass improvements in disease surveillance, the public health infrastructure, and epidemiologic and laboratory training.

Emerging Infectious Diseases represents the scientific communications component of CDC's efforts against the threat of emerging infections. However, even as it addresses CDC's interest in the elusive, continuous, evolving, and global nature of these infections, the journal relies on a broad international authorship base and is rigorously peer-reviewed by independent reviewers from all over the world.

What are the goals of Emerging Infectious Diseases?

1) Recognition of new and reemerging infections and understanding of factors involved in disease emergence, prevention, and elimination. Toward this end, the journal

   - Investigates factors known to influence emergence: microbial adaptation and change, human demographics and behavior, technology and industry, economic development and land use, international travel and commerce, and the breakdown of public health measures.
   - Reports laboratory and epidemiologic findings within a broader public health perspective.
   - Provides swift updates of infectious disease trends and research: new methods of detecting, characterizing, or subtyping pathogens; developments in antimicrobial drugs, vaccines, and prevention or elimination programs; case reports.

2) Fast and broad dissemination of reliable information on emerging infectious diseases. Toward this end, the journal

   - Publishes reports of interest to researchers in infectious diseases and related sciences, as well as to public health generalists learning the scientific basis for prevention programs.
   - Encourages insightful analysis and commentary, stimulating global interest in and discussion of emerging infectious disease issues.
   - Harnesses electronic technology to expedite and enhance global dissemination of emerging infectious disease information.

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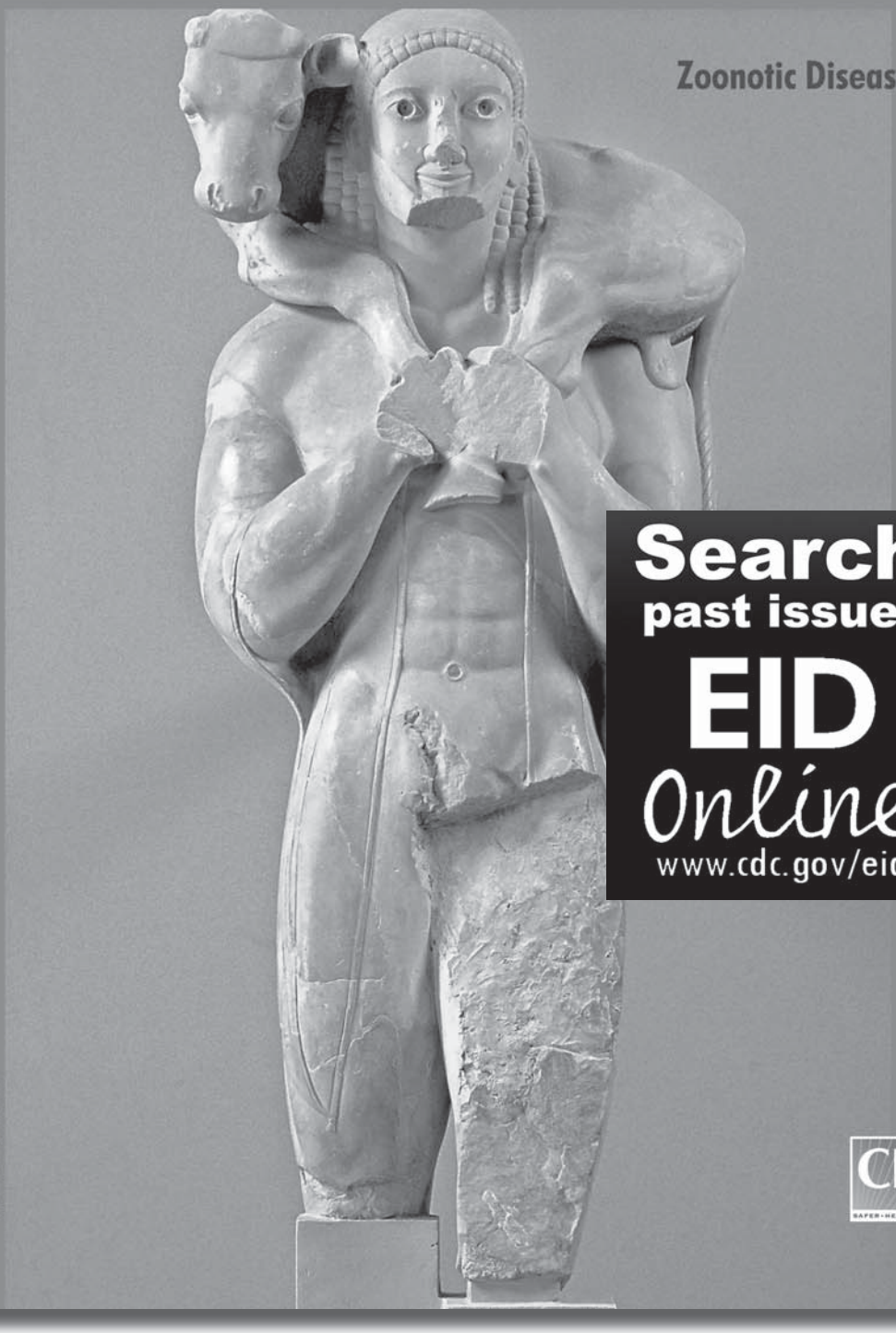
April 2008

Zoonotic Diseases



Acropolis Museum, Athens, Greece

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Emerging Infectious Diseases is a peer-reviewed journal established expressly to promote the recognition of new and reemerging infectious diseases around the world and improve the understanding of factors involved in disease emergence, prevention, and elimination.

The journal is intended for professionals in infectious diseases and related sciences. We welcome contributions from infectious disease specialists in academia, industry, clinical practice, and public health, as well as from specialists in economics, social sciences, and other disciplines. Manuscripts in all categories should explain the contents in public health terms. For information on manuscript categories and suitability of proposed articles see below and visit www.cdc.gov/eid/ncidod/ EID/instruct.htm.

Emerging Infectious Diseases is published in English. To expedite publication, we post articles online ahead of print. Partial translations of the journal are available in Japanese (print only), Chinese, French, and Spanish (www.cdc. gov/ncidod/EID/trans.htm).

Instructions to Authors

Manuscript Preparation. For word processing, use MS Word. List the following information in this order: title page, article summary line, keywords, abstract, text, acknowledgments, biographical sketch, references, tables, figure legends, appendixes, and figures. Each figure should be in a separate file.

Title Page. Give complete information about each author (i.e., full name, graduate degree(s), affiliation, and the name of the institution in which the work was done). Clearly identify the corresponding author and provide that author's mailing address (include phone number, fax number, and email address). Include separate word counts for abstract and text.

Keywords. Include up to 10 keywords; use terms listed in Medical Subject Headings Index Medicus.

Text. Double-space everything, including the title page, abstract, references, tables, and figure legends. Indent paragraphs; leave no extra space between paragraphs. After a period, leave only one space before beginning the next sentence. Use 12-point Times New Roman font and format with ragged right margins (left align). Italicize (rather than underline) scientific names when needed.

Biographical Sketch. Include a short biographical sketch of the first author—both authors if only two. Include affiliations and the author's primary research interests.

References. Follow Uniform Requirements (www.icmje.org/index.html). Do not use endnotes for references. Place reference numbers in parentheses, not superscripts. Number citations in order of appearance (including in text, figures, and tables). Cite personal communications, unpublished data, and manuscripts in preparation or submitted for publication in parentheses in text. Consult List of Journals Indexed in Index Medicus for accepted journal abbreviations; if a journal is not listed, spell out the journal title. List the first six authors followed by "et al." Do not cite references in the abstract.

Tables. Provide tables within the manuscript file, not as separate files. Use the MS Word table tool, no columns, tabs, spaces, or other programs. Footnote any use of boldface. Tables should be no wider than 17 cm. Condense or divide larger tables. Extensive tables may be made available online only.

Figures. Provide figures as separate files, not embedded in MS Word. Use Arial font for text content. Place keys within figure area. Provide footnotes and other information (e.g., source/copyright data, explanation of boldface) in figure legend. Submit figures with text content in native, editable, PC file formats (e.g., MS Excel/PowerPoint). Submit image files (e.g., electromicrographs) without text content as high-resolution (300 dpi/ppi minimum) TIFF or JPG files. Submit separate files for multiple figure panels (e.g., A, B, C). EPS files are admissible but should be saved with fonts embedded (not converted to lines). No PNG or BMP files are admissible. For additional guidance, contact fue7@cdc.gov or 404-639-1250.

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Types of Articles

Perspectives. Articles should be under 3,500 words and should include references, not to exceed 40. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), a one-sentence summary of the conclusions, and a brief biographical sketch. Articles in this section should provide insightful analysis and commentary about new and reemerging infectious diseases and related issues. Perspectives may also address factors known to influence the emergence of diseases, including microbial adaptation and change, human demographics and behavior, technology and industry, economic development and land use, international travel and commerce, and the breakdown of public health measures. If detailed methods are included, a separate section on experimental procedures should immediately follow the body of the text.

Synopses. Articles should be under 3,500 words and should include references, not to exceed 40. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), a one-sentence summary of the conclusions, and a brief biographical sketch. This section comprises concise reviews of infectious diseases or closely related topics. Preference is given to reviews of new and emerging diseases; however, timely updates of other diseases or topics are also welcome. If detailed methods are included, a separate section on experimental procedures should immediately follow the body of the text.

Research Studies. Articles should be under 3,500 words and should include references, not to exceed 40. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), a one-sentence summary, and a brief biographical sketch. Report laboratory and epidemiologic results within a public health perspective. Explain the value of the research in public health terms and place the findings in a larger perspective (i.e., "Here is what we found, and here is what the findings mean").

Policy and Historical Reviews. Articles should be under 3,500 words and should include references, not to exceed 40. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), a one-sentence summary of the conclusions, and a brief biographical sketch. Articles in this section include public health policy or historical reports that are based on research and analysis of emerging disease issues.

Dispatches. Articles should be no more than 1,200 words and need not be divided into sections. If subheadings are used, they should be general, e.g., "The Study" and "Conclusions." Provide a brief abstract (50 words); references (not to exceed 15); figures or illustrations (not to exceed 2); tables (not to exceed 2); and a brief biographical sketch. Dispatches are updates on infectious disease trends and research. The articles include descriptions of new methods for detecting, characterizing, or subtyping new or reemerging pathogens. Developments in antimicrobial drugs, vaccines, or infectious disease prevention or elimination programs are appropriate. Case reports are also welcome.

Commentaries. Thoughtful discussions (500–1,000 words) of current topics. Commentaries may contain references but no figures or tables.

Another Dimension. Thoughtful essays, short stories, or poems on philosophical issues related to science, medical practice, and human health. Topics may include science and the human condition, the unanticipated side of epidemic investigations, or how people perceive and cope with infection and illness. This section is intended to evoke compassion for human suffering and to expand the science reader's literary scope. Manuscripts are selected for publication as much for their content (the experiences they describe) as for their literary merit.

Letters. Letters commenting on recent articles as well as letters reporting cases, outbreaks, or original research are welcome. Letters commenting on articles should contain no more than 300 words and 5 references; they are more likely to be published if submitted within 4 weeks of the original article's publication. Letters reporting cases, outbreaks, or original research should contain no more than 800 words and 10 references. They may have 1 figure or table and should not be divided into sections. All letters should contain material not previously published and include a word count.

Books, Other Media. Reviews (250–500 words) of new books or other media on emerging disease issues are welcome. Name, publisher, number of pages, other pertinent details should be included.

Announcements. We welcome brief announcements (50–150 words) of timely events of interest to our readers. (Announcements may be posted online only, depending on the event date.)

Conference Summaries. Summaries of emerging infectious disease conference activities are published online only. Summaries, which should contain 500–1,000 words, should focus on content rather than process and may provide illustrations, references, and links to full reports of conference activities.