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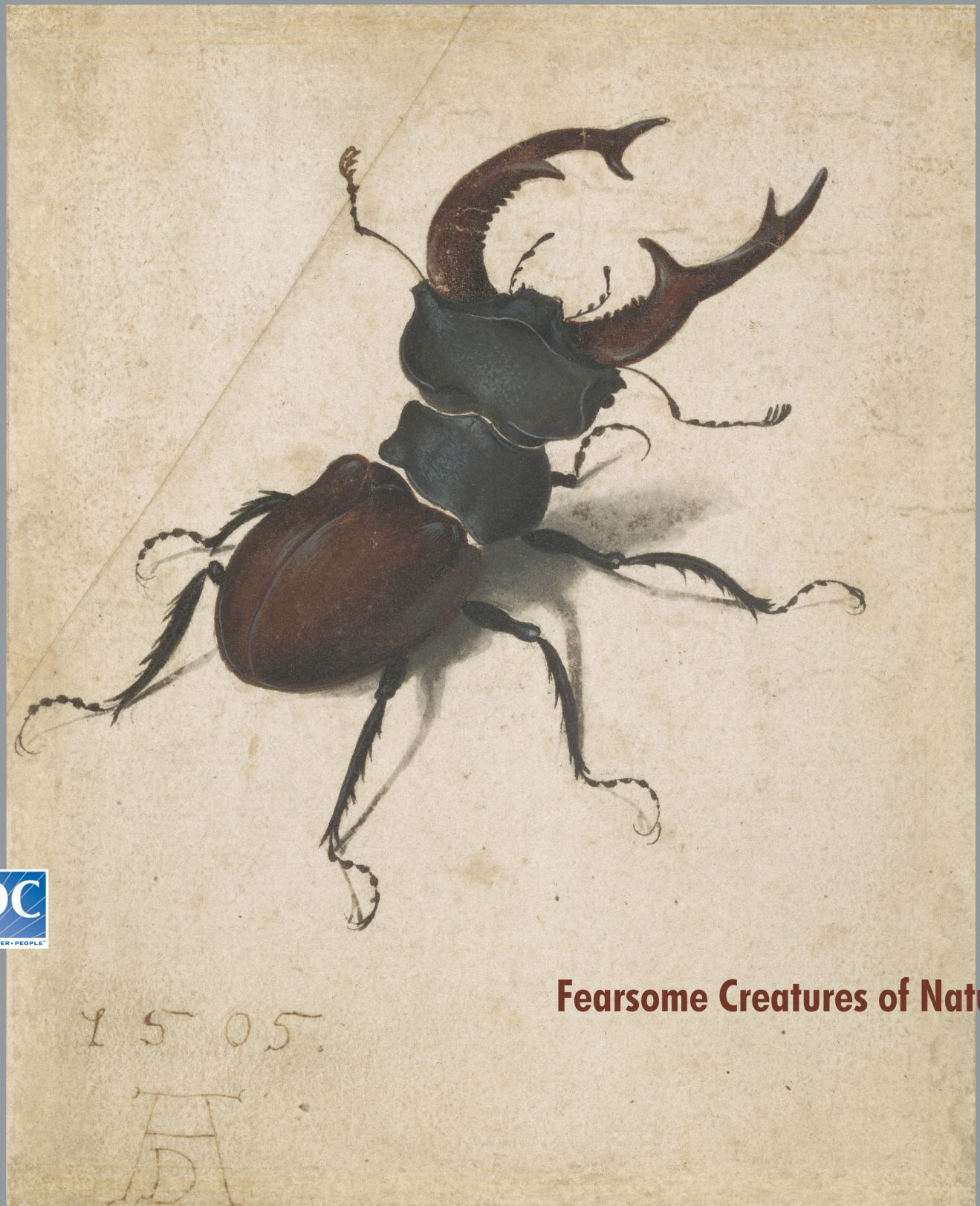

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On the Cover

Albrecht Dürer (1471–1528). Stag Beetle (1505) (detail)
Watercolor and gouache (14.1 cm x 11.4 cm)
The J. Paul Getty Museum,
Los Angeles, California, USA

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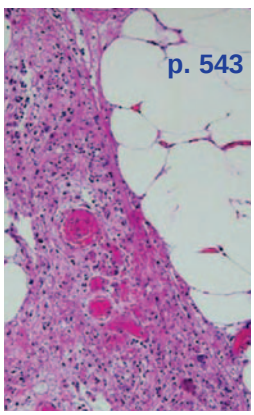
Perspective

Research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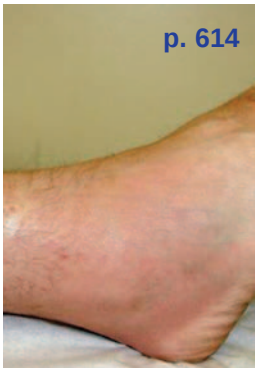

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Emerging Infectious Diseases: a 10-Year Perspective from the National Institute of Allergy and Infectious Diseases

Anthony S. Fauci,* Nancy A. Touchette,* and Gregory K. Folkers*

Although optimists once imagined that serious infectious disease threats would by now be conquered, newly emerging (e.g., severe acute respiratory syndrome [SARS]), reemerging (e.g., West Nile virus), and even deliberately disseminated infectious diseases (e.g., anthrax bioterrorism) continue to appear throughout the world. Over the past decade, the global effort to identify and characterize infectious agents, decipher the underlying pathways by which they cause disease, and develop preventive measures and treatments for many of the world's most dangerous pathogens has resulted in considerable progress. Intramural and extramural investigators supported by the National Institute of Allergy and Infectious Diseases (NIAID) have contributed substantially to this effort. This overview highlights selected NIAID-sponsored research advances over the past decade, with a focus on progress in combating HIV/AIDS, malaria, tuberculosis, influenza, SARS, West Nile virus, and potential bioterror agents. Many basic research discoveries have been translated into novel diagnostics, antiviral and antimicrobial compounds, and vaccines, often with extraordinary speed.

Infectious diseases have been an ever-present threat to mankind. From the biblical plagues and the Plague of Athens in ancient times, to the Black Death of the Middle Ages, the 1918 "Spanish Flu" pandemic, and more recently, the HIV/AIDS pandemic, infectious diseases have continued to emerge and reemerge in a manner that defies accurate predictions (1–3).

The past 10 years (1994–2004) have been no exception, as many new and reemerging microbial threats have continued to challenge the public health and infectious disease research communities worldwide. Since 1994, when Emerging Infectious Diseases made its publication debut, significant strides in the global fight against the HIV/AIDS pandemic have been made. The infectious disease community has confronted several other newly emerging pathogens, such as the severe acute respiratory syndrome–associated coronavirus (SARS-CoV), henipaviruses (Hendra and Nipah), and, most recently, avian influenza viruses that have caused illness and deaths in humans with the threat of evolution into a pandemic (1–3). In addition, historically established infectious diseases, such as West Nile fever, human monkeypox, dengue, tuberculosis, and malaria have reemerged or resurged, sometimes in populations that previously had been relatively exempt from such affronts. Over the past decade, strains of common microbes such as *Staphylococcus aureus* and *Mycobacterium tuberculosis* have continued to develop resistance to the drugs that once were effective against them (1–4). Such antimicrobial-resistant microorganisms, which defy conventional therapies and pose a threat to public health, underscore the need for a robust pipeline of new antimicrobial agents based on innovative therapeutic strategies, new vaccines, and other preventive measures (3,4).

Perhaps most disturbing, the United States has recently experienced a deliberately spread infectious disease in the form of 22 anthrax infections, including 5 anthrax-related deaths resulting from bioterrorism in 2001 (5). These cases were accompanied by widespread psychological sequelae and societal and economic disruptions.

These emerging and reemerging infectious diseases are superimposed on a substantial baseline of established infectious diseases. Although annual deaths and lost years of healthy life from infectious diseases have decreased over the past decade, the worldwide impact from infectious diseases remains substantial. Overall, infectious diseases remain the third leading cause of death in the United

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States each year and the second leading cause of death worldwide (6). As shown in Figure 1, of the estimated 57 million deaths that occur throughout the world each year, ≈15 million, >25%, are directly caused by infectious diseases. Millions more deaths are due to secondary effects of infections (6).

Infectious diseases also lead to compromised health and disability, accounting for nearly 30% of all disability-adjusted life years (DALYs) worldwide (1 disability-adjusted life year is 1 lost year of healthy life) (6). Infectious diseases that contribute to the nearly 1.5 billion total DALYs each year are categorized in Figure 2.

In the United States, the Centers for Disease Control and Prevention has devised strategies to prevent, monitor, and contain disease outbreaks. Within the National Institutes of Health, the National Institute of Allergy and Infectious Diseases (NIAID) is the lead agency for infectious disease research.

Over the past decade, the NIAID budget has quadrupled; spending on emerging infectious diseases has increased from <$50 million in 1994 to >$1.7 billion projected for 2005, a boost due in large part to increases in funding for biodefense research (Figure 3). NIAID-supported intramural and extramural investigators have contributed substantially to the global effort to identify and characterize infectious agents, decipher the underlying pathways by which they cause disease, and develop preventive measures and treatments for many of the world's most dangerous pathogens. This review briefly highlights some of the research strides made by NIAID-supported investigators during the past decade in preventing and combating emerging and reemerging infectious diseases threats.

HIV/AIDS

HIV/AIDS has resulted in the death of >20 million persons throughout the world and is the leading cause of death among persons 15–59 years of age. Approximately 40 million persons are estimated to be living with HIV infection (7). In the United States, an estimated 1 million persons are infected with HIV, and 40,000 new infections occur each year. Since its recognition in 1981, the disease has killed more than half a million people in the United States (8).

Despite these grim statistics, reason for hope exists. Basic research has yielded major insights into the pathogenic mechanisms of HIV disease. This knowledge paved the way for the development of >20 antiretroviral medications approved by the Food and Drug Administration (FDA) that target HIV, as well as novel strategies for prevention and vaccine development (9).

With the use of combinations of drugs that target different proteins involved in HIV pathogenesis (a treatment strategy known as highly active antiretroviral therapy [HAART]), rates of death and illness in the United States and other industrialized countries have been dramatically reduced (7,8) (Figure 4). Although the death rate due to HIV/AIDS in Europe and North America has fallen by 80% since HAART was introduced, relatively few people in poor countries have reaped these benefits. New initiatives such as the Global Fund to Fight AIDS, Tuberculosis, and Malaria and the President's Emergency Plan for AIDS Relief promise to greatly reduce the disparity between rich and poor countries with regard to access to HIV treatment, care, and prevention services (7).

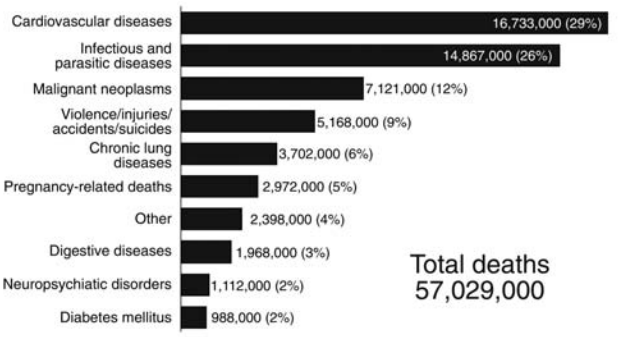

Figure 1. Leading causes of death worldwide (estimates for 2002). Nearly 15 million (>25%) of the 57 million annual deaths worldwide are caused by infectious disease (6).

The greatest challenge in HIV/AIDS research remains developing a vaccine that can either prevent the transmission of the virus or, failing that, halt progression to AIDS. Since 1987, NIAID has funded >70 clinical trials evaluating >50 different HIV vaccine candidates. Unfortunately, the first large-scale phase 3 trial of an HIV vaccine reported in 2003 had disappointing results (10). Many different vaccine strategies, including viral and bacterial vectors, DNA vaccines, viruslike particle vaccines, and peptide vaccines are being investigated, and ≈15 clinical trials in

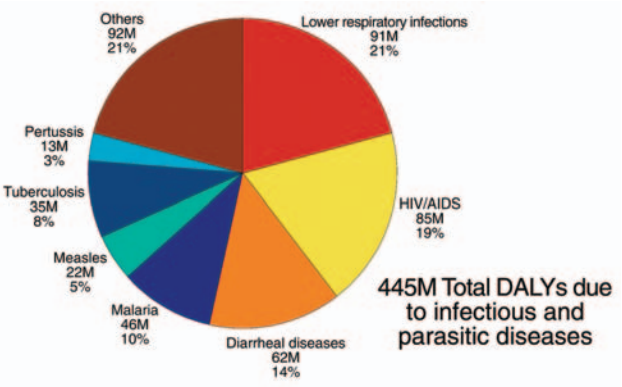

Figure 2. Leading causes of disability life years (DALYs) due to infectious and parasitic diseases (2002 estimates). Lower respiratory infections, HIV/AIDS, diarrheal diseases, and malaria are among the infectious diseases that contribute to the most DALYs lost each year throughout the world (6).

humans are under way. The effects of various adjuvants and different routes of administration also are being tested.

HIV vaccine developers face formidable scientific obstacles, including the virus's genetic diversity and the lack of a clear understanding of the correlates of protective immunity in HIV infection (9). A critical and so far elusive milestone is the discovery of a stable and immunogenic conformational epitope of the HIV envelope that would elicit broadly reactive neutralizing antibodies against primary isolates of HIV (9). To overcome these challenges, collaborations involving government, academia, industry, and philanthropies and new cross-sector partnerships such as the Global HIV Vaccine Enterprise, a virtual consortium of independent organizations, are being established to advance HIV vaccine research and foster greater collaboration among HIV vaccine researchers worldwide (11).

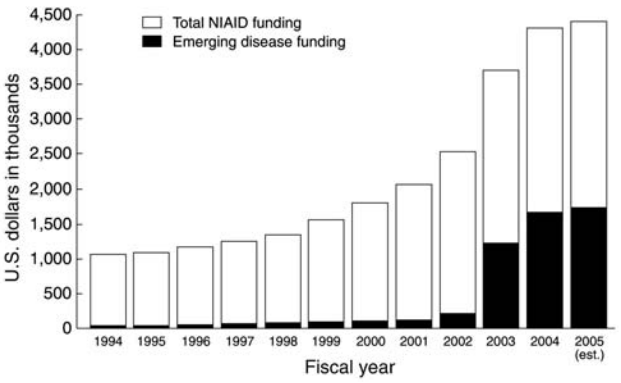

Figure 3. Budget of the National Institute for Allergy and Infectious Disease (NIAID), FY1994–2005. The overall NIAID budget rose from $1.06 billion in FY1994 to $4.4 billion (estimated) in FY2005. Funding for emerging infectious diseases rose from $47.2 million in FY1994 to $1.74 billion in FY2005 (est.).

Malaria

The social, economic, and human toll exacted by malaria globally is widespread and profound. Each year, acute malaria occurs in >300 million people and results in >1 million deaths worldwide. Most of these deaths occur in young children who live in sub-Saharan Africa (3,6).

In humans, the disease is caused by one of 4 species of *Plasmodium*, a single-cell parasite transmitted by anopheline mosquitoes. In 2002, the complete genomic sequence of *Plasmodium falciparum* as well as that of the mosquito vector *Anopheles gambiae* were completed as the result of a multinational effort (12,13). With the genomic sequences of the parasite and its human and mosquito hosts now available, researchers have powerful tools to further characterize the genes and proteins involved in the life cycle of the parasite, and they are using this information to design effective drugs and vaccines.

Drug-resistant *Plasmodium* strains are widespread, as are insecticide-resistant strains of the mosquitoes that carry the parasites. Mutations in both parasites and mosquitoes that confer drug and insecticide resistance have been identified. For example, genetic analysis and molecular epidemiology studies of *P. falciparum* have shown that resistance to chloroquine and other antimalarials is caused by a mutation in a single gene, called *pfcrt* (14,15). This information is being used to track the spread of drug-resistant strains of the parasite and identify new drug targets (16). Researchers also are exploiting the new genomic information to create genetically altered mosquitoes that resist parasite infection and to develop new compounds that overcome or avoid resistance to existing pesticides (17).

Developing an effective antimalarial vaccine has been a challenge; however, an international research team recently developed a vaccine that shows promise in preventing malaria among children in Mozambique. The vaccine prevented infection and severe disease in a substantial percentage of children tested, a breakthrough with the potential of saving millions of lives (18). Specifically, vaccine efficacy for the first clinical episodes was 29.9% (95% confidence interval [CI] 11.0–44.8, p = 0.004). Vaccine efficacy for severe malaria was 57.7% (95% CI 16.2–80.6, p = 0.019) (18). In addition, at least 35 other malaria vaccine candidates have undergone phase 1 clinical trials in humans, and 13 have moved into more advanced clinical development (19). Preclinical development of more than a dozen other candidates is being supported (19).

Tuberculosis

Another ancient microbial scourge that has reemerged in recent years is tuberculosis (TB), caused by infection with the bacterium *Mycobacterium tuberculosis*. This infection is estimated to be prevalent in one third of the world's population. From this reservoir, 8 million new cases of TB develop worldwide each year that carry a death toll of >2 million (3,6). TB is especially prevalent among persons infected with HIV. The only currently available TB vaccine, *M. bovis* bacillus Calmette-Guérin (BCG), offers some protection, but its effect diminishes with time (20). TB drug treatment is effective, but adherence to lengthy therapeutic regimens is difficult to maintain, and multidrug-resistant TB is on the rise in many countries (3,4).

Researchers are applying state-of-the-art genomic and postgenomic techniques to identify key molecular pathways that could be exploited to develop improved TB interventions and vaccines (21,22). In 2004, for the first time in 60 years, 2 new vaccines designed to prevent TB entered phase 1 clinical trials in the United States (23,24). Many promising new anti-TB drug candidates also are

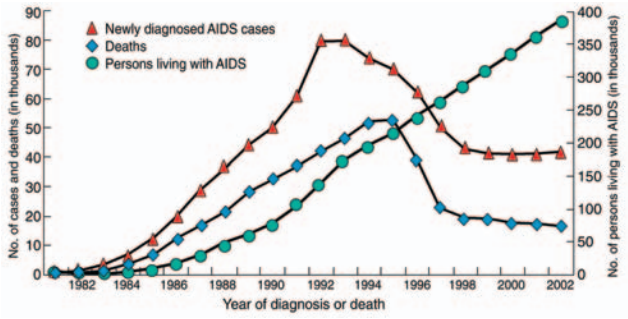

Figure 4. AIDS cases, AIDS deaths, and persons living with AIDS in the United States, 1981–2003. Over the past decade, the number of new AIDS cases and deaths due to AIDS has decreased, while the number of people living with the disease has increased, due in large part to improvements in diagnosis and treatment. Estimates adjusted for reporting delays. Source: CDC (8).

now entering the drug pipeline (25). Derivatives of known anti-TB drugs, such as thiolactomycin and ethambutol, are currently being screened for activity against *M. tuberculosis*. Preclinical development of a highly promising candidate, SQ109 is nearing completion.

Influenza

Each year, influenza develops in up to 20% of all Americans, and >200,000 are hospitalized with the disease. Although influenza is commonplace and generally self-limited, an estimated 36,000 Americans die each year from complications of the disease (26). Worldwide, severe influenza infections develop in 3–5 million people annually, and 250,000–500,000 deaths occur (27).

Outbreaks of avian influenza recently have drawn attention worldwide, particularly in Southeast Asia, where at least 55 persons have been infected and 42 have died since January 2004. The current strain of H5N1 avian influenza is highly pathogenic; it has killed millions of chickens and other birds. Although the virus can cross species to infect humans, few suspected cases of human-to-human transmission have been reported (27). However, the virus could acquire characteristics that allow it to be readily transmitted among humans, which could cause a worldwide influenza pandemic, with the potential for killing millions of people. In 1918, a pandemic of the "Spanish Flu" killed 20–50 million people worldwide (26,27).

Recently, the NIH Influenza Genomics Project was initiated; it will conduct rapid sequencing of the complete genomes of the several thousand known avian and human influenza viruses as well as those that emerge in the future. Approximately 60 genomes are expected to be sequenced each month. This project should also illuminate the molecular basis of how new strains of influenza virus emerge and provide information on characteristics that contribute to increased virulence. Many researchers believe that the H5N1 virus shows the greatest potential for evolving into the next human pandemic strain. Avian H9N2 viruses also have infected humans and have the potential to cause a pandemic. To prepare for this possibility, the development of vaccines to prevent infection with H5N1 and H9N2 viral strains is being supported (28). Researchers also are working to develop a live-attenuated vaccine candidate directed against each of the 15 hemagglutinin proteins that have been isolated, an effort that may speed the development of a vaccine against a potential pandemic strain.

Using reverse genetics, researchers developed a genetically engineered vaccine candidate (called a reference virus) against H5N1 in a matter of weeks, demonstrating the power of this technology (29). The new H5N1 candidate was tested in animals to confirm that it was no longer highly pathogenic (29), and vaccine manufacturers are using the reference virus to develop inactivated vaccines that will be evaluated in phase 1 and 2 clinical trials. Reverse genetics also has been used to identify a specific genetic mutation in a H5N1 viral gene, called PB2, which makes the virus especially lethal. This discovery may be useful in designing antiviral drugs and vaccine candidates (30).

Experiments also are being conducted in which genes isolated from the 1918 influenza strain are cloned into avirulent influenza strains. Researchers recently showed that the hemagglutinin gene from the 1918 virus conferred a high degree of pathogenicity to avirulent influenza strains when introduced into mice (31). These recombinant viruses and others are being evaluated in various animal models, including nonhuman primates, to further determine how genes of the 1918 virus contributed to its ability to spread so rapidly and cause so many deaths, and to understand the molecular basis for its unprecedented virulence. Previous research (32) established the foundation for developing a live-attenuated nasal flu vaccine that was approved by FDA in 2003 for use in healthy adults and children 5–49 years of age (33).

West Nile Virus

West Nile virus (WNV), long endemic in Africa, West Asia, Europe, and the Middle East, represents a reemerging disease that only recently arrived in the United States. The virus first appeared in the New York City area in 1999, where WNV-related disease was reported in 62 persons. It has continued to spread throughout the United States in subsequent summers, infecting ever larger populations, particularly in 2003 (34). Research has led to several promising vaccine candidates against WNV (35). One of these, based on a licensed yellow fever vaccine virus that contains 2 WNV genes, has been tested in nonhuman

primates; it is currently being evaluated in human clinical trials. A second vaccine developed at NIH uses an attenuated dengue virus into which WNV genes have been inserted. This vaccine protects monkeys and horses against WNV infection, and a clinical trial is now underway. Subunit and DNA vaccines against WNV are also in various stages of development and testing.

Several innovative therapies also are being tested to treat persons already infected with WNV. In a clinical trial at >60 sites across the United States and Canada, the protective effect of an immunoglobulin product is being tested in hospitalized patients who are at high risk for or who have WNV encephalitis (36). Technology also has been developed to screen large numbers of chemical compounds for antiviral activity. As of February 28, 2005, 1,500 compounds had been screened in vitro, and 2%–3% were shown to have antiviral activity against WNV. These compounds are undergoing further evaluation in hamster and mouse models of disease. Partnerships with small biotechnology companies have been formed to develop more sensitive and rapid tests for detecting WNV infections. Other studies are ongoing to evaluate the roles of various mosquito vectors and animal reservoirs in virus transmission, to test novel mosquito control methods, and to limit the impact of insecticide resistance on mosquito control (37).

SARS

The emergence of SARS in Asia in late 2002, and the speed with which it was characterized and contained, underscores the importance of cooperation between researchers and public health officials (38). NIAID is focusing its resources on developing diagnostics, vaccines, and novel antiviral compounds to combat SARS-CoV. Basic research on the pathogenesis of the disease to identify appropriate targets for therapeutics and vaccines, as well as clinical studies to test new therapies, is also being supported. Among many projects that have received support are the development of a "SARS chip," a DNA microarray to rapidly identify SARS sequence variants, and a SARS diagnostic test based on polymerase chain reaction technology (38).

Researchers have developed 2 candidate vaccines, based on the SARS-CoV spike protein, that protect mice against SARS (39,40). Another promising vaccine protects against infection in monkeys when delivered intranasally (41). Passive immunization as a treatment for SARS patients is also being investigated. Both mouse and human antibodies against SARS can prevent infection when introduced into uninfected mice (42,43), and an international collaboration has developed a rapid method of producing human anti-SARS antibodies (43). In 2004, in vitro screening of >20,000 chemicals identified ≈1,500 compounds with activity against SARS-CoV, at least 1 of which has been selected by industry as a candidate for further clinical development (38).

Potential Bioterror Agents

The September 11, 2001, attacks on the World Trade Center and Pentagon, and the subsequent anthrax attacks that infected 22 people and killed 5, propelled the U.S. government to expand its biodefense research program (44). These studies are based on 3 approaches: basic research aimed at understanding structure, biology, and mechanisms by which potential bioweapons cause disease; studies to elucidate how the human immune system responds to these dangerous pathogens; and development of the technology to translate these basic studies into safe and effective countermeasures to detect, prevent, and treat diseases caused by such pathogens (44).

At least 60 major NIAID initiatives involving intramural and extramural scientists and industrial partners were funded in fiscal years 2002–2004. Among them are funding for 8 Regional Centers of Excellence for Biodefense and Emerging Infectious Diseases Research and construction of 2 National Biocontainment Laboratories and 9 Regional Biocontainment Laboratories. These facilities will provide the secure space needed to carry out the nation's expanded biodefense research program (44).

The genomes of all biological agents considered to pose the most severe threats have been sequenced by researchers (45). In addition, programs have been expanded and contracts awarded to screen new chemical compounds as possible treatments for bioterror attacks. New animal models have been developed to test promising drugs, and repositories have been established to catalog reagents and specimens (44).

In addition, research to understand the body's protective mechanisms against pathogens is being pursued. The Cooperative Centers for Translational Research on Human Immunology and Biodefense will focus on studies of the human immune response to potential agents of bioterror, while other programs are focused on the innate immune system and the development of ways to boost innate immunity (44).

NIAID also has been very active in vaccine development as a biodefense countermeasure (44). The Institute has supported the development of a next-generation anthrax vaccine, known as recombinant protective antigen (rPA); it is undergoing clinical trials, and contracts for the Strategic National Stockpile to acquire it have recently been awarded. Several new smallpox vaccines also are being tested for safety and efficacy. Preliminary studies in mice and monkeys show that one of these, modified accinia Ankara (MVA), protects against poxvirus infections (46,47). Clinical trials of the MVA vaccine are ongoing at NIAID Vaccine Research Center and elsewhere (44).

A clinical trial of a novel DNA vaccine against Ebola virus also is under way; human testing of an adenovirus-vectored Ebola vaccine is planned for 2005 (48). Vaccine manufacturing and clinical trials also are planned for a new, recombinant vaccine against plague that is highly effective in mice and nonhuman primates (49).

Challenges for the Future

Scientists—government and academic, together with their industrial partners and international collaborators—have made great strides over the past 10 years in understanding many of the pathogenic mechanisms of emerging and reemerging infectious diseases. Many of these discoveries have been translated into novel diagnostics, antiviral and antimicrobial compounds, and vaccines, often with extraordinary speed.

However many challenges remain. Paramount among these is developing a safe and effective HIV vaccine. The evolution of pathogens with resistance to antibacterial and antiviral agents continues to challenge us to better understand the mechanisms of drug resistance and to devise new ways to circumvent the problem. These efforts will pave the way for developing countermeasures against deliberately engineered microbes.

If history is our guide, we can assume that the battle between the intellect and will of the human species and the extraordinary adaptability of microbes will be never-ending. To successfully fight our microbial foes, we must continue to vigorously pursue research on the basic mechanisms that underlie microbial pathogenesis and develop novel strategies to outwit theses ingenious opponents. The past 10 years have been challenging but no more so than will be the future.

Dr. Fauci is director of the National Institute of Allergy and Infectious Diseases (NIAID) of the National Institutes of Health, which is part of the Department of Health and Human Services, Bethesda, Maryland. At NIAID, Dr. Fauci oversees basic and applied research to prevent, diagnose, and treat infectious and immune-mediated illnesses such as HIV/AIDS and other sexually transmitted diseases, illness from potential agents of bioterrorism, tuberculosis, malaria, autoimmune disorders, asthma, and allergies.

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Recurring Methicillin-resistant *Staphylococcus aureus* Infections in a Football Team

Dao M. Nguyen,*† Laurene Mascola,* and Elizabeth Bancroft*

An outbreak of community-associated methicillin-resistant *Staphylococcus aureus* (MRSA) skin and soft tissue infection (SSTI) occurred in a college football team from August to September 2003. Eleven case-players were identified, and boils were the most common sign. Linemen had the highest attack rate (18%). Among 99 (93% of team) players with cultured specimens, 8 (8%) had positive MRSA nasal cultures. All available case-players' MRSA isolates characterized had the community-associated pulsed-field type USA300. A case-control study found that sharing bars of soap and having preexisting cuts or abrasions were associated with infection. A carrier-control study found that having a locker near a teammate with an SSTI, sharing towels, and living on campus were associated with nasal carriage. Successful outbreak control measures included daily hexachlorophene showers and hygiene education.

Football-related skin infections have gained national notoriety and public interest (1,2). Media coverage of high-profile athletes and teams with skin and soft tissue infection (SSTI) has provided more impetus for research of these infections. Annually, 60,000 college football players compete among 600 teams (3). The community-associated methicillin resistant *Staphylococcus aureus* (CA-MRSA) strains have been a cause of SSTI outbreaks among athletes participating in football, wrestling, rugby, soccer, fencing and canoeing (4–7; Jon Rosenberg, pers. comm.; Los Angeles County Department of Health Services, unpub. data). SSTIs (pustules, "insect bites," boils, and abscesses) are the hallmarks of CA-MRSA infections (8,9). CA-MRSA causes disease in young, otherwise healthy persons without the usual risk factors for MRSA infections (9). In addition, CA-MRSA has unique molecular markers (SCC*mec*IV and Panton-Valentine leukocidin) and fewer resistance genes to non β-lactam antimicrobial drugs than healthcare-associated MRSA strains (10,11).

In August 2002, the Los Angeles County Department of Health Services (LACDHS) received reports of 2 college football players (players X and Y) on team A hospitalized for SSTIs due to MRSA, which was later identified as a community-associated strain (USA300) (12). No other MRSA SSTI was reported on team A until 1 year later. On August 25, 2003, an infectious disease physician notified LACDHS of the hospitalizations of 4 different players on team A with MRSA SSTIs. Despite the lack of background SSTI data on this team, the recurrence of infections prompted an investigation with objectives of identifying players with MRSA SSTIs and nasal carriage, conducting epidemiologic studies, implementing outbreak-control measures, and determining the genotype of the outbreak strain.

Team A was a college football program with 107 players on the roster at the time of the outbreak. The team practiced and played 11 of their 13 games on grass fields. Players began their football season with training camp from August 5 to 18, 2003. In camp, players were sequestered and lived together, in suites of 4 per dormitory, to foster camaraderie among teammates. Rigorous practices were held twice daily in the hot, summer weather.

Methods

Case Finding

Case-players were defined as team A members with MRSA culture-confirmed SSTIs or SSTIs presumably caused by the USA300 strain in the outbreak period August 5 to September 5, 2003. Because we suspected that disease exposure occurred during camp, we chose the study period from the start of training camp to ≈2 weeks after the end. Our experience with other SSTI outbreaks

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found that in most persons lesions develop within 2 weeks postexposure to CA-MRSA. To find case-players, we reviewed the trainer's treatment log to identify players with skin lesions who required medical or surgical interventions. We asked the staff to conduct skin inspections of all players. Players were encouraged to report any skin lesion. In addition, we queried the student health center to determine if these infections were prevalent on campus.

Nasal Carriage Study

As soon as the current outbreak was recognized, a returning player (player X) was suspected to be the source of infection. Player X had 1 of the 2 cases of CA-MRSA SSTIs discovered in 2002. His locker was directly across from the index case-player, and he was a roommate, during camp, of another case-player. Trainers obtained a nasal culture from player X on August 25. On September 3, trainers obtained cultures from the anterior nares of 99 available team members for a nasal carriage study.

Laboratory Study

MRSA isolates from case-players and nasal carriers were characterized by using pulsed-field gel electrophoresis (PFGE) with the *Sma*I and *Eag*I restriction enzymes (12,13). PFGE patterns of the isolates were compared with the USA300 strain responsible for other SSTI outbreaks in Los Angeles County (14). This strain was previously determined to contain SCC*mec*IV by the Centers for Disease Control and Prevention (L. Yasuda, pers. comm.). We also characterized a sample of methicillin-susceptible *Staphylococcus aureus* (MSSA) isolates from players' nasal cultures.

Case-control and Carrier-control Studies

On the basis of anecdotal reports of players sleeping in the locker room on used towels and delaying treatment of cuts and abrasions, we hypothesized that poor hygiene habits and compromised skin integrity might predispose players to infection. We designed a standard questionnaire to collect data on player demographics, living situation, football activities, exposure to persons with skin infections, hygiene practices, histories of skin lesions, and clinical symptoms. Trained health department employees administered the questionnaires in person.

We conducted unmatched case-control and carrier-control studies. Controls were selected by jersey numbers, by using a random-number generator, from asymptomatic teammates without nasal carriage of MRSA. Teammates with positive nasal cultures for MRSA were considered carriers. Carrier-players were defined as carriers with matching PFGE pattern to the USA300 strain. We excluded non-USA300 carriers who might represent the background prevalence of MRSA in the community. Players who were not available for interviews were not included. Bivariate analysis was completed by using Fisher exact test in Epi Info version 3.3 (CDC, Atlanta, GA, USA). Statistical significance was defined as p values <0.05. Because of the small sample size and zero-valued cells, similar risk factors from the bivariate analysis were grouped into categories. Multivariate analysis was completed by using the conditional exact test in SAS version 8 (SAS Institute Inc., Cary, NC, USA).

Outbreak Control Interventions

Upon recognition of the outbreak on August 25, team A instituted daily hexachlorophene showers for all players, increased the frequency of cleaning the facilities and athletic gear, disinfected the whirlpool tubs, provided more towels, and posted hand-hygiene signs in the locker room. Once nasal culture results were available, team physicians attempted to decolonize carriers with intranasal mupirocin (15). We recommended improving the timeliness of wound care, barring case-players from playing unless wounds were covered, discouraging the sharing of personal items and tubs, prohibiting sleeping in the locker room, and checking laundry procedures. We also disseminated CA-MRSA educational materials to staff and team members (16).

Results

Characteristics of Case-players

We identified 11 case-players out of 107 team members for an attack rate of 10%. Cases were diagnosed during or within 2 weeks of the end of training camp (Figure 1). The first case was diagnosed on August 15, the last on September 1. With 1 exception, infections occurred before the first scheduled game on August 30. The most common sign was a boil (Table 1). The elbow was the most common body site infected. No infection was at a current site of skin trauma or occurred at >1 body location simultaneously. Before hospitalization, the index and second case-players were given cephalexin and levofloxacin, respectively, for their infections without any clinical improvement. In total, 4 case-players were hospitalized and treated with parenteral vancomycin. Subsequent nonhospitalized case-players were treated with doxycycline and rifampin. Lesions of 9 players required surgical incision and drainage. All case-players ultimately responded to treatment with resolution of their infections. The median age of case-players was 20 years, with a median tenure of 2 years on team A. Linemen had the highest attack rate (18%) among all field positions (Figure 2, Table 2). No quarterbacks, wide receivers, or special team players (kickers, punters) were affected. All were healthy men without underlying illnesses. Eight (80%) case-players interviewed reported having never

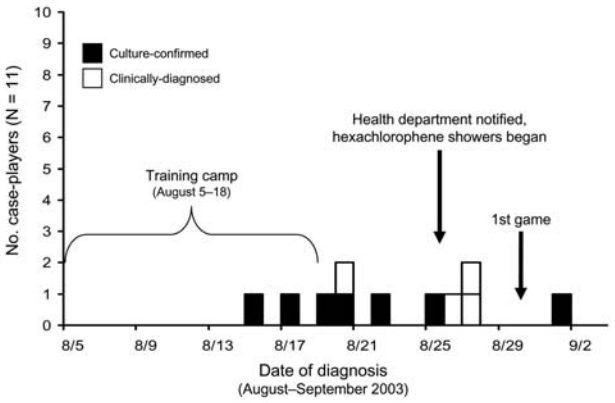

Figure 1. Epidemic curve of clinical and methicillin-resistant *Staphylococcus aureus* skin and soft tissue infections among players on a college football team by date of diagnosis, Los Angeles County, August–September 2003.

worn elbow pads, and 6 (60%) usually did not have cuts or abrasions covered until >1 hour postinjury.

Characteristics of Carriers

Nasal cultures were obtained from 99 (93%) of 107 team members. Twenty-six (26%) cultures were positive for *Staphylococcus aureus*, among which 8 (8%) were positive for MRSA, including player X. Player Y's nasal culture was negative. The median age of carriers was 20 years (range 18–21 years), and median tenure on the team was 2.5 years (range 1–5 years). MRSA carriage was highest in linemen (38%). We identified 1 case-player with nasal carriage of MRSA. However, trainers obtained nasal cultures after all case-players had begun antimicrobial treatment. Locker room assignments showed clustering of case-players and carrier-players, notably the proximity of the potential source player (player X) to the index case-player (Figure 3). Among MSSA carriers (n = 18), no clustering of locker locations was seen. MSSA carriage was highest among linemen (28%) and cornerbacks/safeties (28%).

Laboratory Results

Four (57%) of seven MRSA isolates from culture-confirmed case-players were available for PFGE analysis. All were indistinguishable from each other, the USA300 strain found in Los Angeles County, and the isolates from 2 cases (players X and Y) in 2002. We denoted this genotype as strain A. Of 6 (75%) available MRSA isolates from 8 carriers, 4 (67%) were indistinguishable from strain A. Two carriers had unique MRSA genotypes (strains B and C) with ≥7 bands difference between them and between strain A. Strains A, B, and C, player X and Y's isolates, demonstrate community-associated antimicrobial susceptibility phenotypes (Table 3). Among 5 MSSA isolates characterized, all had ≥7 bands difference among themselves as well as from the USA300 strain.

Case-control and Carrier-control Study Results

Ten of 11 case-players were enrolled in the study; 1 was unavailable for interview. During camp, case-players were 15 times more likely than controls to have shared bars of soap with teammates and more likely to have had preexisting cuts or abrasions (Table 4).

Five of 6 carrier-players were available for interviews. Carrier-players were 60 times more likely than controls to have had a locker adjacent to or across from a teammate with an SSTI and 47 times more likely to have shared towels with teammates (Table 4). Carrier-players were more likely than controls to lived on campus in a dormitory or fraternity house. Among carrier-players and controls, players who lived on campus had a higher mean number of roommates than those who lived in off-campus apartments (2.3 vs. 1.5, p = 0.046).

Table 1. Characteristics of case-players (N = 11)*

Case-player	Age (y)	Field position	No. y on team	Date of diagnosis	Presenting sign	Site of infection	MRSA+ culture	MRSA genotype†
1	20	Fullback‡	2	8/15/03	Boil§	Knee	Y	A
2	21	Cornerback‡	2	8/17/03	Boil§	Elbow	Y	A
3	20	Linebacker	2	8/19/03	Boil§	Elbow	Y	NA
4	18	Lineman	1	8/20/03	Folliculitis	Leg	Y	NA
5	18	Lineman¶	1	8/20/03	Folliculitis	Knee	NC	–
6	21	Lineman‡	3	8/22/03	Insect bite§	Foot	Y	A
7	18	Lineman‡	1	8/25/03	Boil§	Elbow	Y	A
8	20	Lineman	3	8/26/03	Boil§	Elbow	NC	–
9	19	Lineman	1	8/27/03	Boil§	Forearm	NC	–
10	20	Tight end	2	8/27/03	Insect bite§	Forearm	NC	–
11	20	Cornerback	2	9/1/03	Boil§	Elbow	Y	NA

*MRSA, methicillin-resistant *Staphylococcus aureus*; Y, yes; NA, not available for PFGE analysis; NC, not cultured (clinical diagnosis).
†Genotype A is the community-associated MRSA strain (USA300).
‡Hospitalized.
§Required incision and drainage.
¶MRSA nasal carrier.

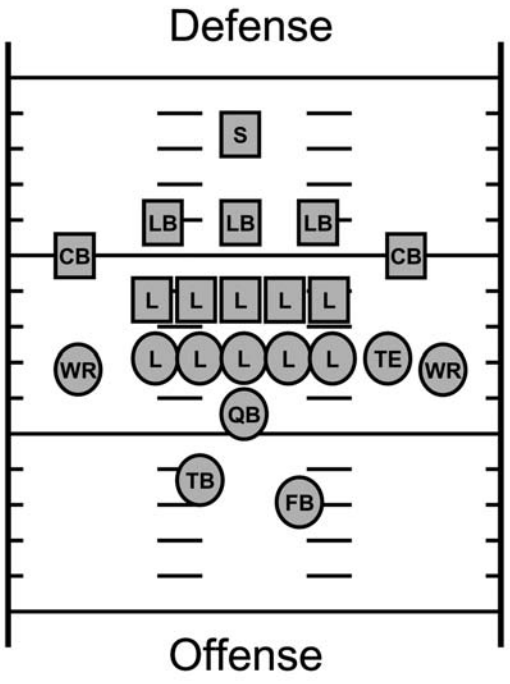

Figure 2. Football field positions; see Table 2 for position-specific attack rates. S, safety; LB, linebacker; CB, cornerback; L, lineman; WR, wide receiver; TE, tight end; QB, quarterback; TB, tailback, FB, fullback.

Potential risk factors were grouped into 3 categories: "sharing" (sharing soap/towels with teammates), "skin injury" (cuts, abrasions), and "close contact" (locker adjacent to case-players, living on-campus). Multivariate analysis including these categories indicated that sharing was a significant risk factor for CA-MRSA infection (OR 12.1, 95% CI 1.83–108, p = 0.006) and carriage (OR 17.4, 95% CI 1.03–undefined, p = 0.047).

Postintervention Surveillance

Daily hexachlorophene showers were in use from August 25 to September 19. No new infections were reported during the 4 weeks after the discontinuation of the hexachlorophene showers. From October 20 to November 9, MRSA SSTI developed in 4 players: a lineman with a chin abscess, a linebacker (player Y from 2002) with an elbow boil, a quarterback (player Z) with folliculitis on a leg, and a tight end with a gluteal boil. Three MRSA isolates (except from the tight end) were available for PFGE; all matched strain A. The lineman in this cluster shared bars of soap with his roommate, a case-player.

Because of ongoing disease transmission and to identify potential reservoirs of MRSA, all 28 staff and student trainers and managers were nasally cultured on November 3; 11 (39%) were positive for MSSA. None was positive for MRSA. On November 22, we observed an official game. Previously unidentified lapses in hygiene practices occurred on the sidelines. We observed that student trainers reused hand towels between players, and players shared towels among themselves. Subsequently, the team switched to single-use towels on the sidelines. No new infections were reported for the remainder of the 2003 season. In the following season (August–December 2004), no MRSA SSTI outbreak occurred on team A. However, player Z had a recurrence of MRSA pustules on the forearm and leg in October 2004. He responded to outpatient treatment with doxycycline, rifampin, and incision and drainage of the lesions. His MRSA isolate was not available for PFGE. Throughout the last 3 football seasons, we received no reports of SSTI outbreaks among opposing athletes after playing this team.

Discussion

This report is the first of recurring CA-MRSA SSTIs in a football team during consecutive seasons. From 2 cases in 2002 to an outbreak involving 11 players in 2003 and then 1 case in 2004, we have shown that eradicating these infections is difficult once they become established in a football team. Infections were likely propagated year to year from previously infected players, and they appear to be susceptible to recurring colonization and infection themselves.

Consistent with other reports, our findings implicate sharing personal items and improper wound care as risk factors for CA-MRSA infections (17,18). While the

Table 2. Position-specific attack rates of clinical and methicillin-resistant *Staphylococcus aureus* skin and soft tissue infections among players on a college football team

Position (Figure 2)	No. case-players (%), N = 11	No. controls (%), N = 32	Total no. on team (%), N = 107	Position-specific attack rate (%)*
Lineman (L)	6 (55)	11 (34)	33 (31)	18
Tight end (TE)	1 (9)	3 (9)	6 (6)	17
Cornerback (CB), Safety (S)	2 (18)	4 (13)	21 (19)	10
Linebacker (LB)	1 (9)	4 (13)	12 (11)	8
Fullback (FB)/Tailback (TB)	1 (9)	3 (9)	12 (11)	8
Wide receiver (WR)	0	4 (13)	12 (11)	–
Quarterback (QB)	0	2 (6)	6 (6)	–
Special team	0	1 (3)	5 (5)	–

*Attack rate = no. case-players/total no. on team, per position. The overall attack rate = 10%.

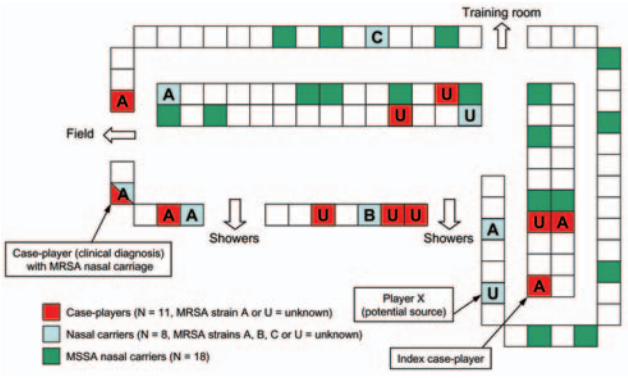

Figure 3. Distribution of locker locations for case-players, methicillin-resistant *Staphylococcus aureus* (MRSA) nasal carriers, and methicillin-susceptible *S. aureus* (MSSA) nasal carriers.

concept is counterintuitive, soap sharing was also associated with MRSA infections in a prison outbreak (19). Therefore, teams should consider switching to liquid soaps in an outbreak situation and always provide prompt wound care.

Linemen were identified as a high-risk subgroup. They engage in frequent and aggressive skin-to-skin contact during games, similar to hand-to-hand combat maneuvers as reported in a military MRSA outbreak (20). In addition, linemen tend to be physically larger than their teammates. Increased body mass index and lineman position were risk factors for CA-MRSA infection in another football team outbreak (18).

Two recent reported CA-MRSA outbreaks in football teams detected no nasal carriage in their combined cohort of 182 football players (17,18). In contrast, we document a high MRSA nasal carriage rate (8%) among team A players even while hexachlorophene showers were provided. The actual carriage rate might be higher, since we obtained nasal cultures after all case-players had begun antimicrobial treatment. Additional case-players may have been carriers as well, but they may have been decolonized before culture. Further research is needed to study the association between nasal carriage of CA-MRSA and SSTI to develop decolonization guidelines. The data facilitated a carrier-control study. Similar to risk factors for infection, nasal acquisition of CA-MRSA is associated with sharing personal items, particularly in the locker room.

Crowded living conditions during training camp appear to facilitate the acquisition of CA-MRSA, which then propagates in on-campus housing. Investigators of an outbreak among military recruits found an association between having a roommate with an SSTI and MRSA infection (21). Consequently, players' living arrangements should be as dispersed as possible.

Unique to our investigation are 1 confirmed and 2 presumed community-associated strains of MRSA. We presented laboratory results indicating that the outbreak strain was likely the USA300 genotype. Since we do not have PFGE results from 6 case-players, different strains could have caused those infections. However, a multiclonal outbreak is unlikely, since other MRSA SSTI outbreaks in Los Angeles County among soccer players, men who have sex with men, jail inmates, and newborns have been exclusively due to the USA300 strain (14,22; Los Angeles County Department of Health Services, unpub. data). In contrast, our limited data do not suggest a clonal spread of MSSA on this team. Multilocus sequence typing was not available locally, which prevented further characterization of the isolates.

Selection bias of case-players and controls is a limitation of this study. Enrollment of players with uncultured infections and those without PFGE results introduces the possibility of misdiagnosis and misclassification. Most football teams assign jersey numbers on the basis of field position. Therefore, our control selection method might not have captured a representative sample of the team. However, the distribution of field positions among controls

Table 3. Comparison of antimicrobial susceptibility patterns for *Staphylococcus aureus* isolates from case-players, carriers, and players X and Y*

		Nasal carriage strains (2003)			Player X		Player Y	
Antimicrobial drug	Case-players (2003)	A†	B	C	Wound (2002)	Nasal (2003)	Wound (2002)	Wound (2003)
Penicillin	R	R	R	R	R	R	R	R
Oxacillin	R	R	R	R	R	R	R	R
Gentamicin	S	S	NT	S	S	NT	S	S
Levofloxacin	I	I	S	R	I	I	I	I
Vancomycin	S	S	S	S	S	S	S	S
Clindamycin	S	S	S	R	S	S	S	S
Tetracycline	S	S	S	S	S	NT	S	S
Rifampin	S	S	S	S	S	NT	S	S
Trimethoprim-sulfamethoxazole	NT	NT	S	S	NT	S	NT	NT

*R, resistant; S, susceptible; I, Intermediate; NT, susceptibility was not tested for that particular antimicrobial drug.
†Strain A is indistinguishable on pulsed-field gel electrophoresis from a community-associated MRSA strain (USA300).

Table 4. Comparison of selected potential risk factors and characteristics of case-players and carriers, versus controls

Risk factor or characteristic	Case-players (%), N = 10*	Controls (%), N = 32	OR†	95% CI†	p value†
Shared bars of soap with teammates	5 (50)	2 (6)	15.0	1.69–180	**0.005**
Had preexisting cuts or abrasions	10 (100)	20 (63)	Undef	1.08–undef	**0.02**
Shared towels with teammates	2 (20)	1 (3)	7.8	0.34–471	0.14
Had recent "boil"	3 (30)	2 (6)	6.4	0.58–86	0.08
Shared whirlpool tubs with teammates	8 (80)	15 (47)	4.5	0.72–49	0.07
Used whirlpool tubs	8 (80)	16 (50)	4.0	0.63–43	0.09
Had recent "insect bites"	4 (40)	6 (19)	2.9	0.44–17	0.17
Shaved body	4 (40)	12 (38)	1.1	0.19–6	0.59
Used antimicrobial drugs in prior 3 months	1 (10)	6 (19)	0.5	0.01–5	0.46
Chafed skin from athletic equipment	1 (10)	9 (28)	0.3	0.01–3	0.23
	Carriers (%), N = 5‡	Controls (%), N = 32			
Had locker adjacent/across from teammate with skin infection	4 (80)	2 (6)	60.0	3.05–3042	**0.001**
Shared towels with teammates	3 (60)	1 (3)	46.5	2.02–2511	**0.005**
Lived in dormitory, fraternity, or on-campus housing	5 (100)	8 (25)	Undef	2.12–undef	**0.003**
Shared bars of soap with teammates	2 (40)	2 (6)	10.0	0.49–170	0.08
Had recent "insect bites"	3 (60)	6 (19)	6.5	0.57–88	0.08
Slept in locker/training room	5 (100)	19 (59)	Undef	0.52–undef	0.10
Shared whirlpool tubs with teammates	4 (80)	15 (47)	4.5	0.38–236	0.19

*Not including 1 case-player who was unavailable for interview.
†Fisher exact test. Values in **boldface** are significant ($p < 0.05$). OR, odds ratio; CI, confidence interval; Undef, undefined or incalculable value.
‡Not including 3 carriers; 1 was unavailable for interview, and 2 had non–strain A genotypes.

and the entire team appears similar (Table 2). The small sample size produces less precise (wide confidence intervals) results and prohibits more in-depth multivariate analyses. Reporting bias is possible, since players and the team fear negative publicity, and we do not have data on risk factors during the off-season. In order to maintain confidentiality, we were unable to interview several players because of high media scrutiny.

As CA-MRSA strains become more prevalent in the community (23), SSTIs will likely continue to afflict football players. Despite comprehensive infection control interventions, sporadic cases of MRSA SSTIs continue to occur on this team. However, a recurrent outbreak was averted in the latest season likely because of increased vigilance to proper hygiene practices and awareness of this disease among the staff and players.

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Bed Bug Infestations in an Urban Environment

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Until recently, bed bugs have been considered uncommon in the industrialized world. This study determined the extent of reemerging bed bug infestations in homeless shelters and other locations in Toronto, Canada. Toronto Public Health documented complaints of bed bug infestations from 46 locations in 2003, most commonly apartments (63%), shelters (15%), and rooming houses (11%). Pest control operators in Toronto (N = 34) reported treating bed bug infestations at 847 locations in 2003, most commonly single-family dwellings (70%), apartments (18%), and shelters (8%). Bed bug infestations were reported at 20 (31%) of 65 homeless shelters. At 1 affected shelter, 4% of residents reported having bed bug bites. Bed bug infestations can have an adverse effect on health and quality of life in the general population, particularly among homeless persons living in shelters.

The common bed bug (*Cimex lectularius*) is a wingless, red-brown, blood-sucking insect that grows up to 7 mm in length and has a lifespan from 4 months up to 1 year (Figure 1) (1). Bed bugs hide in cracks and crevices in beds, wooden furniture, floors, and walls during the daytime and emerge at night to feed on their preferred host, humans.

The common bed bug is found worldwide. Infestations are common in the developing world, occurring in settings of unsanitary living conditions and severe crowding (2,3). In North America and Western Europe, bed bug infestations became rare during the second half of the 20th century and have been viewed as a condition that occurs in travelers returning from developing countries (4). However, anecdotal reports suggest that bed bugs are increasingly common in the United States, Canada, and the United Kingdom (5–10). This study was conducted to document the magnitude and adverse effects of bed bug infestations in homeless shelters and other locations in Toronto.

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Ethical and scientific approval was obtained from Toronto Public Health and the St. Michael's Hospital Research Ethics Board. Shelter staff and residents gave informed consent before participation.

Methods

Toronto Public Health and Pest Control Operators Survey

The log of telephone calls made in 2003 to Toronto Public Health was reviewed to identify calls related to bed bug infestations, the types of locations affected, and the regions of the city where infestations were reported. Toronto is divided into 4 public health regions (north, east, south, and west); the south region includes the downtown core of the city. The population of each region was determined from 2001 census data and ranged from 500,000 to 700,000.

A telephone survey of all pest control operators listed in the Toronto telephone directory was conducted using a structured interview. The survey documented the number of bed bug–related calls received, the number of treatments provided by pest control operators in 2003, and the types of insecticides used to treat bed bug infestations. To protect the confidentiality of persons and establishments affected by bed bugs, we asked each pest control operator to report the number of different locations treated for bed bug infestations by general type (e.g., apartment, single-family dwelling, shelter) and not by specific name or address.

Survey of Homeless Shelter Staff

A telephone interview of the director or supervisor at each homeless shelter in Toronto was conducted to determine which shelters had experienced bed bug infestations. Interviewees were assured that the information they provided would be reported in a way that would not identify their shelter. At affected shelters, follow-up, in-person interviews were conducted with staff from December 2003 to May 2004. A predefined strategy was used to select

Figure 1. Dorsal and lateral views of a bed bug (*Cimex lectularius*).

shelter managers, front-line staff, and healthcare professionals for interviews. The questionnaire included items on time course, manifestations, and extent of the infestation; control measures undertaken; and effects of the infestation on shelter residents and staff. Bed bug infestations were considered confirmed if an entomologist or pest control operator identified a specimen collected at the shelter as *C. lectularius*. Infestations were considered probable if shelter staff reported resident complaints consistent with bed bug infestations.

Homeless Shelter Resident Survey

As part of a separate study of bacterial colonization among shelter residents, a sample of 243 residents at 1 shelter affected by bed bugs was surveyed in July and August 2003. Participants were selected at random from among persons registered at the shelter, and 80% of those contacted agreed to participate. Participants were asked if they currently had any skin-related illness, injury, or condition, and if so, what type. We obtained permission from the principal investigator of this study to review participant responses to determine the prevalence of self-reported bed bug bites (G. Bargh, pers. comm.).

Results

Calls to Toronto Public Health

In 2003, Toronto Public Health received insect-related calls from 82 different street addresses; 46 were complaints of bed bug infestations, 11 were requests for information about bed bugs, and 25 were unrelated to bed bugs. The 46 separate locations where infestations were reported are shown in Table 1. In response to these calls, public health staff spent a total of 27 hours providing information, and health inspectors spent a total of 78 hours conducting site visits to confirm complaints and offer assistance. More complaints of infestations were received in the last 6 months (31 calls) than in the first 6 months (15 calls) of 2003. In the south region, which includes the downtown core of the city, 4.7 complaint calls were received per

Table 1. Reports of bed bug infestations in Toronto, 2003

	Calls to pest control operators			Calls to Toronto Public Health
Type of location	No. locations treated (%)*	No. treatments (%)	Mean no. treatments per location	No. locations (%)
Single-family dwelling	588 (70)	641 (49)	1.1	2 (4)
Apartment unit	155 (18)	297 (23)	1.9	29 (63)
Homeless shelter	68 (8)	218 (17)	3.2	8 (17)
Hotel	19 (2)	96 (7)	5.1	1 (2)
Rooming house	6 (0.7)	16 (1)	2.7	5 (11)
Community center	5 (0.5)	5 (0.4)	1.0	1 (2)†
University dormitory	4 (0.5)	36 (3)	9.0	0 (0)
Restaurant	1 (0.1)	1 (0.1)	1.0	0 (0)
Other residential institution	1 (0.1)	5 (0.4)	5.0	0 (0)
Total	847 (100)	1,315 (100)	1.6	46 (100)

*Figures in this column may reflect some double counting of locations (see details in Methods section).
†Infestation located at the clothing bank in a community center.

100,000 population; this rate was 6.1 times higher (95% confidence intervals [CI] 3.3–11.4) than the rate in the rest of the city. A total of 32 complaints (70%) were from locations in the south region.

Survey of Pest Control Operators

We interviewed 34 (89%) of 38 pest control operators listed in the Toronto phonebook; 20 (59%) had provided treatments for bed bugs in 2003. Among these pest control operators, 17 (85%) of 20 reported that they had received an increased number of calls related to bed bugs and had provided more treatments for bed bugs in 2003 than in 2002. The number of locations treated by pest control operators in 2003 and the number of treatments required are shown in Table 1. The mean number of treatments required per affected location was highest at dormitories, hotels, homeless shelters, and rooming houses.

Homeless Shelter Staff and Resident Survey

We contacted all 65 homeless shelters in Toronto and found that 20 (31%) shelters reported previous or current bed bug infestations. Permission was obtained to survey staff at 17 (85%) of 20 affected shelters. Three shelters reporting bed bug infestations either declined or did not respond to our request to interview shelter staff to obtain further information. Forty-three staff members (1–9 per shelter) were interviewed. The time course of the infestation at these shelters is shown in Figure 2. The number of shelters with active bed bug infestations increased steadily from spring 2001 to winter 2003 and then declined. At the end of spring 2004, infestations persisted at 10 shelters. These 10 shelters accounted for 30% of the total shelter bed capacity in the city of Toronto.

At the 17 affected shelters, staff became aware of bed bugs through resident complaints (16 shelters, 94%), visual sightings (14 shelters, 82%), and bite marks on residents (13 shelters, 76%). Staff at 1 shelter (6%) reported that a healthcare provider alerted them to the infestation. A pest control operator or entomologist made a positive identification of the common bed bug at 13 affected shelters (76%). At 3 shelters (18%), residents contacted Toronto Public Health and requested a visit by a health inspector. At 5 locations (29%), shelter staff complained of bed bug bites. Of 243 residents interviewed at an affected shelter in the summer of 2003, 9 persons (4%) had a skin condition that they described as bed bug bites.

The affected locations at homeless shelters and the chemical control measures implemented are shown in Table 2. Professional pest control operators applied insecticides (most commonly, cyfluthrin, bendiocarb, propoxur, and permethrin) at 12 shelters (71%). Shelter staff applied insecticides (most commonly, pyrethrin and propoxur) at 13 shelters (76%).

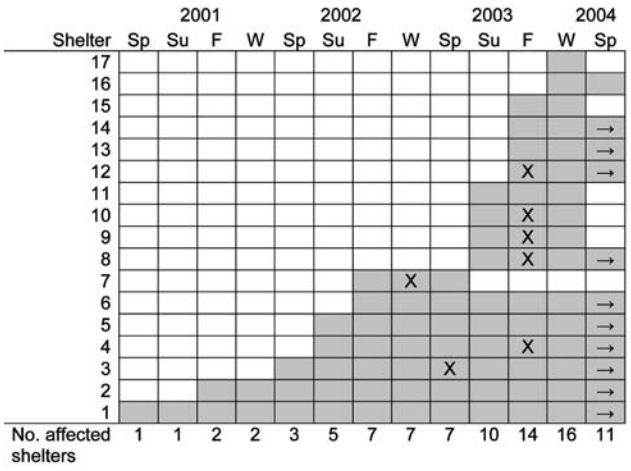

Figure 2. Time course of bed bug infestations in homeless shelters in Toronto. Shaded boxes indicate periods of infestation, X indicates peak period (if reported), and → indicates infestation ongoing as of spring 2004. Sp, spring (March, April, May); Su, summer (June, July, August); F, fall (September, October, November); W, winter (December, January, February).

Shelters implemented a number of environmental control measures (Table 2). To control bed bugs, 6 shelters (35%) made substantial building repairs, including removing floorboards, baseboards, or wood trim; replacing carpets; sealing floor cracks; and painting wooden floors. Two shelters (12%) installed additional washers and dryers to deal with increased laundry demands. The total cost incurred for bed bug control efforts at affected shelters was U.S.$150–$15,000, with a mean of U.S.$3,085 per affected shelter.

Discussion

This study delineates the broad extent of a recent resurgence of bed bug infestations in an urban environment. In light of anecdotal reports from other localities (5–10), we believe that this phenomenon is likely occurring in cities across North America and Europe. The reasons for this resurgence are unknown, although some reports have suggested a role for increasing world travel, reluctance to use insecticides because of concerns regarding toxicity, and insecticide resistance (9,10). Although initial reports in Toronto indicated that bed bug infestations were occurring primarily in homeless shelters, our study showed that bed bugs are found in a wide variety of locations in the urban environment, including single-family dwellings, apartments, and rooming houses.

Data from public health officials and pest control operators provided markedly different perspectives on the extent and localization of infestations. This difference may reflect a tendency for persons experiencing bed bug infestations in single-family dwellings to rely on pest control

Table 2. Locations at homeless shelters affected by bed bugs and chemical and environmental control measures implemented

Locations and control measures	No. shelters (%), N = 17
Affected locations	
Sleeping rooms	15 (88)
Bed or bed frames	15 (88)
Mattresses	13 (76)
Sheets	13 (76)
Floorboards or walls	9 (53)
Lockers	3 (18)
Other*	11 (65)
Nonsleeping rooms†	11 (65)
Chemical control measures (insecticides)	
Spot treatment only	4 (24)
Treatment of affected rooms	5 (29)
Treatment of entire building‡	8 (47)
All beds dismantled and treated	5 (29)
Environmental control measures	
Residents encouraged to shower and wash belongings	17 (100)
Increased room inspections to detect infestations	13 (76)
Ripped or torn mattresses discarded	8 (47)
Limits on amount of personal belongings	8 (47)
Beds and bedding steam cleaned and vacuumed	6 (35)
Building renovations§	6 (35)
Adhesive boards on the legs of beds to trap bugs	4 (24)
Replacing wooden beds with steel beds	3 (18)

*Other areas consisted of personal belongings, light fixtures, electrical switches and plugs, baseboards, carpeting, and other furniture.
†Affected nonsleeping rooms were the lounge, cafeteria, intake office, or storage room.
‡Treatment of the entire building entailed closing the shelter for 6 to 72 hours.
§See text for details.

operators, whereas apartment dwellers and homeless shelter staff may be more likely to contact public health authorities. The Toronto experience indicates that these calls place a substantial time demand on public health personnel, who in many cities are already struggling with limited resources.

Our data suggest that bed bugs can spread from shelter to shelter, presumably transported in the personal belongings of residents. At an affected homeless shelter, 4% of residents reported having bed bug bites; given the constant turnover of shelter residents, bed bugs could potentially affect a large number of homeless people over the course of a year. In our clinical experience, homeless persons with bed bug bites suffer a substantial degree of emotional distress.

Infestations in shelters are difficult and costly to eradicate. Our observation of a high mean number of pest control treatments per affected location (Table 1) points to the likelihood that infestations will be difficult to control in other communal living settings and in hotels. The pest control literature emphasizes the importance of combining insecticide treatments with environmental measures such as daily laundering of bed linens, vacuuming rooms, and steam cleaning and vacuuming mattresses. Bed bugs can be destroyed by freezing or by heat treatments at temperatures >50°C, but these methods are inconvenient to implement (9,10)

Bed bug bites can result in clinical manifestations; the most common are small clusters of extremely pruritic, erythematous papules or wheals that represent repeated feedings by a single bed bug (1). Less common but more severe manifestations include grouped vesicles, giant urticaria, and hemorrhagic bullous eruptions (11). Bites should be managed symptomatically with topical emollients, topical corticosteroids, oral antihistamines, or some combination of these treatments.

Health professionals should be aware of this reemerging urban pest to facilitate prompt diagnosis of affected patients and treatment of the underlying environmental infestation. Bed bug bites must be differentiated from scabies, body lice, and other insect bites. Diagnostic clues include clustering and timing of bed bug bites. Unlike body lice, bed bugs are rarely found on affected persons or their clothing, and persons with good personal hygiene who enter an infested area are likely to be bitten.

Although bed bugs could theoretically act as a disease vector, as is the case with body lice, which transmit *Bartonella quintana* (the causal agent of trench fever) among homeless persons (12), bed bugs have never been shown to transmit disease in vivo (13). Hepatitis B viral DNA can be detected in bed bugs up to 6 weeks after they feed on infectious blood, but no transmission of hepatitis B infection was found in a chimpanzee model (14–19). Transmission of hepatitis C is unlikely, since hepatitis C

viral RNA is not detectable in bed bugs after an infectious blood meal (18). Live HIV can be recovered from bed bugs up to 1 hour after they feed on infected blood, but no epidemiologic evidence for HIV transmission by this route exists (20–22).

This study has certain limitations. Shelter data were based on self-reports from staff at affected shelters. Although we obtained data from multiple informants at each shelter when possible, we did not independently verify the accuracy of these reports. Affected shelters represented 30% of shelter beds in Toronto, but our methods did not determine how many rooms or beds within each shelter were affected. Shelter residents' reports of having bed bug bites were not independently confirmed, and some of these persons may have had other types of insect bites or delusions of parasitosis. The method we used to survey pest control operators may have resulted in double-counting locations that obtained treatments for bed bugs from >1 pest control operator in 2003. As a result, the number of affected locations may be overestimated. Furthermore, the reliability of reports from pest control operators is uncertain. Finally, our results, based on calls to public health and pest control operators, reflect self-initiated complaints from affected locations and therefore do not provide population-based data on the prevalence of bed bug infestations.

In conclusion, our study documents the broad extent of bed bug infestations in an urban environment. This problem could have a substantial adverse effect on health and quality of life, particularly among persons who use homeless shelters. Physicians should be aware of the typical dermatologic signs and symptoms of bed bug bites, which may become increasingly common in the future. Further research is needed to determine the geographic extent of the current reemergence of bed bugs in the industrialized world and the prevalence and risk factors for bed bug infestations in the general population, including those living in both congregate and noncongregate housing.

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Experimental Infection of Prairie Dogs with Monkeypox Virus

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Studies of experimental infection of prairie dogs (*Cynomys ludovicianus*) with monkeypox virus are described. After intraperitoneal infection, all of the animals died within 11 days. Virus was cultured from their blood and oropharynx several days before death; at necropsy, most of the organs tested contained monkeypox virus. Marked hepatic and splenic necrosis were observed, along with mild inflammatory changes in the lungs. After intranasal (IN) infection, the primary pathologic changes were in the lungs and pleural cavity. Some of the IN infected animals (40%) survived, and monkeypox virus could be cultured from their nasal discharge and oropharynx for ≤22 days. Ulcerative lesions also developed on the lips, tongue, and buccal mucosa of the surviving animals. Our findings support an earlier report, which suggested that infected prairie dogs can transmit monkeypox virus by respiratory and mucocutaneous contact with susceptible animals and persons.

In the summer of 2003, an outbreak of monkeypox virus infection occurred among humans living in the midwestern United States (1,2). A total of 32 human cases were confirmed. Most of the patients experienced a brief febrile illness with vesicular skin eruptions; no deaths resulted. Most patients with this illness had a history of contact with sick pet prairie dogs (*Cynomys* spp.), originally obtained from a single pet distributor (1,2). The epidemiologic investigation, clinical findings, and control of this outbreak were described (1).

A recent publication (2) reported the pathologic findings at necropsy in 2 sick prairie dogs confiscated during the 2003 outbreak. The animals showed necrotizing bronchopneumonia, conjunctivitis, and tongue ulceration. Virus isolation and electron microscopy examination of tissue samples from the animals demonstrated active viral replication in lungs and tongue, which suggested that both respiratory and mucocutaneous exposures are potentially important routes of monkeypox virus transmission from rodents to humans. To learn more about the nature of monkeypox virus infection in prairie dogs, we experimentally infected 10 of the animals with a human isolate from the 2003 U.S. outbreak.

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Materials and Methods

Animals

Ten wild-caught, adult prairie dogs (*Cynomys ludovicianus*) were obtained from a commercial trapper, with permission of the Food and Drug Administration. Animals were housed in pairs in large (61 cm × 61 cm × 45 cm) metal cages within a Duo-Flow biosafety cabinet (Biochem Lab Products, Seaford, DE, USA) in an isolation room of an animal biosafety level 3 facility. All persons handling the animals had recently received a smallpox (vaccinia) vaccination and used appropriate personal protection. Animals were cared for in accordance with the guidelines of the Committee on Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Research Council) under an animal use protocol approved by the Institutional Animal Care and Use Committee at the University of Texas Medical Branch.

Virus

The strain of monkeypox virus used (provided by the Centers for Disease Control and Prevention, Atlanta, Georgia, USA) was designated MPX 2003. This virus was originally isolated from a skin lesion of a person with monkeypox during the 2003 outbreak in the United States (1). A stock of the virus was prepared from infected Vero cells and was used to infect the rodents; the unsonicated frozen cell lysate had a titer of $10^{6.1}$ PFU/mL.

Virus Assay

Tissues and other samples for virus assay were stored at –80°C. For analysis, the tissue samples were thawed and

triturated in sterile TenBroeck glass tissue grinders in phosphate-buffered saline (PBS), pH 7.4, containing 30% heat-inactivated (56°C for 30 min) fetal bovine serum (FBS) to prepare a 10% tissue homogenate. After centrifugation at 5,000 rpm for 5 min to clarify the suspension, serial 10-fold dilutions from 10^{-1} to 10^{-8} were prepared in PBS containing 10% FBS. Similar dilutions were made with blood and throat swab suspensions for virus assay.

Dilutions of the tissue homogenates, blood, and throat swab suspensions were titrated in 24-well cultures of Vero cells, 4 wells per dilution, as described before (3). Cultures were incubated at 37°C, and plaques were counted 6 days later, as they were sharp and easy to read at that time. Virus titers were defined as the log of PFU per milliliter of sample.

Experimental Infection of Animals

Since the response of prairie dogs to monkeypox virus infection was uncertain when these experiments were conducted, the animals were infected by the intraperitoneal (IP) or intranasal routes. Four rodents (MPX-1 to MPX-4) were injected IP with $10^{5.1}$ PFU of MPX 2003 virus. The other 6 animals (MPX-5 to MPX-10) were infected by the intranasal route; under Halothane (Halocarbon Laboratories, River Edge, NJ, USA) anesthesia, 2 drops (≈100 μL) of the stock virus solution containing $10^{6.1}$ PFU/mL were instilled into each nostril. After infection, all rodents were observed daily for signs of illness; if an animal died, a necropsy was performed, and tissues (liver, spleen, kidney, adrenal, pancreas, lung, heart, and brain) were taken for histopathologic examination and virus titration. Blood (100 μL from the retroorbital sinus) and an oropharyngeal swab were also taken daily from each animal for virus assay. The whole blood and the swab were expressed in 900 μL of PBS with 10% FBS. Twenty-five days after infection, the surviving animals were exsanguinated and euthanized, and a necropsy was performed to obtain tissue samples.

Serologic Tests

Complement fixation (CF) tests were performed by microtechnique (4) with 2 full units of guinea pig complement and antigen titers ≥1:32. The antigen used in the CF tests was prepared from brains of infected baby mice injected intracerebrally with vaccinia virus; infected brains were treated by the sucrose acetone extraction method (4). CF antibody titers were recorded as the highest serum dilution giving +3 or +4 fixation of complement.

Plaque reduction neutralization (PRN) tests were conducted in 24-well microplate cultures of Vero cells, by using a technique described previously (5). The MPX 2003 virus was used at a dose of ≈20 PFU. Serial 10-fold dilutions of each serum specimen were incubated overnight at 5°C with the virus dose, before inoculation. Plaques were read on day 6 after inoculation; a 50% reduction of virus plaques, compared to uninfected control prairie dog serum, was used as the endpoint (6). PRN antibody titers were recorded as the highest serum dilution that produced ≥50% plaque reduction.

Histopathologic and Immunohistochemical Methods

At necropsy, tissue samples were taken from the animals and preserved in 10% buffered formalin for 24 to 48 h, followed by storage in 70% ethanol. After fixation, the samples were processed for routine embedding in paraffin. Four- to 5-μm-thick tissue sections were made and stained by the hematoxylin and eosin (H&E) method (5).

Selected tissue sections were also studied immunohistochemically, by using vaccinia hyperimmune mouse ascitic fluid (1:100 dilution) as the primary antibody. A mouse-on-mouse IHC-ISO labeling kit (InnoGenex, San Ramon, CA, USA) was used, according to the manufacturer's instructions and a published protocol (7). The primary antibody was incubated with the sections at 4°C overnight. Tissue sections from an uninfected animal were used as negative controls.

Results

Clinical Manifestations

Two animals, MPX-2 and MPX-10, died of respiratory arrest during anesthesia and sample collection on days 5 and 6, respectively. They were omitted from the results.

Beginning on approximately day 4 after infection, most of the prairie dogs became lethargic and anorexic. The 3 animals that were infected IP (MPX-1, MPX-3, and MPX-4) died 8–11 days after infection. Visible lesions did not develop on the skin or mucous membrane of any of these animals.

Three of 5 intranasally infected animals (MPX-6, MPX-7, and MPX-9) also died; their deaths occurred 11–14 days after infection. Their clinical symptoms (increasing lethargy and anorexia) were the same as those observed in the IP infected group. Two of the intranasally infected prairie dogs (MPX-5 and MPX-8) survived infection, although they were lethargic and anorexic for several days between weeks 1 and 2 after infection. In the latter 2 animals, vesicular lesions developed on their lips and tongue, along with nasal congestion and a mucopurulent nasal discharge. Inoculation of fresh samples of the nasal discharge into Vero cell cultures yielded monkeypox virus; and smears of the discharge, stained by immunofluorescence with a vaccinia hyperimmune mouse ascitic fluid, demonstrated swollen macrophages containing multiple fluorescent inclusion bodies, characteristic of poxvirus infection (Figure 1). The nasal congestion and discharge

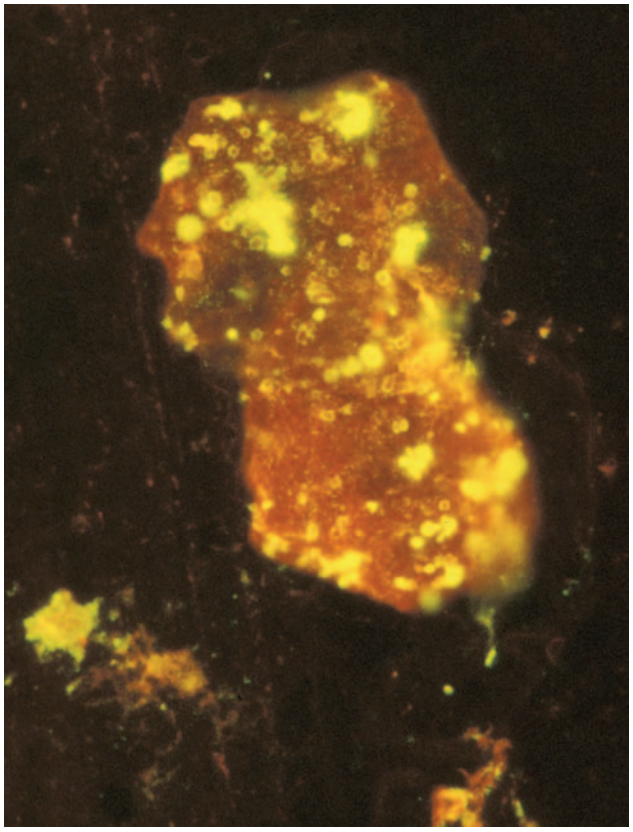

Figure 1. Photomicrograph of a smear of mucopurulent nasal discharge from a monkeypox virus–infected prairie dog (MPX-8), showing a swollen macrophage containing multiple fluorescein-labeled bodies (viral proteins) characteristic of poxvirus infection. Magnification ×1,400.

continued for ≈10 to 14 days, but it gradually diminished, and the 2 surviving prairie dogs appeared healthy when they were euthanized 25 days after infection.

Virus Titrations

In the IP infected group (MPX-1, MPX-3, and MPX-4), monkeypox virus was first detected simultaneously in the blood and throat on day 5 or 6 after infection (Table1). The amount of virus in these samples increased daily until death. In the IP infected group, the highest virus titers were found in liver and spleen; lower titers were observed in kidney and lung (Table 2).

The temporal appearance, organ distribution, and amount of virus present in the blood, throat, and tissues of the intranasally infected prairie dogs were quite different. Among the 3 animals with fatal infections (MPX-6, MPX-7, and MPX-9) in this group, monkeypox virus appeared in the throat several days before it was detected in the blood (Table 1). At death, animal MPX-9 had $10^{8.0}$ PFU/g of virus in lung; smaller amounts of virus were detected in liver, spleen, kidney, and heart (Table 2). In contrast, no virus could be detected in these same organs in animals MPX-6 or MPX-7 at death.

Likewise, monkeypox virus was detected in the throats of the 2 surviving animals (MPX-5 and MPX-8) for 19 and 22 consecutive days, respectively (Table 1). No samples were taken on day 24; but throat swabs taken from MPX-5 on days 23 and 25 were negative; and samples from MPX-8 were negative on day 23 but positive ($10^{0.7}$) on day 25 (data not shown). In these 2 animals, a comparable level of viremia developed; but the duration was uncertain, since no blood samples were taken after day 15. However, blood and organ cultures from the 2 survivors were negative when they were euthanized and underwent necropsy 25 days after infection (Table 2).

Antibody Formation

All of the blood samples taken from the infected prairie dogs (Table 1) were examined by CF test for monkeypox virus antibodies, by using a vaccinia antigen. Only 2 samples gave a positive reaction. Blood (serum) samples from the 2 survivors, MPX-5 and MPX-8, both had a CF antibody titer of 1:64 on day 25 after infection, when the animals were euthanized. The last previous blood samples from these animals were taken on day 15 and were negative, so seroconversion occurred during week 2 or 3 after infection.

Serum specimens from MPX-5 and MPX-8, collected 25 days after infection, were also tested by PRN. Using 50% plaque inhibition as an endpoint, both animals had a neutralizing antibody titer of 1:320 against monkeypox virus.

Histopathologic Changes

No histologic abnormalities were noted in the heart, pancreas, kidneys, or adrenal glands of any of the animals, regardless of the route of infection. The gastrointestinal tracts of a few animals were examined and were likewise unremarkable.

Animals Infected Intraperitoneally

At necropsy, firm nodular changes were observed in the abdominal adipose tissue (omentum) of the animals in this group (MPX-1, MPX-3, and MPX-4). Microscopically, multifocal necrosis of adipose tissue occurred, with vasculitis, prominent fibroblast proliferation, and infiltration by macrophages and other inflammatory cells (Figure 2A). Their spleens showed moderate to severe necrosis, mainly in the lymphoid areas as described before (3). The livers showed centrilobular necrosis, with some inflammatory cellular infiltration and prominent inclusion bodies in the hepatocytes (Figure 2B). The lungs exhibited mild-to-moderate thickening of the interstitium, with increased infiltration by mononuclear inflammatory cells.

Table 1. Results of virus titration of serial blood and throat swab samples taken from prairie dogs after experimental monkeypox infection

Day after infection	Animal no.*															
	MPX-1		MPX-3		MPX-4		MPX-5		MPX-6		MPX-7		MPX-8		MPX-9	
	B	TS	B	TS	B	TS	B	TS	B	TS	B	TS	B	TS	B	TS
1	0	NT	0	NT	0	NT	0	NT	0	NT	0	NT	0	NT	0	NT
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0.7	0	0	0	0	0	1.0	0	0
4	0	0	0	0	0	0	0	0.7	0	0	0	2.4	0	0.7	0	1.6
5	0	0	0.7	0.7	1.9	1.9	0	2.2	0	0	0	2.6	0	1.5	0	1.7
6	1.2†	1.4	1.7	1.5	3.9	3.2	0	3.2	0	2.4	0	1.4	0	1.2	0	1.4
7	2.4	3.0	2.3	2.5	5.4	4.2	0	3.6	0	1.9	0	2.7	0	2.0	0	2.0
8	2.5	3.2	3.5	1.7	D	D	0	3.6	0	1.6	0	2.7	0	2.3	1.5	3.2
9	4.0	4.3	D	D			1.4	4.6	1.6	2.7	1.4	2.6	3.4	3.5	1.2	4.5
10	5.3	5.1					1.0	4.8	3.5	3.0	2.1	3.7	4.4	2.5	3.6	5.0
11	D	D					1.4	5.0	D	D	1.9	3.9	4.0	4.4	3.8	5.1
12							1.2	5.1			2.3	4.7	4.4	4.7	4.3	5.2
13							2.4	5.1			2.0	3.5	4.0	5.1	D	D
14							NT	NT			D	D	NT	NT		
15							4.5	4.4					4.0	5.0		
16							NT	4.3					NT	5.0		
17							NT	3.2					NT	5.2		
18							NT	2.1					NT	5.0		
19							NT	2.9					NT	4.8		
20							NT	1.7					NT	4.2		
21							NT	1.0					NT	4.0		
22							NT	0					NT	2.9		

*Animals MPX-1, MPX-3, and MPX-4 were infected intraperitoneally; animals MPX-5 to MPX-9 were infected intranasally.
†Indicates titer of monkeypox virus in sample; $\log_{10}$ PFU/mL of sample (blood [B] or throat swab diluent [TS]); 0, $<10^{0.7}$ PFU/mL; NT, not tested; D, animal dead.

Animals Infected Intranasally

No evident hepatic lesions or splenic necrosis was observed in the 5 animals infected intranasally (MPX-5 to MPX-9). One of the animals (MPX-9) had marked inflammation and necrosis of adipose tissue and skeletal muscle, with proliferation of large fibroblasts and macrophages. The mediastinal lymph nodes, and thymus in some animals, exhibited marked lymphoid necrosis and depletion, with the infiltration by plump inflammatory cells containing dense eosinophilic material (Figure 2C). The lungs of animals MPX-6, MPX-7, and MPX-9 showed marked edema, hemorrhage, and necrosis, which also involved the pleura and muscle of the diaphragm. Extensive adhesions and a proliferation of swollen cells containing globules of eosinophilic material, later shown by immunohistochemical tests to be viral antigen, were evident in these structures (Figure 2D).

The 2 surviving animals in this group (MPX-5 and MPX-8) were euthanized on day 25 in apparent good health. Few histologic abnormalities were noted at necropsy, except for focal inflammation in the skin (lymphocytic infiltration in dermis). An immunostain of the skin for viral antigen was negative. Animal MPX-8 also had multifocal inflammation of the lung with epithelial and giant cell granulomas.

Immunohistochemical Analysis

Selected tissue sections were studied immunohistochemically (IHC), by using a vaccinia mouse hyperimmune mouse and an IHC-ISO labeling kit. Tissue sections

Table 2. Monkeypox virus titers in selected tissues of experimentally infected prairie dogs at death

Route of infection	Animal no.	Virus titer ($\log_{10}$ PFU/g of tissue)*					
		Liver	Spleen	Kidney	Lung	Heart	Brain
Intraperitoneal	MPX-1	8.1	7.2	4.3	6.7	0	0
	MPX-3	7.6	7.4	5.4	6.8	3.5	3.6
	MPX-4	7.7	8.4	5.4	7.1	5.5	NT
Intranasal	MPX-5	0	0	0	0	0	NT
	MPX-6	0	0	0	0	0	4.7
	MPX-7	0	0	0	0	0	0
	MPX-8	0	0	0	0	0	NT
	MPX-9	3.0	2.4	2.4	8.0	4.5	0

*0, <0.7 ($<10^{0.7}$); NT, not tested.

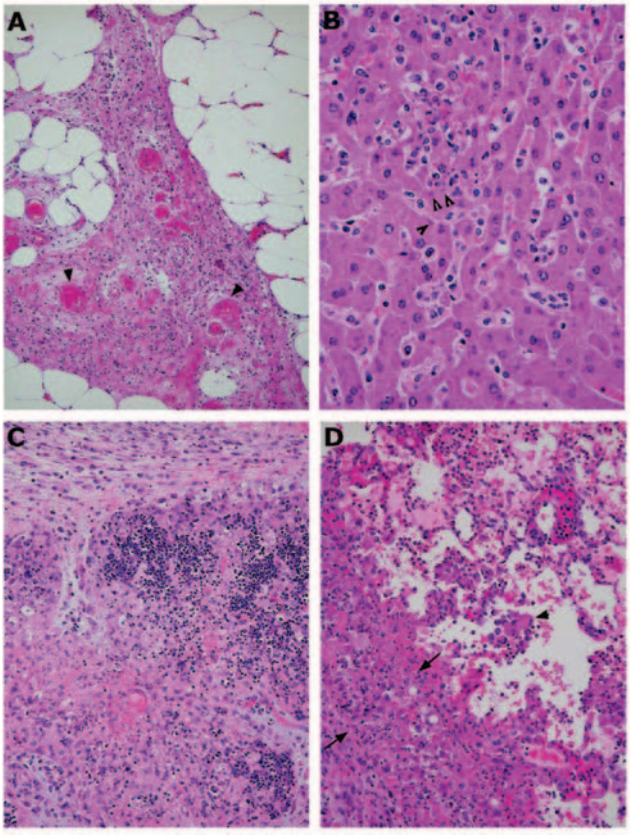

Figure 2. Photomicrographs of pathologic changes in tissues of prairie dogs infected with monkeypox virus. A) Abdominal adipose tissue from an intraperitoneally infected animal showing focal vasculitis (arrowheads), necrosis, and proliferation of fibroblasts. B) Mild hepatitis, characterized by focal inflammatory cell infiltration in the lobules and hepatocytes containing cytoplasmic inclusion bodies (arrowheads). C) Severe necrosis of the thymus from an animal infected intranasally. The necrotic areas contain many swollen macrophages with cytoplasm full of intensively eosinophilic material (viral proteins). D) Lung from the same animal, showing inflammation with swollen cells (arrowheads), alveolar edema, and necrosis of the pleura. The latter is infiltrated by many inclusion-filled macrophages and proliferating fibroblasts (the thick layer between the 2 arrows). Magnification: A, 10× objective; B, 40× objective; C, 10× objective; D, 20× objective. Hematoxylin and eosin stain.

from 2 uninfected prairie dogs were used as negative controls. No positive staining was observed in the control animals. Likewise, tissues from the 2 survivors (MPX-5 and MPX-8) were also IHC-negative.

Animals Infected Intraperitoneally

In the liver of IP infected animals (MPX-1, MPX-3, and MPX-4), most of the inclusion bodies were strongly IHC-positive for poxvirus antigen. Depending on the severity of the histologic abnormality, this positive staining sometimes involved the surrounding cytoplasm and cellular membranes. Some of the smaller inclusion bodies were negative. The spleen was also IHC-positive, corresponding to the severity of pathologic changes. In some animals, the cells lining the surface of the splenic capsule (mesothelial cells) were enlarged or tall and stained strongly positive for viral antigen. In these animals, the positive staining also appeared to involve the superficial zones of the neighboring organs such as the pancreas and adrenals, which were otherwise negative and without pathologic changes. This pattern suggested direct virus spread between adjacent sites when the boundaries (capsules) were broken. Necrotic areas in the perisplenic and periadrenal fat also stained strongly positive.

Animals Infected Intranasally

In animal MPX-9, viral antigen was present in the liver, lungs, mediastinum, and bronchus. The mediastinal lymph nodes were also strongly positive, accompanied by central necrosis. An antigen-positive area in a bronchus of this animal showed distinctive epithelial cell proliferation and squamous metaplasia, as opposed to a negative zone, which was lined by normal columnar epithelium (Figure 3).

Although no evidence of hepatocytic degeneration or necrosis was seen in the H&E-stained sections of liver from the other 4 animals in this group (MPX-5 to MPX-8), the immunostain for viral antigen highlighted rare inclusion bodies in the hepatocytes of animals MPX-6 and MPX-7, which indicated a small amount of monkeypox virus replication in the liver. The spleens of these 4 animals were negative for monkeypox virus antigen by IHC. Scattered antigen-positive interstitial cells (mostly macrophages) were observed in lungs of animal MPX-6; but lungs in the other animals (MPX-5, MPX-7, and MPX-8) were negative by IHC. Sections of the adrenals, kidneys, and heart of all of the infected animals were likewise negative.

Discussion

Results of this study confirm that prairie dogs are highly susceptible to infection with monkeypox virus, although the observed death rate and pathologic changes were less severe than in 13-lined ground squirrels (*Spermophilus tridecemlineatus*) that had been infected IP and intranasally with the same virus dose (3). In contrast to ground squirrels, the pathogenesis and severity of monkeypox virus infection in prairie dogs varied, depending on the route of infection. The IP infected prairie dogs all died after infection; at necropsy, marked hepatic and splenic necrosis was seen, along with mild-to-moderate inflammatory changes in the lungs. Only 3 of the 5 intranasally infected prairie dogs died. In 1 of these animals (MPX-9), the virologic, histopathologic, and immunohistochemical findings were similar to those observed in the IP infected prairie dogs. However, in the other 2 animals with fatal infections

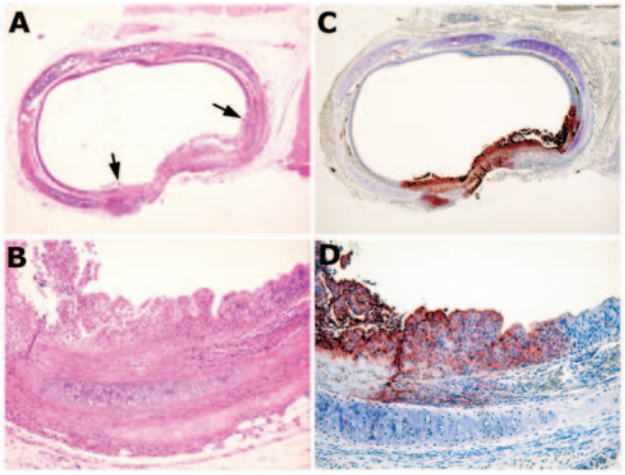

Figure 3. Bronchus from animal MPX-9, which was infected intranasally. A) Cross-section of a bronchus, showing focal metaplasia and proliferation (between the arrows) of the luminal epithelium. B) Higher magnification showing the details of the metaplastic epithelium, accompanied by focal necrosis. Compare to the adjacent unaffected area, which is lined by normal ciliated columnar epithelial cells. C and D) Immunohistochemical staining of the corresponding field shows presence of viral antigen limited to the region of epithelial abnormality. A and B, hematoxylin and eosin stain; C and D, immunoperoxidase staining with vaccinia antibody. Original magnification: A and C, 4× objectives; B and D, 20× objectives.

(MPX-6 and MPX-7), few pathologic changes were observed in the liver, spleen, or other abdominal organs, although marked edema, hemorrhage, and necrosis were observed in the lungs. The reason for this different response is unknown, but our sample size was small.

The pattern of monkeypox virus infection seen in the 2 surviving prairie dogs (MPX-5 and MPX-8) was potentially the most important. These 2 animals continued to have infectious monkeypox virus in their throat and nasal discharge for several weeks after infection.

The pattern of experimental infection in the intranasal group of prairie dogs concurs with the clinical and pathologic observations made during the 2003 monkeypox virus outbreak in the United States. Guarner et al. (2) reported that 10 (67%) of 15 prairie dogs in 1 affected pet store died rapidly; the other 5 animals exhibited anorexia, wasting, sneezing, coughing, swollen eyelids, and nasal discharge. Our intranasally infected animals manifested similar symptoms and had a comparable death rate (60%). Two of the sick prairie dogs at the affected pet store were euthanized, and necropsies were performed (2).

Apart from ulcerative lesions on the tongue and eyelids, these animals had bronchoalveolar pneumonia with edema, necrosis, and a marked infiltrate of macrophages containing many poxvirus particles. Mild inflammatory changes occurred in the liver and spleen, and other organs appeared normal. Although direct culture of monkeypox virus from tissues of these 2 animals was not attempted, the authors noted difficulty detecting monkeypox virus DNA by standard polymerase chain reaction (PCR) and by PCR followed by restriction-endonuclease fragment length polymorphism, even though viral antigen was easily detected by IHC in the same tissue samples (2).

Our experience was similar with animals MPX-6 and MPX-7. Despite the presence of viral antigen in the liver of these 2 experimentally infected prairie dogs, cultures of their lungs, livers, and spleens did not yield infectious virus. Yet on the day before death, monkeypox virus was isolated from their blood and throat swabs. Thus the exact cause of death in these 2 animals is uncertain.

In animals MPX-5 and MPX-8, cutaneous ulcerative lesions developed on the lips, tongue, and buccal mucosa, along with nasal congestion and discharge. The same clinical manifestations were reported from the affected pet prairie dogs (2). In view of the prolonged shedding of monkeypox virus in the throat and nasal discharge of animals MPX-5 and MPX-8, such pets likely could easily transmit virus by direct contact or by bite to cagemates or to humans handling them. Guarner et al. (2) concluded that both respiratory and direct mucocutaneous exposures are potentially important routes of transmission of monkeypox virus between rodents and humans. Our findings with experimentally infected prairie dogs support those conclusions.

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Image created by Curt Wommack

Antimicrobial-resistant Invasive *Escherichia coli*, Spain

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To address the public health problem of antimicrobial resistance, the European Union founded the European Antimicrobial Resistance Surveillance System. A network of 32 Spanish hospitals, serving ≈9.6 million persons, submitted antimicrobial-susceptibility data on 7,098 invasive *Escherichia coli* species (2001–2003). Resistance to ampicillin, cotrimoxazole, ciprofloxacin, gentamicin, and tobramycin was found at rates of 59.9%, 32.6%, 19.3%, 6.8%, and 5.3%, respectively. Resistance to multiple drugs increased from 13.8% in 2001 to 20.6% in 2003 ($p < 0.0001$). Antimicrobial consumption data were obtained from the Spanish National Health System. In spite of decreased cephalosporin and β-lactam use, overall extended-spectrum β-lactamase production increased from 1.6% (2001) to 4.1% (2003) ($p < 0.0001$), mainly due to the rising prevalence of cefotaximases. Resistance to ciprofloxacin significantly increased, mostly in community-onset infections, which coincided with a rise in community quinolone use. Cotrimoxazole resistance remained stable at ≈30%, even though its use was dramatically reduced.

Antimicrobial resistance is a well-known clinical and public health problem (1). For example, in the United States in 2002, resistance to ampicillin and ciprofloxacin among 5,192 *Escherichia coli* blood isolates was 47.8% and 13.3%, respectively (2). The World Health Organization (WHO), the European Commission, and the U.S. Centers for Disease Control and Prevention (CDC) have recognized the importance of studying the emergence and determinants of resistance as well as the need for control strategies (1,3,4).

The European Antimicrobial Resistance Surveillance System (EARSS) is an international network of national surveillance systems that attempts to collect reliable and comparable antimicrobial resistance data of invasive pathogens. The International Network for the Study and Prevention of Emerging Antimicrobial Resistance has similar goals (3). The purpose of EARSS is to document variations in antimicrobial resistance over time and space to provide the basis for developing prevention programs, making policy decisions, and assessing the effectiveness of both.

E. coli is one of the main causes of both nosocomial and community-acquired infections in humans (5) and one of the microorganisms most frequently isolated from blood (2,6–8). Pathogenic isolates of *E. coli* have a relatively large potential for developing resistance (2,5,7,9). In recent years, fluoroquinolone resistance has increased in some countries (2,10,11), CTX-M-type extended-spectrum β-lactamase (ESBL) dissemination has been described (12,13), and reports of multidrug resistance are not infrequent (9,14,15).

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Among western countries, Spain has one of the highest rates of antimicrobial consumption (16,17) and antimicrobial resistance (15). The goal of this prospective study was to describe and analyze the evolution of antimicrobial resistance in comparison to antimicrobial use. Using 7,098 blood or cerebrospinal fluid (CSF) isolates of *E. coli* collected by Spanish hospitals participating in the EARSS network from 2001 to 2003, we found that antimicrobial resistance, particularly to fluoroquinolones and third-generation cephalosporins, was increasing in *E. coli*.

Materials and Methods

Selection of Participating Hospitals

To fulfill the goal of obtaining representative data, participating hospitals were chosen to meet the following criteria: 1) coverage of at least 20% of the Spanish population, 2) different areas of the country covered, and 3) different kinds of hospitals (size and category) represented. The official catalog of all available Spanish hospitals, as published by Spanish Ministry of Health, was used to randomly select hospitals involved in this surveillance system; 3 hospitals refused to participate and were replaced by 3 other hospitals of similar characteristics.

Strains Studied

All clinical isolates of *E. coli* obtained from blood and CSF samples in microbiology laboratories of Spanish hospitals that participated in EARSS from 2001 to 2003 were included. Only the first invasive isolate per patient was reported. Invasive infection was defined as infection with an *E. coli* isolate from blood or CSF. Nosocomial infections were defined as infections acquired at least 48 h after hospital admission. Patients with community-acquired infections were those who had positive cultures by *E. coli* at the time of or within 48 h of hospitalization.

Data Collection

A questionnaire concerning hospital characteristics (coverage, hospital type, number of beds, number of patients admitted per year, hospital departments), methods of antimicrobial susceptibility study, and interpretation criteria was completed by each participating center. One isolate record form was completed for each patient. This form included personal patient data (code, age, sex), hospital and departmental data, and antimicrobial susceptibility data.

Participating hospitals sent prospectively standardized results to the Ministry of Health, where results were analyzed and validated by using the laboratory-based WHONET 5 program (WHO Collaborating Center for the Surveillance of Antibiotic Resistance). A medical microbiologist carefully reviewed all records.

Only the first isolate per patient and year was included. Discrepancies and atypical results were resolved by telephone inquiry, and the corresponding database records were updated if necessary. At the end of each year, an annual report of all data stored in the central database was sent to each participating laboratory to avoid possible discrepancies.

Antimicrobial Susceptibility Studies

The protocol for *E. coli* susceptibility testing included the following antimicrobial agents: ampicillin, aminoglycosides (gentamicin and tobramycin), fluoroquinolones (ciprofloxacin), and third-generation cephalosporins (cefotaxime and ceftazidime). Data on antimicrobial susceptibility to additional antimicrobial agents were also considered when this information was available for at least 5,900 isolates**.** For this reason, the number of strains studied for each antimicrobial agent in some cases was not the same as the total number of strains.

Each laboratory identified strains and tested their susceptibility according to standard microbiologic procedures. In 29 laboratories, identification and antimicrobial susceptibility tests were performed by using the following commercial microdilution systems: 14 used MicroScan (Dade-Behring, Deerfield, Illinois, USA); 8, Wider (Fco. Soria Melguizo S.A., Madrid, Spain); 5, Vitek (bioMérieux, Marcy l'Etoile, France); and 2, Sensititre (Radiometer/ Copenhagen Company, Denmark). The 3 remaining laboratories used the disc-plate diffusion method combined with E test strips (AB-Biodisk, Solna, Sweden). Results were scored as susceptible, intermediate, or resistant according to criteria established by the National Committee for Clinical Laboratory Standards (NCCLS, now the Clinical and Laboratory Standards Institute) (18).

Based on NCCLS criteria, a consensus guideline for detecting ESBL production was recommended by EARSS to all participants (18). ESBL producers were considered resistant to both cefotaxime and ceftazidime independent of their MIC in accordance with NCCLS criteria (18). Multidrug resistance was defined as resistance to $\geq$3 of the antimicrobial agents tested.

Quality Control

To assess the comparability of susceptibility test results, a quality assurance exercise was performed yearly among the 32 participating laboratories. The U.K. National External Quality Assessment Scheme designed the quality controls. Altogether, 24 well-characterized control invasive strains, including 6 *E. coli* strains with different resistance phenotypes, were tested. All these external quality control strains were recommended to be included in the regular internal quality control procedures performed by each laboratory. Data on susceptibility to ampicillin, ciprofloxacin, gentamicin, cefotaxime, and ceftazidime

were required. In addition, each laboratory completed a questionnaire concerning the methods used for determining susceptibility and applying interpretation criteria.

Community Antimicrobial Use

The Ministry of Health and Consumer Affairs maintains a drug database of retail pharmacy sales of all medicines acquired with National Health System prescriptions, covering nearly 100% of the Spanish population (17,19). These data reflect the outpatient antimicrobial use in Spain. This database was used to gather information on sales for the period 1998–2003. The information was tabulated, and the number of units sold was converted into defined daily doses (DDD) of the active drug ingredients in accordance with WHO guidelines (20). We then calculated the number of DDD per 1,000 inhabitants per day for each of the active drug ingredients. This information was not available in relation to patient age.

Statistical Analyses

Differences in the prevalence of antimicrobial resistance between different groups were assessed by Fisher exact test. Association was determined by calculation of the odds ratio (OR) with 95% confidence intervals (CI). The null hypothesis was rejected for values of $p < 0.05$. Statistical analyses were performed with EpiInfo version 6.04 software (CDC, Atlanta, GA, USA).

Results

Characteristics of Participating Laboratories

From 2001 to 2003, a total of 32 laboratories reported data on invasive *E. coli* isolates. The estimated average coverage of the Spanish population was 23%, which corresponds to ≈9.5 million persons. The median annual numbers of hospital beds and patients admitted were ≈14,500 and 550,000, respectively. Four hospitals (12.5%) had >1,000 beds, 8 (25%) had 500–1,000 beds, 15 (46.9%) had 250–499, and 5 (15.6%) had <250. Twelve (37.5%) were university or tertiary-care hospitals, and 20 (62.5%) were general or secondary-care hospitals.

Quality Control Results

Among participating laboratories, the overall concordance of susceptibility to ampicillin, gentamicin, and ciprofloxacin in the 6 *E. coli* control strains was 100%, 89%–100%, and 92%–100%, respectively. ESBL production was detected by 85.2%–97% of the laboratories. The participating laboratories used NCCLS-recommended procedures for ESBL detection (18).

In the few cases of disagreement between the expected quality control results and the actual performance of individual laboratories, individual cases were analyzed and discussed with participants. Measures to improve laboratory procedures were proposed when necessary, including the dispatch of isolates to the Spanish *E. coli* reference laboratory (352 [4.9%] strains submitted during the study period).

Patient Data

Data on 7,098 isolates of *E. coli*, corresponding to the same number of patients, were reported, including 3,484 (49.1%) male patients, 3,581 (50.5%) female patients, and 33 of unknown sex. All isolates were collected from blood except 9 from CSF. Of the total number of isolates, 309 (4.4%) were from children ≤14 years of age, 2,145 (30.2%) were from patients ≥15 and ≤64 years of age, and 4,644 (65.4%) were from patients >64 years of age. A total of 3,339 (47.3%) isolates were implicated in nosocomial infections (1,465 from internal medicine, 442 from surgery, 309 from pediatrics, 290 from intensive care units, 81 from infectious diseases, 75 from obstetrics and gynecology, and 677 from other departments), and 3,735 (52.6%) isolates were implicated in community-acquired infections; in 24 cases this information was missing.

Antimicrobial Susceptibility

The antimicrobial susceptibility of the *E. coli* isolates studied is shown in Table 1. In the *E. coli* isolates, resistance to ampicillin, cotrimoxazole, ciprofloxacin, gentamicin, and tobramycin was found at rates of 59.9%, 32.6%, 19.3%, 6.8%, and 5.3%, respectively. Of the 7,098 isolates tested for cefotaxime, 234 isolates (3.3%) were nonsusceptible, including 19 (0.3%) intermediate and 215 (3%) resistant. ESBL producers totaled 204 (2.9% of all strains tested for cefotaxime) isolates. Ceftazidime susceptibility data were available for 5,960 isolates. Of these, 209 (3.5%) were nonsusceptible, including 10 (0.2%) intermediate and 199 (3.3%) resistant.

Among the 185 *E. coli* ESBL producers in which susceptibility data to both cefotaxime and ceftazidime were reported, nonsusceptibility to cefotaxime according to MIC data was found in 113 (61.1%) cases, while nonsusceptibility to ceftazidime was reported in 68 cases (36.8%). Resistance figures to other antimicrobial agents were as follows: imipenem, 0% of 4,504 isolates tested; amikacin, 0.3% of 4,484 isolates tested; and amoxicillin/clavulanic acid, 6% intermediate and 4.5% resistant of 3,023 isolates tested.

The prevalence of antimicrobial resistance was higher in male patients than in female patients (Table 2), particularly for ciprofloxacin, gentamicin, and cotrimoxazole. Nosocomial isolates were significantly more resistant to ampicillin, ciprofloxacin, cotrimoxazole, gentamicin, and cefotaxime than community-acquired isolates (Table 3). Of the 204 ESBL producers, 66 (32.4%) were implicated in community-onset infections. Resistance to ciprofloxacin

Table 1. Antimicrobial susceptibility in invasive isolates of *Escherichia coli*, Spain, 2001–2003*

Antimicrobial agent	N	S (%)	I (%)	R (%)
Ampicillin	7,098	2,884 (40.6)	34 (0.5)	4,180 (59.9)
Cefotaxime	7,098	6,830 (96.7)	19 (0.3)	215 (3.0)†
Ceftazidime	5,960	5,751 (96.5)	10 (0.2)	199 (3.3)‡
Ciprofloxacin	7,078	5,673 (80.1)	33 (0.6)	1,372 (19.3)
Gentamicin	7,074	6,558 (92.7)	34 (0.5)	482 (6.8)
Cotrimoxazole	6,597	4,432 (67.2)	11 (0.2)	2,154 (32.6)
Tobramycin	6,135	5,688 (92.7)	122 (2.0)	325 (5.3)

*S, susceptible; I, intermediate; R, resistant.
†204 extended-spectrum β-lactamase (ESBL) producers.
‡185 ESBL producers.

was higher in nosocomial isolates from hospitals with >500 beds than in those from hospitals with ≤500 beds, 24.6% vs. 21.3% (p = 0.02, OR 1.2, 95% CI 1.02–1.42). No statistical differences were found in the resistance figures to other antimicrobial agents according to hospital size. In general, antimicrobial resistance did not vary in relation to hospital departments; however, resistance to gentamicin was more prevalent in intensive care units than in internal medicine, 10.5% vs. 6.8% (p = 0.04, OR 1.57, 95% CI 1.02–2.40).

Isolates from children ≤14 years of age were significantly more resistant to ampicillin than those from patients >14 years of age, 63% vs. 57.4% (p = 0.047, OR 1.27, 95% CI 1–1.62). In contrast, ciprofloxacin resistance was less prevalent in children than in adults, 8.8% vs. 20% (p < 0.001, OR 0.38, 95% CI 0.25–0.58). In the other antimicrobial agents tested, no differences relating to patient age were apparent. Among the 27 ciprofloxacin resistance isolates from children, 3 (11.1%) were also ESBL producers.

Resistance to cotrimoxazole, ciprofloxacin, and gentamicin was more prevalent in ampicillin-resistant (46.7%, 27.7%, and 10.8%, respectively) strains than in ampicillin-susceptible strains (9.9%, 8.5%, and 1.8%, respectively) (p < 0.001). Also, *E. coli* ESBL-producing strains were significantly more resistant to other non–β-lactam antimicrobial agents than nonproducing strains, as was the case for ciprofloxacin (57.4% vs. 18.4%; p < 0.001), cotrimoxazole (56.8% vs. 30.3%; p < 0.001), and gentamicin (22.5% vs. 6.5%; p < 0.001). Of the 1,372 ciprofloxacin-resistant isolates, 113 (8.2%) were also ESBL producers. In contrast, of 5,673 ciprofloxacin susceptible isolates, only 91 (1.6%) were ESBL producers (p < 0.001, OR 5.59, 95% CI 4.21–7.42).

Of the 5,018 (70.7%) strains tested for simultaneous susceptibility to ampicillin, ciprofloxacin, gentamicin, cotrimoxazole, cefotaxime and ceftazidime, multidrug resistance was present in 863 (17.2%) isolates. The most prevalent phenotypes included resistance to ampicillin, cotrimoxazole, and ciprofloxacin, which was detected in 382 isolates (44.3% of multidrug-resistant strains and 7.6% of strains overall) and resistance to ampicillin, cotrimoxazole, ciprofloxacin, and gentamicin, detected in 151 strains (17.5% of multidrug-resistant strains and 3% of strains overall).

Trends in Antimicrobial Resistance

Ampicillin and cotrimoxazole resistance did not significantly vary over the study period, from 58.4% (2001) to 57.9% (2003) and from 32.9% (2001) to 31.9% (2003), respectively (Figure 1). However, resistance to ciprofloxacin increased from 17.2% in 2001 to 21.1% in 2003 (3.9% change) (p < 0.001, OR 1.29, 95% CI 1.11–1.50) (Figure 2).

The prevalence of ciprofloxacin resistance in community-acquired isolates increased from 13.3% in 2001 to 19.3% in 2003 (6% change) (p = 0.0002, OR 1.56, 95% CI 1.22–1.98), a higher increase than that observed for all strains. Figures 2 and 3 show the evolution of community quinolone and cotrimoxazole use compared with resistance to ciprofloxacin and cotrimoxazole, respectively, in invasive community-acquired *E. coli* infections. In the first case (Figure 2), both parameters increased, but cotrimoxazole use was strongly reduced from 1965 to 2003, while resistance figures remained near 30% (2001–2003) (Figure 3).

The global rates of invasive *E. coli* ESBL producers increased from 1.6% (2001) to 4.1% (2003) (2.5% change)

Table 2. Prevalence of antimicrobial resistance in invasive isolates of *Escherichia coli* in relation to patient sex*

	Male		Female			
Antimicrobial agent	N	R% (n)	N	R% (n)	p	OR (CI 95%)
Ampicillin	3,484	58.8 (2,049)	3,581	56.5 (2,023)	0.05	1.10 (1.00–1.21)
Ciprofloxacin	3,478	22.8 (793)	3,570	16.3 (582)	≤0.0001	1.52 (1.34–1.71)
Cotrimoxazole	3,240	32.9 (1,066)	3,329	29.5 (982)	0.002	1.17 (1.05–1.30)
Gentamicin	3,474	8.8 (306)	3,570	5.1 (182)	≤0.0001	1.80 (1.48–2.18)
Cefotaxime	3,468	3.5 (121)†	3,566	2.5 (89)†	0.01	1.41 (1.06–1.88)

*R%, percent resistance; OR, odds ration; CI, confidence interval.
†Include isolates with intermediate susceptibility and resistance.

Table 3. Prevalence of antimicrobial resistance in nosocomial and community-acquired invasive isolates of *Escherichia coli**

	Nosocomial		Community-acquired			
Antimicrobial agent	N	R% (n)	N	R% (n)	p	OR (CI 95%)
Ampicillin	3,337	61 (2,036)	3,734	54.6 (2,039)	<0.0001	1.64 (1.49–1.81)
Ciprofloxacin	3,325	22.6 (751)	3,730	16.7 (623)	<0.0001	1.46 (1.29–1.64)
Cotrimoxazole	3,098	34.3 (1,063)	3,484	28.2 (982)	<0.0001	1.33 (1.20–1.48)
Gentamicin	3,328	8.8 (293)	3,721	5.2 (193)	<0.0001	1.76 (1.46–2.14)
Cefotaxime	3,315	4.4 (146)†	3,724	1.9 (71)†	<0.0001	2.37 (1.76–3.19)
Multiresistance	2,414	19.3 (466)	2,586	13.1 (339)	<0.0001	1.59 (1.36–1.85)

*R%, percent resistance; OR, odds ratio; CI, confidence interval.
†Includes isolates with intermediate susceptibility and resistance.

(p < 0.0001, OR 2.70, 95% CI 1.77–4.15) (Figure 1). Community-acquired ESBL producers increased from 0.4% (2001) to 1.5% (2003) (1.1% change) (p < 0.001, OR 3.74, 95% CI 1.68–8.67).

Regarding susceptibility to third-generation cephalosporins, the number of strains nonsusceptible to cefotaxime (MIC >8 μg/mL) but susceptible to ceftazidime (MIC ≤8 μg/mL), increased from 26.5% in 2001 to 39.8% in 2003 (13.3% change) (p < 0.0001, OR 1.83, 95% CI 1.59–2.12). The prevalence of multidrug resistance among isolates tested for ampicillin, ciprofloxacin, gentamicin, cotrimoxazole, cefotaxime, and ceftazidime was 13.8% in 2001, 16.1% in 2002, and 20.6% in 2003 (p < 0.0001, OR 1.62, CI 95% 1.33–1.97) (Figure 1).

Antimicrobial Use

Total β-lactam use decreased from 13.34 DDD/1,000 inhabitants/day in 1998 to 11.44 DDD/1,000 inhabitants/day in 2003 (14.5% change). Consumption of broad-spectrum penicillins and cephalosporins decreased from 6.02 to 4.52 DDD/1,000 inhabitants/day (24.9% change) and from 2.65 to 2.20 DDD/1,000 inhabitants/day (17% change), respectively. In contrast, the use of amoxicillin/clavulanate (4.67 DDD/1,000 inhabitants/day in 1998 to 6.54 DDD/1,000 inhabitants/day in 2003) and quinolones (1.96 DDD/1,000 inhabitants/day in 1998 to 2.69 DDD/1,000 inhabitants/day in 2003) increased by 40% and 37.2%, respectively. Ciprofloxacin use remained stable; levofloxacin and moxifloxacin use increased. From 1998 to 2003, cotrimoxazole consumption was very low and decreasing. However, when analyzed from 1985, cotrimoxazole use decreased by 89.4%, from 3.2 DDD/1,000 inhabitants/day to 0.34 DDD/1,000 inhabitants/day (Figure 3).

Discussion

Epidemiologic surveillance of antimicrobial resistance is indispensable for empirically treating infections, implementing resistance control measures, and preventing the spread of antimicrobial-resistant microorganisms (21). The EARSS network, which includes >700 laboratories, is the official European network of national surveillance systems. It aims to collect comparable and reliable antimicrobial resistance data, with susceptibility data provided by each microbiology laboratory according to standard methods, mainly based on NCCLS rules.

This European network has some important characteristics as a surveillance system for resistance to antimicrobial agents (22). These characteristics include the following: 1) aggregation of data by each individual country and overall European countries, 2) rapid analysis and diffusion of data, 3) early detection systems for antimicrobial resistance in pathogens of clinical and public health relevance, and 4) basic decision support for public health.

Use of the information generated by the primary clinical laboratory has several disadvantages, namely, the possible variability in the antimicrobial agents assayed, the study methods used, and the interpretative criteria employed. In our experience, however, most laboratories used NCCLS-recommended methods. Previous validation of antimicrobial susceptibility results from 22 European countries, including Spain, has been performed by EARSS researchers (23). In addition, cross-validation of routine data gathering and centralized surveys has been implemented previously (24).

In this study, *E. coli* ESBL producers were infrequent (2.9%) but much higher than the 0.36% found in 1,918 European clinical blood isolates of *E. coli* isolated from 1997 to 1998 (7). One of the ESBL producer strains included in the quality control was undetected by 15% of

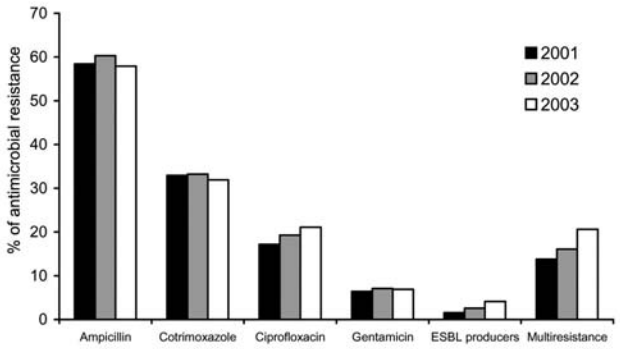

Figure I. Annual evolution of antimicrobial resistance in invasive *Escherichia coli* isolated by Spanish laboratories participating in European Antimicrobial Resistance Surveillance System, 2001–2003.

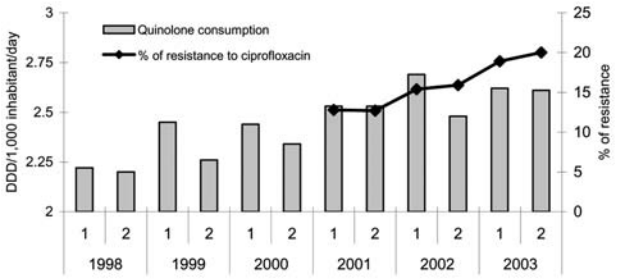

Figure 2. Evolution of community quinolone consumption and prevalence of resistance to ciprofloxacin in invasive community-acquired *Escherichia coli* infections (European Antimicrobial Resistance Surveillance System, Spain 2001–2003). DDD, defined daily doses. 1, January–June; 2, July–December.

the laboratories; this potential misclassification could lead to underestimates of the prevalence of ESBL isolates in this study. When ceftazidime nonsusceptibility was used as a surrogate marker for ESBL, 1.2% of 71,800 *E. coli* isolated from blood in the United States were nonsusceptible to ceftazidime (25).

In addition, 32.4% of ESBL producers were implicated in community-acquired infections. Although no data about possible previous healthcare contact of the persons infected with ESBL in the community were available, the spread of these types of β-lactamases outside hospitals is a matter of great concern.

We found a significant increase in ESBL production in recent years in Spain, which affected both total and community-acquired isolates. In addition, the increased prevalence of isolates showing nonsusceptibility to cefotaxime but susceptibility to ceftazidime (26.5% in 2001 vs. 39.8% in 2003) suggests that ESBL cefotaximases were increasing quickly, as described by other studies (12,13). In 2003 the first report from the United States appeared; it documented the isolation of *E. coli* isolates producing CTX-M–like ESBL (9 strains from 5 U.S. states) (26). The emergence of this ESBL-type has important implications for the detection of ESBL *E. coli* producers in clinical and epidemiologic surveys and emphasizes the need for ESBL screening to include both cefotaxime and ceftazidime.

Fluoroquinolone use has increased in many European countries (11,17), with Spanish consumption increasing from 1.96 DDD/1,000 inhabitants/day in 1998 to 2.69 DDD/1,000 inhabitants/day in 2003 (37.2%). In comparison with other European countries participating in EARSS that provided susceptibility results for at least 750 *E. coli* invasive isolates in 2003, ciprofloxacin resistance in Spain (21.1%) was among the highest in Europe. This figure is lower than that in Portugal (25.8%) and Italy (25.3%) but higher than percentages in such countries as Germany (15.2%), Belgium (11.6%), Greece (9.9%), Ireland (9.6%), France (9.4%), and the Netherlands (6.8%).

Isolates from children had a relatively high prevalence of ciprofloxacin resistance (8.8%), although ciprofloxacin was not used by children. This resistance could be due to the transmission of resistant isolates between adults and children in families, daycare, or school settings and to the use of fluoroquinolones in poultry populations (10).

In a recent survey of 494 U.S. hospitals, the prevalence of ciprofloxacin resistance was 6%; it had increased in 40% of the participant hospitals (27). Also, among 286,187 isolates of *E. coli* from urinary tract infections in female outpatients in the United States, ciprofloxacin was the only agent studied that demonstrated a consistent stepwise increase in resistance from 1995 (0.7%) to 2001 (2.5%) (28). In our study, a significant increase in ciprofloxacin resistance, principally in community-onset infections, coincided with rising community quinolone use. Association between fluoroquinolone use and quinolone-resistant *E. coli* has been described recently (29).

Cotrimoxazole resistance remained stable in this study, ≈30%, and similar to the 27% reported in urinary tract infection isolates in 1993 in Spain (30), in spite of the great reduction (89.4%) found in community cotrimoxazole use in the last 18 years. A similar situation was described previously with sulfonamide resistance in the United Kingdom (31). In areas with high resistance rates maintained over long periods of time, reduction in antimicrobial pressure may have a slower effect, especially in the presence of multidrug resistance (32). This may be due to genetic linkage between resistance mechanisms and, therefore, co-selection by using only 1 antimicrobial agent (31), or to the reservoir of molecular resistance mechanisms in species of commensal flora (33).

Antimicrobial resistance, principally to ciprofloxacin and gentamicin, varied between the sexes, with isolates from male patients more resistant than those from female patients. Similar trends have been described recently in the United States (9) and the Netherlands (11). Nosocomial isolates were also more resistant than community-acquired

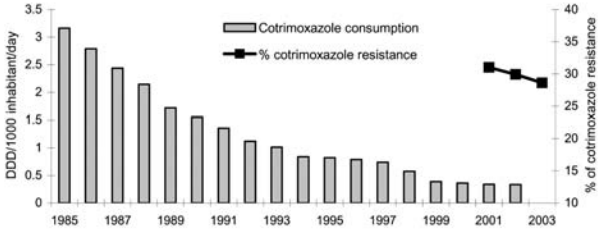

Figure 3. Annual evolution of community cotrimoxazole consumption and prevalence of resistance to cotrimoxazole in invasive community-acquired *Escherichia coli* infections, European Antimicrobial Resistance Surveillance System, Spain, 2001–2003. DDD, defined daily dose.

ones, similar to findings from a recent study in South Korea (34). In both cases, these data probably reflect the tendency for male patients and hospitalized patients to more frequently have complicated urinary tract infections, the principal source of invasive *E. coli*, which may be associated with more chronic pathologic conditions and more antimicrobial treatments. Possibly the most important determining factor in resistance is use of antimicrobial agents, as described for ciprofloxacin (Figure 2) (29).

In our study, multidrug resistance was frequent (17.2%) and increased by 50% during the study period (2001–2003). Multidrug resistance in the United States among 38,835 urinary tract infection isolates was 7.1% in 2000 (9). Such multidrug resistance has important implications for the empiric therapy of infections caused by *E. coli* and for the possible co-selection of antimicrobial resistance mediated by multidrug resistance plasmids (35), as described above.

Because antimicrobial resistance patterns are continually evolving and *E. coli* invasive isolates undergo progressive antimicrobial resistance, continuously updated data on antimicrobial susceptibility profiles will continue to be essential to ensure the provision of safe and effective empiric therapies. Moreover, results obtained from these surveillance systems must be used to implement prevention programs and policy decisions to prevent emergence and spread of antimicrobial resistance.

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Staphylococcus aureus Bacteremia, Australia

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Staphylococcus aureus bacteremia (SAB) is common and increasing worldwide. A retrospective review was undertaken to quantify the number of cases, their place of acquisition, and the proportions caused by methicillin-resistant *S. aureus* (MRSA) in 17 hospitals in Australia. Of 3,192 episodes, 1,571 (49%) were community onset. MRSA caused 40% of hospital-onset episodes and 12% of community-onset episodes. The median rate of SAB was 1.48/1,000 admissions (range 0.61–3.24; median rate for hospital-onset SAB was 0.7/1,000 and for community onset 0.8/1,000 admissions). Using these rates, we estimate that ≈6,900 episodes of SAB occur annually in Australia (35/100,000 population). SAB is common, and a substantial proportion of cases may be preventable. The epidemiology is evolving, with >10% of community-onset SAB now caused by MRSA. This is an emerging infectious disease concern and is likely to impact on empiric antimicrobial drug prescribing in suspected cases of SAB.

Bacteremia caused by *Staphylococcus aureus* continues to be a common problem worldwide. In the preantibiotic era, most cases occurred in young patients without underlying disease. The associated death rate was 82% (1). Even with antimicrobial drug treatment, death rates remain high; in a recent meta-analysis of 31 studies, estimates of death rates for methicillin-resistant strains (MRSA) varied from 0.0% to 83.3% (median 34.2%), while those for methicillin-sensitive strains (MSSA) varied from 3.6% to 51.7% (median 25.0%) (2). Many of these infections are healthcare associated and thus are potentially preventable.

Antimicrobial drug resistance in *S. aureus* arose early after the development of antimicrobial agents and continues to evolve. In Australia, hospital strains are frequently methicillin resistant and resistant to several other antimicrobial drugs (3). This resistance limits the choice of potentially efficacious agents and results in frequent use of glycopeptides, such as vancomycin. The reliance on vancomycin causes difficulties because vancomycin has been shown to be less effective than isoxazolyl penicillins (e.g., flucloxacillin) in treating severe infections caused by *S. aureus* (4,5). This may be 1 explanation for the higher death rate associated with bacteremia caused by MRSA, compared with that caused by MSSA (2,6). Although MRSA tends to be the bacterium discussed most often in relation to healthcare-associated infections, MSSA strains are responsible for the largest proportion of hospital-acquired infections (3).

S. aureus remains a common cause of bloodstream infections of community onset. Increasing numbers of these community-onset infections are being caused by MRSA. Some of these infections may be caused by hospital strains carried into the community by patients or healthcare workers, but others are caused by true community strains in patients who have had no recent healthcare contact (7–9). These strains have emerged in many countries, including Australia, New Zealand, the United States, Canada, France, Switzerland, Greece, Denmark, Finland, Scotland, and the Netherlands. They are susceptible to most or all non–β-lactam antimicrobial drugs, are highly pyogenic, and are often associated with indigenous populations (10,11).

Although *S. aureus* is a well-known major cause of bacteremia, population-based estimates of its incidence are lacking. This study used hospital data to estimate the incidence of *S. aureus* bacteremia in Australia. In addition, we

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classified episodes on the basis of community or hospital onset and on the basis of methicillin susceptibility.

Methods

S. aureus bacteremia data were obtained from microbiology departments that prospectively collected information for >12 months on episodes of laboratory-confirmed bacteremia for the hospitals they serviced from January 1, 1999, to December 31, 2002. Information retrieved from existing databases included the total number of episodes of community- and hospital-onset bacteremia, the number of episodes of community- and hospital-onset MRSA and MSSA bacteremia, the total number of hospital separations (defined as completed hospital admissions), and the mean length of stay. Multiple positive blood cultures in the same patient within 14 days were considered a single episode. Episodes were considered to have a hospital onset when the first positive blood culture was collected >48 hours after admission to hospital. All other infections were designated community onset (for example, day-only dialysis related episodes were defined as community onset, as were infections with their onset in nursing homes). Organism identification and susceptibility testing were by standard methods. All these laboratories participate in external quality assurance programs as well as AGAR national surveys (3,12), which have quality control procedures to ensure these laboratories accurately detect methicillin resistance. Published data were used for the details on the number of hospital beds and separations for Australia and for the classification of different types of hospitals (13). The term separations, rather than admissions, is used in the published data because hospital abstracts for inpatient care are based on information gathered at the time of discharge. We have used the more commonly applied term of admissions, however, for these episodes.

In Australia, most healthcare-associated MRSA is caused by 1 clone defined by multilocus sequence type (ST) 239; this clone is characteristically resistant to multiple antimicrobial agents, including gentamicin (3,12). Most of the remaining healthcare-associated infections are caused by a recently introduced strain, ST22, which is indistinguishable from epidemic MRSA-15 in the United Kingdom. It is invariably resistant to ciprofloxacin (12). Thus, in Australia, MRSA that is acquired in the community and is sensitive to both ciprofloxacin and gentamicin is not likely to be associated with healthcare facility acquisition. We used this pattern as a surrogate marker for community acquisition of MRSA without healthcare-associated risk factors.

Results

We detected 12,771 bloodstream infections in the 17 hospitals participating in this study (12 principal referral metropolitan, 3 large metropolitan, 1 private hospital, and 1 medium-sized public hospital, and 1 private hospital with 2,013,534 total separations; Table 1). There were 3,192 episodes of *S. aureus* bacteremia identified (i.e., 25% of the total true bloodstream infections). The median rate of *S. aureus* bacteremia was higher in the principal referral metropolitan hospitals (1.59/1000 admissions) than in large metropolitan hospitals (1.3) or the private hospital (0.6). The range varied from 0.60 to 3.24 (Table 2). The median rate of community-onset bacteremia episodes was 0.80/1000 admissions (range 0.11–0.99). The median rate of hospital-onset bacteremia was 0.72 episodes/1,000 admissions (range 0.13–1.30). The median rate of hospital-onset MRSA episodes was 0.22/1,000 admissions (range 0–0.89). When expressed as MRSA episodes per 1,000 occupied bed days (OBDs), the rates varied from 0 to 0.30 with a median rate of 0.08. If day-only cases are removed from the denominator then the median rate was 0.10 per 1,000 OBDs (range 0–0.39).

Of these 3,192 SAB episodes, 1,621 (51%) were of hospital onset, and 1,571 (49%) had their onset in the community. Of those with a hospital onset, 40% were MRSA in comparison to 12% with a community onset. Of all MRSA bacteremia episodes, 23% had a community onset, and 77% had hospital onset. Of the 193 community-onset episodes of MRSA that occurred, only 47 (24%) had a sensitivity pattern (sensitive to gentamicin and ciprofloxacin) that suggests that they were community acquired.

When both MRSA and MSSA were considered, data were available for 560 community-onset SAB infections (but only from 4 hospitals). The proportions of these episodes that were noninpatient, healthcare-associated were 35%, 42%, 18% and 16%, respectively (from hospitals A, D, E, and N). In those hospitals, the percentage of *S. aureus* episodes that were healthcare associated overall (i.e., all hospital-onset cases and those community-onset cases associated with healthcare exposure) were 75%, 69%, 64%, and 36%, respectively.

Mortality data were available for 526 patients from 2 hospitals. At hospital E, the mortality rate at day 7 was 10% (27 of 267 patients). When a subgroup of these patients at hospital E (52 patients) was followed for a longer period (2001–2002), the mortality rate was 23% at 30 days and 35% at 6 months. For those 24 patients with a community-onset episode of bacteremia that was not healthcare associated, mortality rates were 6% at day 7, 17% at 1 month, and 21% at 6 months, respectively. At hospital H (259 patients), the mortality rate at 30 days was 19%. At hospital H, the mean length of stay for those with SAB was 25.6 days compared to 6.2 days in matched controls. The mean length of stay was longer for MRSA infections (39.2 days) than for MSSA infections (23.3 days).

Table 1. Bacteremia episodes at individual hospitals*

	Hospital								
	A	B	C	D	E	F	G	H	I
Classification†	a	a	a	a	a	a	a	a	a
Beds	723	587	551	525	504	468	455	394	391
Years studied	4‡	4‡	3§	4‡	4‡	3§	4‡	4‡	2¶
Admissions over study period	256,251	203,130	150,502	204,116	194,246	132,781	185,680	175,583	67,855
Admissions >24 h over study period	66,035	76,147	49,501	102,361	60,498	42,515	39,758	44,502	47,406
Mean length of stay (day cases included)	3.85	3.48	3.84	3.6	3.43	3.8	3.61	3.32	4.25
OBDs (including day only patients)	986,566	706,892	577,928	781,235	666,264	504, 568	670,305	582, 936	288, 384
OBDs (excluding day only patients)	796,350	579,909	476,927	679,481	532,516	414,302	524,383	451,855	267,935
Total *S. aureus* bacteremia	331	365	333	373	267	107	426	259	115
Total BSIs over study period (all orgs)	1,531	1,172	1,294	1,546	196	605	1,689	1,120	472
Total BSI rate per hosp admissions (x1,000)	5.97	5.76	8.60	7.57	6.67	4.50	9.01	6.38	6.95

	Hospital								
	J	K	L	M	N	O	P	Q	Total
Classification†	a	a	a	b	b	b	c	d	–
Beds	368	297	276	199	170	162	72	52	6,194
Years studied	4‡	2¶	4‡	4‡	4‡	4‡	4‡	4‡	–
Admissions over study period	104,534	58,549	92,114	64,311	41,690	48,900	18,223	15,069	2,013,534
Admissions >24 h over study period	50,018	25,617	36,322	31,259	10,556	31,681	13,055	2,894	730,125
Mean length of stay (day cases included)	4.1	4.49	3.06	3.37	5.4	3.60	5.10	2.88	–
OBDs (including day only patients)	428,589	262,592	281,869	216,728	225,126	176,040	92,937	43,399	7,491,240
OBDs (excluding day only patients)	374,073	229,660	226,077	183,676	192,874	158,821	87,769	31,224	6,207,832
Total *S. aureus* bacteremia	155	123	72	44	135	62	11	14	3,192
Total BSIs over study period (all orgs)	653	338	351	282	881	274	67	63	12,771
Total BSI rate per hosp admissions (x1,000)	6.25	5.77	3.81	4.39	21.13	5.60	3.68	4.18	–

*MSSA, methicillin-susceptible *Staphylococcus aureus*; MRSA, methicillin-resistant *S. aureus*; OBDs, occupied bed days; BSI, bloodstream infection; orgs, microorganisms.
†Hospital classification: a, principal referral: metropolitan (>20,000 acute weighted separations per year) and rural (>16,000 acute weighted separations); b, large metropolitan (>10,000 acute weighted separations); c, private hospital; d, medium sized (metropolitan and rural 2,000 acute or acute weighted to 5,000 acute weighted separations).
‡1999–2002.
§1999–2001.
¶1999–2000.

The rates of *S. aureus* bacteremia in different hospital populations were used to estimate the incidence for Australia. Using our median bacteremia rate for *S. aureus* bacteremia in different types of public hospitals (1.27/1,000 admissions, range 0.68–3.24) and in private hospitals (0.6/1,000 admissions), we estimated ≈6,900 episodes per year nationally (range 3,826–20,658) or 35/100,000 per year (Tables 3 and 4). Some data are available from other countries for comparison; the lowest annual rates are in Northern Ireland (23/100,000) and the highest in the United States (56/100,000; Table 4). However only 2 countries, Denmark and England, appeared to have comprehensive collection systems, and their rates were 29/100,000 and 37/100,000, respectively (17,20,22).

Discussion

S. aureus bacteremia is very common. Approximately one fourth (26%) of all *S. aureus* bacteremia episodes were caused by MRSA, and, as expected, the onset of most of

Table 2. *Staphylococcus aureus* bacteremia at individual hospitals*

Hospital	Rate†							
	Incidence‡	Community-onset infections‡	Hospital-onset *S. aureus* infection‡	Hospital-onset MSSA‡	Hospital-onset MRSA‡	SAB sepsis§	MRSA SAB§ (including 1 day only)	MRSA SAB§ (excluding 1 day only)
A	1.29	0.51	0.78	0.54	0.24	0.34	0.08	0.10
B	1.80	0.74	1.05	0.58	0.48	0.52	0.17	0.21
C	2.21	0.94	1.27	0.74	0.53	0.58	0.18	0.22
D	1.83	0.99	0.84	0.64	0.20	0.48	0.08	0.09
E	1.37	0.66	0.72	0.46	0.26	0.40	0.09	0.11
F	0.80	0.35	0.46	0.42	0.04	0.21	0.01	0.01
G	2.29	0.99	1.30	0.41	0.89	0.64	0.30	0.39
H	1.48	0.83	0.65	0.55	0.10	0.44	0.05	0.06
I	1.69	0.77	0.93	0.49	0.44	0.40	0.14	0.15
J	1.48	0.88	0.60	0.25	0.35	0.35	0.15	.017
K	2.10	1.06	1.04	0.59	0.44	0.47	0.13	0.15
L	0.78	0.58	0.21	0.10	0.11	0.26	0.05	0.06
M	0.68	0.48	0.20	0.08	0.12	0.20	0.05	0.05
N	3.24	2.40	0.84	0.62	0.22	0.60	0.06	0.07
O	1.27	0.80	0.47	0.41	0.06	0.35	0.02	0.03
P	0.60	0.11	0.49	0.44	0.05	0.12	0.01	0.01
Q	0.93	0.80	0.13	0.13	0.00	0.32	0.00	0.00
Total	1.59	0.78	0.81	0.49	0.32	0.43	0.11	0.13

*MSSA, methicillin-susceptible *S. aureus*; MRSA, methicillin-resistant *S. aureus*; SAB, *S. aureus* bacteremia.
†Data for these calculations presented in expanded online table, available at http://www.cdc.gov/ncidod/eid/vol11no04/04-0772.htm#table2
‡Per hospital admission (×1,000).
§Per occupied bed days (×1,000).

these episodes was in hospitals (77%). Notably, however, 12% of all community-onset *S. aureus* infections were MRSA, which was 23% of all MRSA bloodstream infection episodes. A recent study from the United States similarly showed that 15% of community-onset SAB episodes were MRSA (14). Most of the community-onset strains in our study were multiresistant or phenotypically consistent with UK EMRSA-15 (15) and thus most likely to have been acquired by patients who had previous hospital contact, with nursing home contact a major factor in at least 1 of the hospitals in this study (hospital G). However, approximately one fourth of these community-onset MRSA infections were caused by other phenotypes of non–multiresistant MRSA and thus more likely to be true community-acquired episodes of MRSA bacteremia. Severe cases of MRSA bacteremia not associated with prior healthcare contact have been reported previously in Australia (7,9,16).

Use of the >48 hours postadmission definition of hospital onset underestimates the number of episodes of bacteremia that are healthcare associated. Many patients with chronic conditions are now treated in the community or on a day-only basis. Vascular lines are increasingly used in the community and outpatient settings, providing a potential source of bacteremia. The collection of data on the true association of episodes of bacteremia to health care is time-consuming and was not done by most institutions participating in this study. However, 3 principal referral hospitals (hospitals A, D, and E) did collect these data for 971 episodes, and 64%–75% of their total *S. aureus* bacteremia episodes were healthcare associated. Only 46%–61% of the episodes were acquired while the patient was an inpatient (i.e., >48 h in hospital). This finding means that in these larger hospitals approximately one third of healthcare-associated episodes were acquired by either outpatients or short-stay patients. These episodes are better defined as "noninpatient, healthcare-associated." In a recent study in the United States, 62% of their community-onset SAB infections were healthcare related (with intravenous [IV] catheters the most common clinically apparent site of infection) (14). On the basis of our data, we conclude that in Australia approximately two thirds of all SAB episodes were associated with healthcare or medical procedures (i.e., all hospital-onset and approximately one third of community-onset episodes). A similar situation is evident in Denmark (17), where in 2002, at least 59% of all *S. aureus* infections were associated with healthcare procedures. Clearly, substantial scope exists internationally for interventions in healthcare settings to decrease the numbers of these episodes (especially those related to IV catheters). Interventions to reduce *S. aureus* bacteremia need to target healthcare-associated infections in the broadest sense and include those following non–inpatient-related medical procedures.

Community-onset infections that have no healthcare association are also common and associated with a high death rate (17% and 19% at hospitals E and H at 1 month,

Table 3. Estimated numbers of *Staphylococcus aureus* bacteremia in Australia*

Published data for Australia 2001–2002 (13)	Total hospitals†		
	Acute public‡	Private	Australia-wide
No. hospitals	724	537§	1,306
No. beds	49,004	27,407	75,516
Total admissions (x1,000)	3,950	2,426	6,376
Same day separations (x1,000)	1,886	1,453	–
Average length of stay	4.1	2.9	3.5
S. aureus BSI episodes; (calculated rates from data in this study)			
S. aureus BSI rate/1,000 admissions	0.68–3.24	0.6	0.6–3.24
Estimated episodes/y	2,370–12,798	1,456	3,826–20,658
Median rate/1,000 admissions	1.37	0.6	NA
Estimated episodes/y (based on median)	5,412	1,456	6,867
Hospital-onset MSSA			
Rate/1000 admissions	0.08–0.74	0.44	0.10– 0.97
Estimated episodes/y	316–2,923	1 067	638–4,718
Median rate/1,000 admissions	0.47	0.44	NA
Estimated episodes/y (based on median)	1,769	1,067	2,836
Hospital-onset MRSA			
Rate/1,000 admissions	0.05–0·89	0.05	0.05–0.89
Estimated episodes/y	198–3,516	121	255– 5,675
Median rate/1,000 admissions	0.25	0.05	NA
Estimate episodes/y (based on median)	868	121	1,015

*BSI, bloodstream infection; MSSA, methicillin-sensitive *S. aureus*; MRSA, methicillin-resistant *S. aureus*; NA, not applicable.
†For full details for individual hospitals in this study and hospital grouping, see Table 3 at http://www.cdc.gov/ncidod/eid/vol11no04/04-0772.htm#table3
‡Acute public hospitals exclude psychiatric hospitals.
§Of private hospitals, 246 were day only, 314 others had admissions for >24 h.

respectively). How best to intervene to decrease these infections is difficult to determine. Vaccination is a possibility for the future; a recent trial of a conjugated capsular polysaccharide vaccine in renal dialysis patients estimated efficacy at ≈60% (18). However, vaccination for the general population is unlikely to be available soon. We should therefore concentrate on reducing the number of deaths from established infections. Because the mortality rate associated with community-acquired bacteremia increases with inadequate empiric therapy (19), all efforts should be made to promote compliance with published guidelines for treatment of severe staphylococcal sepsis, including adequate duration of therapy.

Available data suggest that staphylococcal bacteremia is a major global health problem. The median death rate for MSSA infections is 25%, and for MRSA infections, 34% (20). Thus, >1,700 deaths in Australia are likely associated with *S. aureus* bacteremia per year (assuming 6,900 episodes or a bacteremia rate of 35/100,000/year). This estimate of the rate of SAB is similar to England (20,22) but much lower than in the United States on the basis of the rate derived from the figures available in the only comparative study (55/100,000) (14). Our estimated rate in Australia is higher than that in Denmark (17,21). It is also higher that those reported from Wales (22) and Ireland (23) (Table 4); however, all episodes from these last 2 countries likely were not reported in their voluntary reporting schemes. England changed recently from a similar voluntary reporting scheme to a compulsory scheme, and the numbers of reported episodes increased by almost 50% (24).

The rate of MRSA bacteremia in England was higher per 1,000 OBDs than in our figures from Australia (0.17 compared to 0.10 episodes per 1,000 OBDs, respectively). MRSA was a substantial cause of episodes of SAB in this study (26%). However, this percentage was lower than that seen in most other countries (e.g., Wales, 47%; Table 4) with the notable exception of Denmark (0.6% in 2002) (17).

We may have overestimated the number of cases of bacteremia occurring in Australia because of the overrepresentation of larger hospitals in our survey. However, these hospitals participated because they had in place surveillance systems for measuring all episodes of bacteremia. The rates of SAB may have been relatively lower in these hospitals because they were also more likely than were hospitals without surveillance systems to have infection control programs in place to try to decrease the numbers of these episodes. If systems were in place that better captured and reported on all bacteremia episodes in well-defined populations (e.g., all of Australia or a state), then this would give a more accurate rate. Such systems appear only to be in place in Denmark and England (17,21,24). Currently, no such systems are operating in Australia. Limited data are available from a voluntary surveillance

Table 4. International rates and numbers of *Staphylococcus aureus* bacteremia (SAB)*

Country	Y	Population	SAB/y	SAB/10^5/y	% MRSA
Australia					
Present report	1998–2002	19,500,000	6,900	35	27
Victoria (25)†	1990–1999	4,502,000	804	27	28
Denmark					
Northern Jutland (21)	1996–1998	493 000	155	31	ND
Whole of Denmark (17)‡	2002	5,350,000	1,488	28	0.6
Ireland (23)§	1999	3,700,000	ND*	25	36
United Kingdom					
England (20,22)¶	2002–2003	49,200,000	18,403	37	40
	2003		19,244	39	41
Northern Ireland (22,24)#	2002	1,697,000	397	23	38
	2003		569	34	44
Wales (22)#	2003	2,920,000	742	25	47
USA					
Connecticut (14)**	1998	1,124,337	634	56	ND*

*MRSA, methicillin-resistant *Staphylococcus aureus*; ND, no data given.
†In Victoria, 8,036 SAB episodes were reported, resulting in a rate of 17.8/100,000. The final rate (27.0) for the entire state was extrapolated from this figure. The Victorian scheme is estimated to capture about two thirds of all bacteremia episodes that occur in that state per year.
‡System in place in Denmark since 1960, with numbers of episodes continually rising (e.g., in 1966, 400 per year and total population 4.8 million or 8/100,000). Collection data based on reviewing all discharge summaries and laboratory samples (15 of 16 counties). Associated 23% mortality rate in 2002, and 22% of these deaths were directly related to sepsis.
§Rates in different regions varied from 8.9 to 37.1 per 100,000. Likely underreporting (22).
¶Compulsory reporting system. Unclear if all community onset episodes were included. In England, underreporting occurred when a voluntary system was in place (only 13,770 episodes reported for 2003; thus, a 50% increase with compulsory system) (22).
#This rate is based on voluntary reporting system. Real rate might be 50% higher (22,24).
**Retrospective case analysis. Rate increased with age, urban areas, and African American ethnicity. 15% of community-onset SAB episodes were MRSA.

system in Victoria (25) that captures an estimated two thirds of bacteremic episodes that occur in that state. The extrapolated rate (27 episodes/100,000 persons/year; Table 4) was slightly lower than what we estimated for all of Australia in this study.

Substantial illness and increased medical costs are also associated with staphylococcal bacteremia. *S. aureus* bacteremia is often related to serious infections, including endocarditis, osteomyelitis, and septic arthritis. It frequently results in prolonged hospital admission and increased costs. In hospital H, the average length of stay for patients with *S. aureus* bacteremia was 26.5 days. In South Australia, the estimated additional cost of each episode of hospital-acquired *S. aureus* infection was $22,000 in 1998 (26). Nationally, these South Australian costs translate to additional hospital costs of ≈$150 million dollars ($22,000 x 6,900 episodes).

Treatment of *S. aureus* infections is complicated by the high prevalence of antimicrobial drug resistance. Although this has long been the case with multiresistant strains of MRSA in hospitals, the spread of hospital strains into the community, as well as the emergence of unique strains of MRSA unrelated to health care, have made this an issue of general importance. At least 3 community strains of MRSA are currently circulating in Australia (10,27,28). Two of these 3 community strains carry the gene for Panton-Valentine leukocidin, which is associated with subcutaneous abscess formation and necrotizing pneumonia. A number of reports have already highlighted the clinical impact of infection due to these strains (9,28–30). Surveillance data show that their prevalence is increasing in our capital cities, but the situation in rural Australia is not well documented (3). This increase will inevitably affect guidelines for empirical antimicrobial drug prescribing for staphylococcal infections and for patients in the community with suspected SAB. Further surveillance of staphylococcal infections, including bacteremia, is warranted to guide recommendations for empirical therapy and infection control interventions.

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The Australian Group on Antimicrobial Resistance (AGAR) is a group that represents 21 teaching hospital microbiology laboratories and 5 private laboratories. AGAR meets every 6 months. At these meetings, Drs. Gottlieb and Collignon made the initial proposal for this project. All members of AGAR were able to participate in the discussion of the project and

suggest modifications of the project design. Only 10 hospital laboratories had collected details on all their *S. aureus* bacteremia data prospectively, and these formed the AGAR participants able to participate in this study. Archie Darbar and Denise Daley were involved in the collection of data at their hospitals. Jan Roberts was involved in the collection of data at her hospital and also in the spreadsheet analysis of the data of all the participating hospitals.

Drs. Collignon, Nimmo, Gottlieb, and Gosbell were involved in the writing of the manuscript. They made substantial contributions to the conception and design of the study, as well as to the acquisition, analysis, and interpretation of data. They also drafted the article and revised it critically for intellectual content. Additionally, all of the other participants in this AGAR project provided comment and feedback on numerous drafts over a 6-month period. All authors have reviewed this version and given final approval for publication.

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Echovirus 30, Jiangsu Province, China

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An outbreak of aseptic meningitis occurred in the northern area of Jiangsu Province in China from January to July in 2003. A total of 1,681 cases were involved in this outbreak, and 99% of patients were <15 years of age. To identify the etiologic agent, 66 cerebrospinal fluid specimens were tested by cell culture. Eighteen showed an enteroviruslike cytopathic effect on MRC-5 human fetal diploid lung cells. An enterovirus primer-mediated reverse transcriptase–polymerase chain reaction, a standard neutralization assay, and sequencing of the complete capsid-encoding (VP1) gene identified the 18 isolates (FDJS03) as echovirus 30. At least a 10% difference was seen in nucleotide sequences of VP1 between FDJS03 isolates and other global strains of echovirus 30. Phylogenetic analysis based on complete sequences of VP1 was performed to further characterize the FDJS03 isolates. This report is the first to identify a distinct lineage of echovirus 30 as a probable cause of this outbreak.

Enteroviruses circulate worldwide and are the most commonly identified cause of aseptic meningitis, particularly in infants and young children; 30,000–50,000 persons per year are hospitalized with aseptic meningitis in the United States (1). During the past decade, numerous outbreaks of enteroviral meningitis were documented throughout the world (2–5). In China, infections with other enteroviruses are reported more frequently because wild-type polioviruses were eradicated by the expanded immunization program in 1992. Outbreaks associated with nonpolio enteroviruses have been sequentially reported in recent years (6–8). Specific serotypes of etiologic agents were not identified in outbreaks involving nonpolio enteroviruses in China, since serotyping usually had no influence on clinical management of a given patient with an enteroviral infection. However, identification of the serotype and the molecular characteristics of the prevailing virus can provide valuable epidemiologic information in outbreak investigations. The serotype-specific immune status of the population, territorial competition among serotypes, and transmission efficiency of the virus may also be important factors influencing epidemiologic behavior of human enteroviruses (HEV) (9,10). An understanding of circulating virus strains in local regions would be essential in effectively controlling enteroviral infections.

An unusual outbreak of aseptic meningitis in 1,681 patients occurred in the northern area of Jiangsu Province in China from January to July in 2003. In this report, we provide evidence for a distinct lineage of echovirus 30 as the etiologic agent of this outbreak.

Materials and Methods

Specimen Collection

Lumbar puncture on admission was used to obtain 204 cerebrospinal fluid (CSF) specimens from 204 hospitalized patients in prefectural hospitals in whom aseptic meningitis had been diagnosed. After culturing for bacterial growth, 66 CSF specimens were available for virus isolation on 3 cell lines. All CSF specimens (≈2 mL per sample) were sent to our laboratory in sterile containers at 4°C, separated into aliquots, and stored at –80°C for further study.

Cell Culture and Virus Isolation

Human fetal diploid lung (MRC-5), buffalo green monkey kidney (Vero), and human epidermoid carcinoma (HEp2) cells were used in the study. Cells were grown in minimal essential medium (MEM) supplemented with 10% newborn calf serum, 50 U/mL of penicillin, and 50 μg/mL of streptomycin. Viruses were isolated from the original clinical specimens and propagated in cell culture by standard methods (11). Briefly, 200 μL of each CSF specimen was added in duplicate into 24-well plates covered with monolayers of each cell culture. Maintenance medium (MEM plus 2% newborn calf serum) was then added to each well. All cultures were incubated at 37°C in

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an atmosphere of 5% CO_2 and observed daily for 7 days for an enteroviruslike cytopathic effect (CPE). In cultures exhibiting no CPE by the end of observation period, blind passage was performed for another 7 days. Passage was performed twice before the culture was reported as negative. Cultures showing an enteroviruslike CPE were passed once more for confirmation. The primary identification of positive isolates as enterovirus was done by a reverse transcriptase-polymerase chain reaction (RT-PCR) with 2 pairs of enterovirus general primers (12–14), which detect nearly all types of enterovirus (12,15). The reference enterovirus strain used in this study was coxsackievirus B1. Positive isolates were designated Echo30-FDJS03, which was abbreviated as FDJS03.

Neutralization Assays with an Antibody Pool

Serotype identification was performed by neutralization with an antibody pool for enterovirus (Kunming Medical Biology Institute, Kunming, China) and 11 other type-specific monoclonal antibodies (Kunming Medical Biology Institute) not included in the antibody pool. Each positive strain was tested by a neutralization assay, according to standard procedures (16), and viral titers were simultaneously determined.

Extraction of Viral RNA

Viral RNA was extracted from 200 μL of positive culture supernatants by using RNAex reagent (Huashun Co., Shanghai, China), according to the manufacturer's instructions, and diluted in 20 μL of diethylpyrocarbonate-treated sterile distilled water. The viral RNA solution was used immediately or stored at –80°C until analysis by RT-PCR. A standard reference virus strain and a normal cell culture supernatant were used as positive and negative controls, respectively.

RT-PCR and Sequencing of DNA

A 2-step RT-PCR was used. Synthesis of cDNA was done in a 20-μL reaction volume containing 2 mmol/L deoxynucleotide triphosphates, 20 U of RNAsin (Promega, Madison, WI, USA), 10 pmol of random hexamer, 200 U of Moloney murine leukemia virus reverse transcriptase (Promega), 4 μL of 5× RT buffer, and 5 μL of extracted RNA. The mixture was incubated at 37°C for 60 min and inactivated at 95°C for 5 min.

The highly conserved 5′ untranslated region (UTR) was used to detect enterovirus by PCR amplification (17). Two sets of amplification primers (12–14), UG52 (sense, 5′-CAAGCACTTCTGTTTCCCCGG-3′, nucleotides [nt] 168–188, Bastianni) and primer 2 (sense, 5′-TCCTCCG-GCCCCTGAATGCG-3′, nt 445–464, coxsackie B1) (12), and a common antisense primer (UC53 5′-TTGTCAC-CATAACCAGCCA-3′, nt 588–606, Bastianni) were used to produce PCR products of 440 (UG52/UC53) and 155 (primer2/UC53) bp, respectively. The PCR protocol consisted of denaturation at 94°C for 5 min; extension for 35 cycles at 94°C for 45 s, 52°C for 45 s, and 72°C for 45 s; and final extension at 72°C for 7 min.

The capsid-encoding VP3 and VP1 genes and the 2A gene of enterovirus were amplified with PCR primers 008 (sense, 5′-GCRTGCAATGAYTTCTCWGT-3′, nt 2411–2430, PV1-Mahoney) and 011 (antisense, 5′-GCIC-CIGAYTGITGICCRAA-3′, nt 3408–3389, PV1-Mahoney) (9). PCR amplification was done for 35 cycles at 94°C for 30 s, 51°C for 30 s, and 72°C for 30 s. The RT-PCR products were analyzed by electrophoresis on 2% agarose gels containing 0.5 μg/mL of ethidium bromide, and purified by using a gel extraction kit (Bioasia Co., Shanghai, China).

DNA sequencing was performed by using an automated DNA sequencer (ABI 3730, Applied Biosytems, Foster City, CA, USA). Each RT-PCR product was sequenced in both directions to resolve possible ambiguous nucleotides.

Sequence Analysis

The serotypes of the viral isolates were determined by comparing their complete VP1 sequences with those available in the GenBank database. Nucleotide sequence homology was inferred by the identity scores obtained with the BLASTn program (National Center for Biotechnology Information, Bethesda, MD, USA). The pairwise sequence identities of the nucleotide and deduced amino acid sequences among FDJS03 isolates and other serotypes were calculated with the program Omiga 2.0 (Oxford Molecular Ltd., Madison, WI, USA). Four isolates randomly sampled from the 18 positive strains were used for phylogenetic analysis. Multiple-sequence alignments were done with ClustalX 1.83 (European Molecular Biology Laboratory, Heidelberg, Germany), and 2 methods (neighbor-joining and maximum parsimony) of phylogenetic analysis were used to provide a more reliable inference of phylogeny. A neighbor-joining tree was constructed with TREE-PUZZLE 5.0 (Deutsches Krebsforschungszentrum, Heidelberg, Germany) and the Dnapars program in software package PHYLIP 3.573c (University of Washington, Seattle, WA, USA) was used for maximum parsimony analysis. The statistical significance of phylogenetic trees constructed with Neighbor and Dnapars in PHYLIP 3.573c was estimated by bootstrap analysis with 100 pseudoreplicate data sets. The final consensus tree was produced with the Consense program in PHYLIP 3.573c. Treeview 1.6.6 (University of Glasgow, Glasgow, United Kingdom) was used to edit phylogenetic trees. The echovirus 30 sequences reported have been deposited in the GenBank database under accession nos. AY665606–AY665609.

Results

An outbreak of aseptic meningitis in 1,681 patients occurred in the northern area of Jiangsu Province in China from January to July 2003. Most (99%) patients in this outbreak were children <15 years of age. Boys were nearly twice as likely as girls to have aseptic meningitis (ratio 1,145:536). Fever, headache, and vomiting were the most common clinical manifestations, and 204 CSF cultures were negative for bacterial growth. The epidemic was distributed in both urban and rural areas. The peak period was from March to June, 2003, when 1,565 cases occurred, which accounted for 93.1% of all patients.

To investigate the primary etiologic agent in this outbreak, CSF specimens were used for virus isolation and identification. Sixty-six CSF specimens from 66 aseptic meningitis patients were tested by cell culture, and 18 showed positive results (isolation rate 27.3%). An enteroviruslike CPE (data not shown) was observed in MRC-5 cells infected with these isolated strains, but not in Vero and HEp2 cells. Most positive isolates showed a CPE as early as 3–5 days postinoculation. The exception was 1 isolate that did not show any CPE until the third day of the second blind passage. For FDJS03 isolates, typical picornavirus particles (round, nonstructured virus particles ≈30 nm in diameter) were observed by negative staining and electron microscopy (data not shown). All 18 positive isolates were successfully amplified by RT-PCR with primers specific for enterovirus sequences to yield predicted products of 440 bp and 155 bp (data not shown). Specific serotype identification was performed with a standard microneutralization test, but it did not show neutralization of any strains with the antibody pool. This finding prompted serotype identification with additional monoclonal antibodies not present in the pool. The isolates were totally neutralized by monoclonal antibody to echovirus 30, and the other antibodies did not show neutralization. This finding suggests that all 18 positive strains were echovirus 30.

After the complete VP1-encoding genes of 4 randomly sampled isolates were sequenced, molecular identification of the isolates by comparison with corresponding sequences of HEV in GeneBank confirmed the results of neutralization assays. The percentage identity was calculated by pairwise comparisons of aligned nucleotide and amino acid sequences (Table). Among 4 FDJS03 isolates, 99% sequence identity was seen at both nucleotide and amino acid levels. HEV can be clustered into 4 species (HEV-A, HEV-B, HEV-C, and HEV-D) based on genetic relationships of the capsid-encoding region VP1 (1). The nucleotide and amino acid VP1 sequences of 4 FDJS03 isolates showed low identities with the HEV-A, HEV-C, and HEV-D enterovirus species. Identity scores for FDJS03 and other isolates with HEV-B were <70% and

Table. Pairwise comparisons of nucleotide and amino acid sequences among FDJS03 isolates and other human enteroviruses (HEVs)*

		FDJS03_18		FDJS03_30		FDJS03_73		FDJS03_102	
Cluster†	Serotype	nt	aa	nt	aa	nt	aa	nt	aa
HEV-A	CA3	46	34	46	34	46	34	46	34
	CA4	44	32	44	31	44	31	44	32
	CA5	47	34	47	34	47	34	47	34
	CA6	47	33	47	33	47	33	47	33
	CA12	46	33	46	33	46	33	46	33
	CA14	42	29	41	29	42	29	42	29
HEV-B	CB2	60	61	60	61	60	61	60	61
	CB6	63	64	63	64	63	64	63	64
	E1	64	65	64	65	64	65	64	65
	E3	62	62	62	62	62	62	62	62
	E18	63	63	63	63	63	63	63	63
	E21	69	78	69	78	69	79	69	79
	E25	67	75	67	75	67	75	67	75
	Echovirus 30	**82**	**93**	**82**	**92**	**82**	**92**	**82**	**93**
	E32	61	62	61	62	61	62	61	62
HEV-C	CA1	49	43	49	43	49	43	49	43
	CA11	51	42	50	42	49	42	51	42
	CA15	50	42	50	42	50	42	50	42
	CA22	48	44	48	44	48	44	48	44
	CA24	49	40	49	40	49	40	49	40
	PV1	49	40	49	40	49	40	49	40
HEV-D	EV68	48	34	48	34	48	34	48	34

*nt, nucleotide identity score; aa, amino acid identity score; boldface used to highlight echovirus 30 serotype.
†HEV-A was represented by CA3 (AF081294), CA4 (AF081295), CA5 (AF081296), CA6 (AF081297), CA12 (AF081302), and CA14 (AF081304); HEV-B was represented by CB2 (AF081312), CB6 (AF081313), E1 (AF081314), E3 (AF081316), E18 (AF081331), E21 (AF081334), E25 (AF081336), echovirus 30 (AF081340), and E32 (AF081345); HEV-C was represented by CA1 (AF081293), CA11 (AF081301), CA15 (AF081305), CA22 (AF081310), CA24 (AF081311), and PV1 (V01150); HEV-D was represented by EV68 (AF081348).

<80% at the nucleotide and amino acid sequence levels, respectively, except with echovirus 30 strains. According to proposed molecular typing criteria (1), the limits of intraserotypic divergence should be <25% nucleotide sequence differences or <12% amino acid sequence differences. FDJS03 isolates are clustered as echovirus 30 since they share high identities of both nucleotide (82%) and amino acid (92%) sequences with the prototype echovirus 30 Bastianni strain. This finding is in agreement with the results of the serotyping tests.

To investigate the genetic relationships between FDJS03 isolates and other echovirus 30 isolates from different regions and periods, a phylogenetic analysis was conducted on complete VP1 nucleotide sequences (Figure). The tree constructed by the neighbor-joining method is highly similar to that produced by maximum parsimony method (data not shown). According to the bootstrapping support values, echovirus 30 sequences segregate into 3 distinct groups (bootstrap value >80%), with some temporal and regional subclustering, similar to that in a previous study (9). FDJS03 isolates were monophyletic and closely related to each other, which suggests a common origin (bootstrap value 95). Sequences of FDJS03 isolates grouped in subgroup 3c together with those from Europe and America isolated in the 1970s and 1980s. Subgroup 3b was composed of viruses isolated between 1990 and 1998 in Europe and North America. In addition, subgroup 3a contained virus isolated from Japan in 1998 and those from an aseptic meningitis outbreak in Taiwan in 2001 that shared the same genotype with the other 2 subgroups, as observed in other studies (18,19). The prototype strain Bastianni and strains isolated before 1977 were distributed in 2 distinct genotypes (groups 1 and 2), which may have become extinct in recent years (9,18).

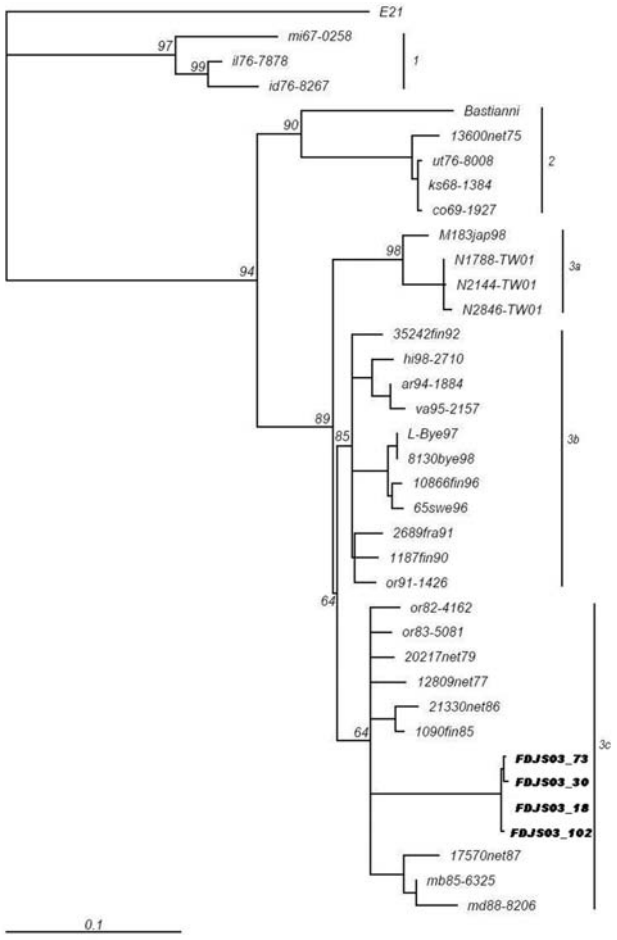

Figure. Phylogenetic tree based on complete VP1 sequences among FDJS03 isolates and other strains of echovirus 30 from different geographic and temporal origins. The neighbor-joining method was used to construct the tree. Numbers at the nodes represent the percentage of 100 bootstrap pseudoreplicates that contained clusters distal to the node. Prototype strain Farina of echovirus 21 (shown as E21) was included as the outgroup, but the tree is unrooted.

Discussion

Echovirus 30 is one of the most frequently isolated enteroviral serotypes that causes aseptic meningitis. Numerous outbreaks of aseptic meningitis caused by echovirus 30 have been reported during the last decade in many countries (20–24). In the spring and summer of 2003, an aseptic meningitis outbreak occurred in the northern part of Jiangsu Province in China. Results of the present study show that a distinct lineage of echovirus 30 is likely responsible for this outbreak. In most cases, knowledge of a specific type of echovirus strain does not contribute to the management of patients. However, in large outbreaks, identifying enteroviral isolates from patient specimens should be of epidemiologic importance.

Enterovirus can be transmitted rapidly and may have caused worldwide infections (25). However, surveillance data for enterovirus are incomplete because of the voluntary nature of reporting and because only a small number of enteroviral isolates have been typed (9). The situation is much worse in many developing countries such as China. The number of reported outbreaks in China caused by nonpolio enteroviruses has increased in recent years, perhaps due to better surveillance (26), but little information is available for these viruses. The temporal dynamics and genetic diversity among strains of the echovirus 30 serotype have been reported in previous studies (1,9,27), but no echovirus isolates from China were included. The present study is the first to show that a distinct lineage of echovirus 30 caused meningitis in China. This finding is an important addition to the worldwide echovirus 30 information database.

In the present study, CSF specimens were obtained from only a small number of patients. These specimens

were then tested by cell culture, largely because of logistic difficulties encountered since the peak period of this outbreak overlapped that of the epidemic of severe acute respiratory syndrome. We obtained a considerable number of echovirus 30 isolates, but no other serotypes of enterovirus. This finding strongly suggests that echovirus 30 was the etiologic agent in this outbreak.

The distinct lineage of echovirus 30 reported in this outbreak is not surprising, given that genomes of enteroviruses are known to evolve by ≈1%–2% per year (28–30), and interserotypic recombination contributes to evolution of these viruses (31–33). The FDJS03 isolates may represent possible recombinants between echovirus 30 and other serotypic enteroviruses. However, large antigenic variations in FDJS03 isolates were not observed, since they could be neutralized by standard antibodies to echovirus 30. A more detailed determination will require the complete sequencing of viral genome.

Phylogenetic analysis based on complete sequences of VP1 genes showed that FDJS03 isolates are closely related to each other, with an overall variation <1%. This finding indicates that this outbreak originated from a single genotype of echovirus 30. Unexpectedly, FDJS03 isolates are more closely related to echovirus 30 strains prevalent in Europe and North America than to those circulating in Japan and Taiwan. Group 3 echoviruses have been isolated in different areas of 3 continents, demonstrating the potential of this viral lineage to be transmitted over a large geographic region. Similarly, the temporal dynamics of echovirus 30 were seen both in Europe and North America, but until now no study reported the corresponding dynamics in Asia. Although we used 4 isolates from China and 3 from Japan and Taiwan in the phylogenetic analysis, this method is not sufficient to determine the overall genetic diversity and molecular dynamics of echovirus 30 in Asia. Nevertheless, pairwise comparison of nucleotide sequences and phylogenetic tree analysis demonstrated that the VP1 sequences of FDJS03 isolates exist in a subcluster composed of sequences from viruses isolated in North America in the early 1980s. However, these affinities seem less closely related between FDJS03 isolates and those circulating during the 1990s. Unfortunately, insufficient data are available to make definitive conclusions about defining characteristics of echovirus 30 in China and elsewhere.

In conclusion, we have characterized a distinct lineage of echovirus 30 strains isolated from CSF specimens of aseptic meningitis patients, which appears to be responsible for an outbreak in the northern area of Jiangsu Province in China in 2003. The VP1 sequences from these isolates are most similar to those of echovirus 30 strains that were common in North America in the early 1980s. This report is the first of the molecular characteristics of echovirus 30 strains circulating in mainland China and their respective phylogenetic relationships. However, we could not provide a comprehensive description of the genetic diversity and dynamics of echovirus 30 in China or Asia. Thus, establishing an enterovirus molecular surveillance system is needed in China to provide a better understanding of virus transmission and evolution.

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Reemergence of Canine *Echinococcus granulosus* Infection, Wales

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As a consequence of large-scale outdoor slaughter of sheep during the 2001 foot and mouth disease (FMD) outbreak in the United Kingdom and the possibility of increased risk for transmission of *Echinococcus granulosus* between sheep and dogs, a large survey of canine echinococcosis was undertaken in mid-Wales in 2002. An *Echinococcus* coproantigen-positive rate of 8.1% (94/1,164) was recorded on 22% of farms surveyed, which compares to a rate of 3.4% obtained in the same region in 1993. Positivity rates between FMD-affected properties and unaffected ones did not differ significantly. Significant risk factors for positive results in farm dogs were allowing dogs to roam free and the infrequent dosing (>4-month intervals) of dogs with praziquantel. When these data are compared to those of a previous pilot hydatid control program in the area (1983–1989), an increase in transmission to humans appears probable.

Echinococcus granulosus infection in sheep and dogs has been known to be endemic in parts of Wales and the English borders for many decades (1–3). An analysis of national hospital records for the period 1974–1983 showed that the incidence of human cystic echinococcosis was 0.2 cases per million in England and 2 cases per million population in Wales, with highest rates (5.6 cases per million) occurring in southern Powys County (4). To reduce the incidence of human cystic echinococcosis (also called cystic hydatid disease), a voluntary hydatid control program of supervised dog dosings at 6 weekly intervals with praziquantel was introduced in south Powys in 1983 and continued until 1989 (5–7). Ovine hydatidosis rates in the intervention area dropped from 23.5% to 10.5% after that period, and experimental use of sentinel lambs confirmed that transmission of *E. granulosus* was significantly reduced by this regime (8). Trend analyses of hospital admissions of human hydatid disease showed that, by 1993, clinical cystic echinococcosis disease in children (<15 years old) had ceased in the intervention area. However, a new focus of human cystic echinococcosis was identified for the period 1984–1990 in an area bordering south Powys, namely, the northern parts of the counties of Gwent and mid-Glamorgan (7). Furthermore, canine echinococcosis rates, measured indirectly with an *Echinococcus*-specific coproantigen enzyme-linked immunosorbent assay (ELISA), reflected the clinical data for intervention and nonintervention areas (9).

In 1989, the supervised dog-dosing program was stopped and replaced by a health education program (T.M. Walters, pers. comm.). A follow-up abattoir and dog coproantigen survey in 1995 to 1996, however, indicated that *E. granulosus* infection had reemerged in sheep and dogs in the previous hydatid-control intervention areas (7). In 2001, the foot and mouth disease (FMD) epidemic in sheep in England and Wales (10) affected some farms in both the former hydatid-intervention and nonintervention areas. Concern was raised that dog access to carcasses of sheep, euthanized as part of the FMD control program and awaiting incineration, could amplify the prevalence of infection in dogs and thereby the subsequent risk for humans. A third coproantigen survey of farm dogs in south Powys and north Gwent was therefore undertaken in 2002 to determine the prevalence of canine echinococcosis in the former hydatid-intervention and nonintervention areas.

Methods

Design

The pre-FMD prevalence of canine echinococcosis was estimated to be 7% from previous surveys. We decided that

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a doubling of this value would represent a clinically important increase in prevalence. To detect an increase in prevalence from 7% in farms without FMD to 15% in farms with FMD, with a power of 80% and significance level of 5%, a sample of 150 dogs on FMD farms and 850 dogs on non-FMD farms was required.

Sampling took place during a 5-month period from July to November 2002. Farmers in the Welsh counties of Powys (previous hydatid-intervention area) and Gwent (nonintervention area) were randomly selected from a government listing of farms and invited by telephone to join the study. Farms were visited, dogs were sampled, and questionnaires were completed.

Coproantigen Testing

Dogs were tethered or muzzled by owners, and a rectal fecal sample was taken from each dog with a plastic loop spatula. The specimen was mixed (1:1) with pre-prepared phosphate-buffered saline (pH 7.2) plus 0.3% Tween 20 (PBS-T) (11,12). Each sample was collected into a 5-mL screw-capped tube labeled with the dog identification and farm reference numbers. Fecal samples were initially stored frozen at –20°C for 5–10 weeks soon after collection, then transported to the pathogen laboratory at University of Salford and frozen at (–80°C) until tested. Before testing, fecal samples were thawed and mixed by hand shaking, then centrifuged at 500 x *g* for 10 min at room temperature. Fecal supernatants were tested for *Echinococcus* coproantigens by using a standard ELISA that used a capture antibody against *E. granulosus* adult somatic antigens (12) as had been used in the previous survey of canine echinococcosis in the Powys area (7).

Risk Factors

Data were collected on previous history of human cases of cystic echinococcosis in the household, knowledge of hydatid disease, dog age and sex, frequency of dog anthelmintic treatment (by owner or veterinarian), nature of dog food, and how dogs were restrained. In addition, farmers were asked whether they had had FMD on their properties or if their livestock were slaughtered for condemnation or because of FMD contiguous culling. Dog owners were also asked if they slaughtered livestock even occasionally at or around their farm, and if so, where and how they disposed of slaughter offal.

The chi-square test was used to determine whether dog coproantigen prevalence differed significantly between the levels of selected possible risk factors. Odds ratios (ORs), p values, and 95% confidence intervals (CIs) were calculated to quantify the magnitude of these differences. Multilevel modeling was used to examine the effect of clustering of dogs within farms. Results showed that variation between farms was not significant. Consequently, a single-level multivariate logistic regression was carried out; all risk factors were entered as explanatory variables. Analyses were performed with SPSS (SPSS Inc., Chicago IL, USA) and MLWin (Institute of Education, London, UK).

Results

In Powys, 473 farmers were eligible to take part; 72 (15%) did not have dogs, 112 (24%) could not be contacted, and 16 (3%) declined to cooperate. Therefore, a total of 273 farmers who were contacted and were eligible agreed to take part. Equivalent figures on response rates were unavailable for Gwent. A total of 1,178 dogs were on these farms (990 from Powys and 188 from Gwent), and fecal samples were obtained from 1,164 (588 dogs and 576 bitches). In 75 farms, sheep, cattle, or both had been slaughtered as part of FMD controls; FMD was recorded on 27 of these farms.

The *Echinococcus* coproantigen ELISA was positive in 94 (8.1%) of 1,164 of farm dogs. Of farms surveyed, 22% contained at least 1 dog with a positive coproantigen result. Prevalence of positive coproantigen tests in the previous hydatid-intervention area was 8.5% (79/928), compared with 6.4% (15/233) in the former nonintervention areas.

Univariate analysis of questionnaire data showed that male and female dog coproantigen-positive rates did not differ significantly. Younger dogs had a tendency for higher positive rates ($\leq$5 years, $p = 0.03$). Prevalence of dog coproantigen positivity was significantly associated with farm onsite slaughter of sheep (home-slaughter), the occurrence of free-roaming dogs, and low frequency of dog anthelmintic treatment by owners (Table 1). Coproantigen-positive rates in dogs for FMD and non-FMD farms were similar. Farms that reported feeding food scraps or offal to dogs, or allowing them to scavenge freely, were also associated with a significantly higher risk of an *Echinococcus* coproantigen-positive result (Table 1).

Multilevel modeling was used to examine risk factors for dogs, farms, and districts, but the variation between farms was not significant. In a single-level multivariate logistic regression model that used backward stepwise variable selection, allowing a dog to roam free and administering anthelmintic treatment infrequently were significant risk factors for *Echinococcus* coproantigen positivity (Table 2). Using this logistic regression model, we calculated, for example, that a dog that had been reportedly prevented from roaming free and that had received anthelmintic treatment 4 times a year had a probability of a positive coproantigen result of 0.07 $\{1/[1+ \exp – (-2.52)]\}$. In contrast, a dog that was allowed to roam and was given anthelmintic treatment only once per year had a probability of 0.4 $\{1/ [1+\exp – (-2.52+1.15+1.07)]\}$ for an *Echinococcus*-positive coproantigen result.

Table 1. Univariate analysis of *Echinococcus* coproantigen ELISA data from Welsh farm dogs and possible risk factors determined by questionnaire*

Risk factor	No. positive/negative for coproantigen† (N =1,164)		OR	95% CI	p value
	Present	Absent			
Farmer slaughters sheep	11/54	83/1,016	2.49	1.26–4.95	0.007
Stock euthanized because of FMD	20/255	74/815	0.86	0.52–1.44	0.576
FMD-affected farm	8/89	85/975	1.03	0.48–2.2	0.937
Dog free roaming	69/504	22/519‡	3.23	1.97–5.3	<0.0001
No disease knowledge	24/230	70/840	1.25	0.77–2.04	0.364
Dosing interval every 4–6 mo	63/554	18/434§	2.7	1.6–4.7	<0.0001
Dosing interval >6 mo	13/81	18/434§	3.87	1.83–8.21	<0.0001
Dog sex (male)	43/543	50/526	0.83	0.55–1.27	0.4
Age of dog ≤5 y	63/602	30/467	1.63	1.04–2.56	0.033
Food: scraps or offal	18/122	76/947¶	1.84	1.06–3.18	0.027

*Sampled in Powys and Gwent, Wales, United Kingdom, July–November 2002. ELISA, enzyme-linked immunosorbent assay; OR, odds ratio; CI, confidence interval; FMD, foot and mouth disease.
†By enzyme-linked immunosorbent assay.
‡Dog chained up.
§Dosing <4 months.
¶Boiled food.

Discussion

We conducted a study to investigate the concern that human cystic hydatid disease, or cystic echinococcosis, could reemerge in mid-Wales as a consequence of FMD control measures in which large numbers of sheep carcasses lying in fields were potentially accessible to dogs and foxes for several weeks. Although we found no statistical association with FMD-affected farms, we did confirm that canine echinococcosis had reemerged in dogs living in previously hydatid-free areas. The coproantigen-positive rate of 8% in the 2002 farm dog survey was significantly higher than the 3.4% prevalence recorded in the same localities in 1989 to 1993 (7). Risk factors for coproantigen-positive dogs were allowing farm dogs to roam free and infrequent anthelmintic treatment.

Why an association between canine echinococcosis coproantigen positivity and FMD-affected properties was not found is not clear. The lack of an association may be because the large piles of FMD-culled sheep carcasses were in designated government-regulated areas with relatively poor access for scavenging dogs. Moreover, dogs from non-FMD farms might have had the same access to culled carcasses as dogs from FMD farms. However, we were not able to identify whether dogs from non–FMD-affected farms had access to carcasses of slaughtered sheep on FMD farms. Dogs that scavenged may also have been already infected.

The absence of a statistical association with FMD and the findings of earlier surveys suggest that the high prevalence of canine echinococcosis is due to failure of the control strategy. The supervised dog-dosing hydatid-intervention program initiated in south Powys under the auspices of the U.K. Ministry of Agriculture, Fisheries and Food from 1983 to 1989 reduced transmission of *E. granulosus* to sentinel lambs. Moreover, the incidence of hospital-treated human cystic echinococcosis disease in the intervention area in Powys fell from 4/100,000 to 2.3/100,000 from 1984 to 1990. In May 1993, prevalence of *Echinococcus* in dogs, as measured by a highly genus-specific coproantigen test, was 0% in the intervention area (lower 95% CI 0%–3.4%). However, 10%–18% of older sheep (>3 years) at slaughter remained infected (7).

In 1989, the supervised dog-dosing program was replaced by a health education program aimed at schoolchildren and farmers in Powys to encourage dog owners to dose their dogs every 6 weeks. Following this, a sentinel

Table 2. Multivariate logistic regression model of possible risk factors for a positive *Echinococcus* coproantigen-test result in farm dogs (n = 1,164)

Risk factor	p value	Regression coefficient	OR	95% CI
Location of dog (compared to chained dogs)				
Free roaming	<0.0001	1.07	2.91	1.77–4.8
Other	0.411	0.53	1.7	0.48–5.91
Frequency of anthelmintic treatment (compared to <4 months)				
4–6 months	0.002	0.84	2.31	1.35–3.95
>6 months	0.004	1.15	3.16	1.46–6.85
Constant	<0.0001	–2.52		

*Sampled July–November 2002, Powys and Gwent Counties, Wales. OR, odds ratio; CI, confidence interval.

lamb study in the former intervention area from 1995 to 1996 (13) found that 5% of sentinel lambs became infected (10% in the adjacent nonintervention areas), indicating that pastures were recontaminated. A coproantigen survey of farm dogs at the same time (1995–1996) showed that 6% of dogs in the former intervention area were positive as were 24% of those in nonintervention areas (13). Our study used the same *Echinococcus*-specific coproantigen ELISA (11,12) and indicated that *E. granulosus* tapeworm infection in dogs was widespread in both the former intervention and nonintervention areas. Because of the latency of human cystic echinococcosis, children and adults exposed to infected dogs since 1989 may not have clinical disease for several decades.

A study conducted to evaluate the effectiveness of the health educational program on control of transmission of *E. granulosus* in south Powys (after 1989) demonstrated that the educational campaign alone was unable or insufficient to prevent transmission of *E. granulosus* in that region. This study also suggested that lifting the "fast-track" attack phase of treating dogs every 6 weeks with praziquantel after only 5 years was premature (13).

Reemergence of human cystic echinococcosis as a public health problem has occurred in other countries when hydatid-control programs ceased. For example, in Cyprus, cystic echinococcosis reemerged after the breakdown of an islandwide hydatid-control program following partitioning of the island (14). In Bulgaria, human cystic echinococcosis rates increased after hydatid campaigns were ended or reduced (15). And in Kazakhstan, human cystic echinococcosis incidence rates increased 4-fold within 10 years after post-Soviet independence, with its dismantling of collectives and changes in organized livestock and farming practices (16).

In conclusion, reemergence of *E. granulosus* in dogs in the last 10 years in south Powys, Wales, appears to be due to the withdrawal of the supervised dog-dosing control scheme and a reversion to risky practices of farmers (e.g., allowing farm dogs to roam free and infrequent or no anthelmintic treatment). The FMD outbreak in 2001 did not appear to increase the risk for canine echinococcosis. Urgent efforts are needed to address farm dog owner–associated risk factors if hydatid disease is to be brought back under control in this area.

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European Bat Lyssavirus in Scottish Bats

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We report the first seroprevalence study of the occurrence of specific antibodies to European bat lyssavirus type 2 (EBLV-2) in Daubenton's bats. Bats were captured from 19 sites across eastern and southern Scotland. Samples from 198 Daubenton's bats, 20 Natterer's bats, and 6 Pipistrelle's bats were tested for EBLV-2. Blood samples (N = 94) were subjected to a modified fluorescent antibody virus neutralization test to determine antibody titer. From 0.05% to 3.8% (95% confidence interval) of Daubenton's bats were seropositive. Antibodies to EBLV-2 were not detected in the 2 other species tested. Mouth swabs (N = 218) were obtained, and RNA was extracted for a reverse transcription–polymerase chain reaction (RT-PCR). The RT-PCR included pan lyssavirus-primers (N gene) and internal PCR control primers for ribosomal RNA. EBLV-2 RNA was not detected in any of the saliva samples tested, and live virus was not detected in virus isolation tests.

Rabies is a public health problem in most parts of the world. In Europe, in addition to classic carnivore-based rabies virus strains, 2 European bat lyssaviruses (EBLV-1 and EBLV-2) have been identified (>700 cases) in several European bat species (1). In 2003, a new bat virus, West Caucasian bat virus, was reported in Europe (2). Classical rabies virus is the archetype virus of the *Lyssavirus* genus that with 5 other genera make up the family *Rhabdoviridae* within the order Mononegavirales. The *Lyssavirus* genus is differentiated into 7 genetically divergent lineages, Rabies virus (genotype 1), Lagos bat virus (genotype 2), Mokola virus (genotype 3), Duvenhage virus (genotype 4), EBLV-1 (genotype 5), EBLV-2 (genotype 6), and Australian bat lyssavirus (genotype 7). With 1 exception (Mokola virus), all remaining genotypes have been isolated from bats (3). EBLVs are generally not transmissible to terrestrial animals other than bats (4), although 3 cases in humans occurred in an 18-year period, 1 case in a Stone marten (*Martes foina*), and 5 cases in sheep (*Ovis aries*) (5). However, underreporting occurs throughout parts of Europe, and in some circumstances rabies is confirmed without genetic typing of the virus. This underreporting was demonstrated in a confirmed case of rabies that occurred after a 15-year-old girl was bitten on the finger by a bat of unknown species in Voroshilovgrad (now Lugansk), Ukraine, in 1977 (6). A lyssavirus was isolated from the girl's brain, but the virus was not genetically typed (5).

EBLV-2 is the only lyssavirus that has been detected in the United Kingdom (5). Four cases of infection with this virus in England have been reported in Daubenton's bats (*Myotis daubenton'sii*): a pregnant female in 1996 in Sussex (7), a juvenile female in 2002 and an adult male in 2003 in Lancashire (8,9), and a juvenile female in 2004 in Surrey (10) (Figure 1). In November 2002 in Scotland, the first human case of rabies (with suspected bat involvement) since 1902 was reported (11) (Figure 1). These suspected cases were all confirmed as EBLV-2 infections by laboratory diagnosis. Rabies was previously reported in quarantined animals and in humans with the classical form of this disease from foreign countries (12).

The exact prevalence of EBLVs in bats in the United Kingdom is not known. From 1987 to 2004, a total of 4 of 5,030 bats were found to be infected with EBLVs in the United Kingdom through surveillance programs funded by the Department for Environment, Food and Rural Affairs. However, during this period of surveillance, only 99 Daubenton's bats were submitted for testing. Thus, the proportion of Daubenton's bats tested is underrepresented compared with estimates of Daubenton's bats in the population in the United Kingdom. Nineteen cases of infection

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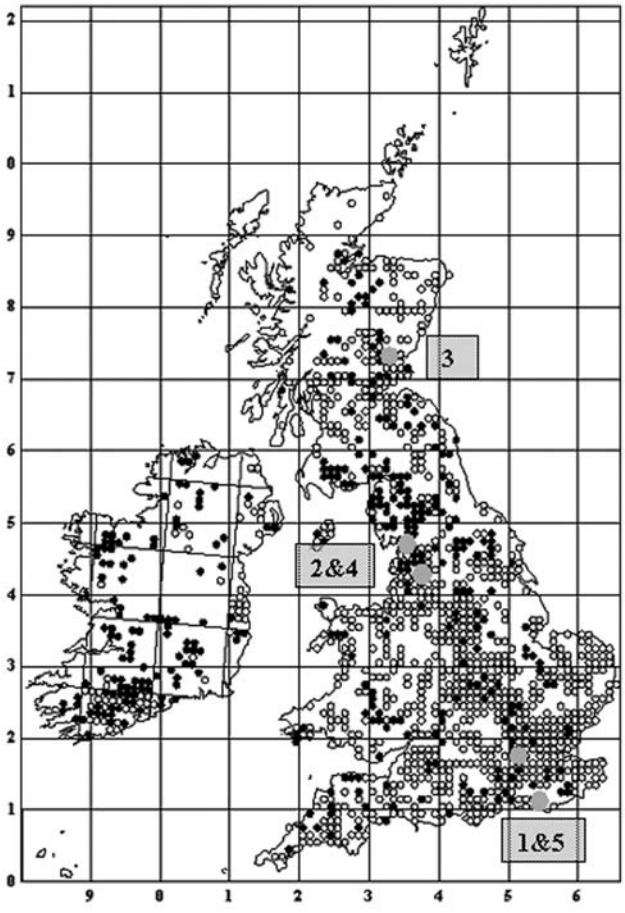

Figure 1. Distribution of Daubenton's bats in the United Kingdom and Ireland showing 5 cases of infection with European Bat lyssavirus type 2 (EBLV-2). Open circles are sites where Daubenton's bats were observed away from their roosts, and the closed circles are roosts of Daubenton's bats (summer and winter). The 5 numbered gray circles are sequential sites where EBLV-2-positive cases were found. Reprinted with permission of The Bat Conservation Trust (London, United Kingdom) from Distribution of Bats in Britain and Ireland 1980–1999.

with EBLV-2 in European bats have been documented (5). All (with 1 exception) were in European myotid species: Daubenton's bats and Pond bats (*M. dasycneme*). However, the latter is not indigenous to the British Isles, and Pond bat roosts have not been reported anywhere in the United Kingdom. Similar surveillance strategies have been used in other European countries (13).

The active surveillance described in this study investigated the prevalence of EBLV-2 in bats across southern and eastern Scotland by detecting antibodies to EBLV-2 in blood by using a modified fluorescent antibody virus neutralization (mFAVN) test and assessing oral swabs for the presence of lyssavirus RNA. The mFAVN test for EBLV-2 and polymerase chain reaction assays were developed as research techniques and used as surveillance tools after the death of a bat conservationist in 2002 (11). Previous studies have successfully demonstrated this approach to detect EBLV-1 in bat colonies from Spain (14–16). The emergence of new virulent bat lyssaviruses in Europe (2) emphasizes the need for continual appraisal and surveillance for the presence of lyssaviruses in European bats.

Methods

Sample Collection

From April to October 2003, a total of 229 bats were caught on 22 nights at 19 locations in Scotland (Figure 2). Each bat was assessed for health, sex, age, and reproductive status. Features examined in each bat for signs of poor health included unusual posture, matted fur, discharges from the orifices, thin appearance, excessively injured wings (rips, tears, and punctures), and excessive parasite burden. In addition, both weight and forearm measurements were taken, and bats that were unusually light for their skeletal size were examined more closely. Behavioral signs also noted included loss of coordination, seizures, and persistent aggression. Bats were required to fly at the end of sampling. An inability to fly after feeding, rehydration, and warming indicated debilitation. Any unusual observations were recorded.

A 2.9-mm, uniquely numbered bat ring (Mammal Society, London, UK) was fitted for individual recognition. Mouth swabs (saliva) were taken from each bat with either a dry sterile swab or a combination of dry and wet sterile swabs. These were stored individually in 500-μL sterile transport buffer (L15 medium [Sigma, St. Louis, MO, USA] containing 2 mmol L-glutamine, 50 μg/mL of penicillin, 2 μg/mL of streptomycin, 2 μg/mL of nystatin, and 2% fetal calf serum).

Blood (up to 100 μL) was taken by puncture from the antebrachial or uropatagial veins (in some bats after application of a local anesthetic cream [Lignocaine gel, Dunlop's Vet Supplies, Dumfries, UK]) by using a 26-gauge needle and then collected by using 10- to 50-μL heparinized glass capillary tubes (Statspin, Norwood, MA, USA). A proprietary antibleeding product (Hemablock veterinary wound powder, Dunlop's Vet Supplies) was then applied to each puncture site. The capillary tubes were then emptied into a sterile screw-topped tube. To increase blood volume obtained, 50 bats were attached by elastic bands to a thin cork board that was placed on a heated surface (≈43°C) to ensure vasodilatation of the peripheral veins. All samples were refrigerated and stored at ≈4°C until testing (1–5 days later). Capturing, handling, ringing, and sampling of bats were done under guidelines approved by the Home Office (UK Project Licenses PPL 60/3122 and PPL 30/1948).

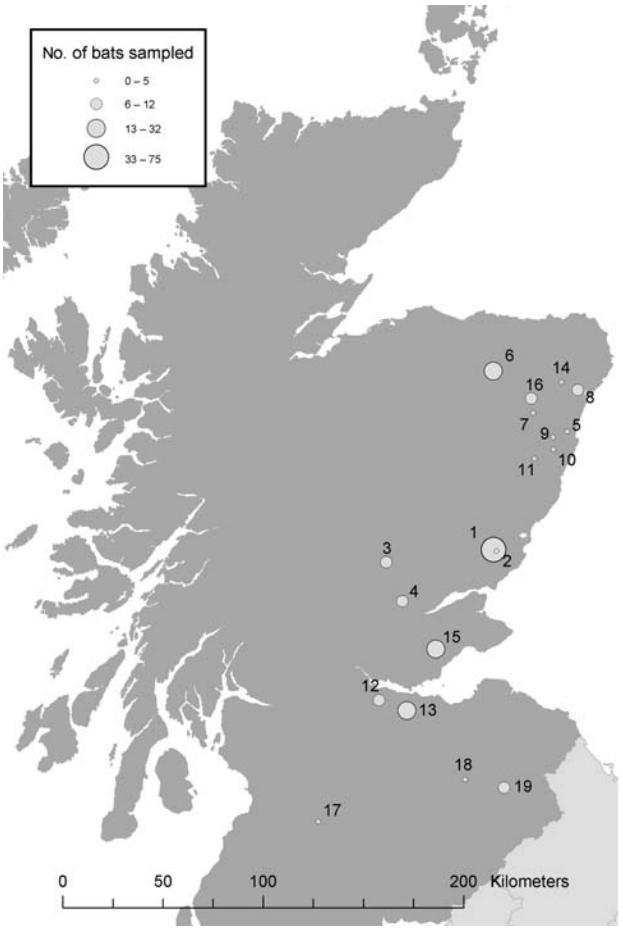

Figure 2. Bat sampling locations in southern and eastern Scotland. The circles indicate both the location (number) and an estimate of the number (size) of bats sampled.

From 25% to 35% of sampled bats were recaptured bats. Recapturing occurred within days of original sampling and also during a 1-year period from the date of initial sampling. This finding suggests that the sampling procedure was not harmful to bats. Ethical constraints in the United Kingdom restrict the resampling of wild bats within 3 months of capture. For this reason, additional sampling opportunities were limited.

Sample Analysis

mFAVN Test

A fluorescent antibody test (a virus neutralization assay) is routinely used to measure levels of antibodies to rabies virus in sera from vaccinated animals by using rabies virus strain CVS. The mFAVN test used in this study was based on the routine test but used an EBLV-2 virus (RV628, a Daubenton's bat isolate from the United Kingdom in 1996, EBLV-2a GenBank U89478/AY721613) (7), instead of rabies virus. A conjugate (Centacor, Fujirebio Diagnostics Inc., Malvern, PA, USA) was used at a dilution of 1:40. Samples were analyzed in duplicate because of their small volume and serially diluted using a 3-fold series (representing reciprocal titers of 9, 27, 81, and 243–19,683). No significant difference was observed in a comparison of duplicate versus quadruplicate tests on rabies-vaccinated pet sera (Veterinary Laboratories Agency, Weybridge, UK, unpub. data).

This assay was monitored for reproducibility with positive controls (anti-rabies virus sera [Office International des Epizooties, Agence Française de Sécurité Sanitaire des Aliments, Nancy, France] and a pooled anti-EBLV-2 [inactivated RV628] serum from rabbits) and negative controls (normal pooled dog and normal pooled rabbit sera, Harlan, Loughborough, UK). A positive serum sample from an EBLV-2–infected bat was not available for full validation of this test.

The mFAVN test is a quantitative procedure requiring a threshold to separate positive from negative results. To eliminate false-positive results, studies in Spain (16) used a reciprocal titer of 27 as a cutoff for EBLV-1, while others (14) used a threshold titer of 9. We used a reciprocal titer ≥27 as a positive cutoff level; samples with lower titers were considered negative. When applied to pooled samples, this threshold may underestimate the actual number of EBLV–2-positive bats; with a cutoff value of 1:27, weakly positive samples might have been overlooked. Further studies during consecutive years in a longitudinal study would provide confirmatory data indicating the prevalence of EBLV-2 in Scotland.

Where necessary, samples were combined to give the minimum volume (50 μL) needed for the test. Blood was pooled only across 1 species at any given site and with samples of a similar volume, such that plasma from an individual bat contributed equally to the pooled sample. As a result of pooling and being unable to determine the number of bats that were antibody positive or negative in a pooled sample, the 95% confidence intervals (CIs) were broader than if no pooling had taken place.

Reverse Transcriptase–Polymerase Chain Reaction (RT-PCR)

The presence of virus can be determined directly by using an RT-PCR (17) that detects the RNA of all lyssavirus genotypes (including EBLV-1 and EBLV-2). The sensitivity of this RT-PCR and a hemi-nested PCR is of the order of 0.1 and 10^{-3} 50% tissue culture infectious doses of rabies virus (18). Similar values have been obtained for EBLVs (Veterinary Laboratories Agency, Weybridge, UK, unpub. data). The interpretation of PCR results assumes that each swab contained saliva or cells from the oral cavity. To determine this, a separate ribosomal RNA (rRNA) PCR (18) was conducted to detect host

oral rRNA. The result for the lyssavirus RNA PCR was reported as unknown if rRNA was not detected. In contrast, if the swab was positive for rRNA, the lyssavirus PCR result was reported as either positive or negative. Methods used in this study were as previously described (17,18), with 250 μL of transport medium for the initial RNA extraction.

Isolation of Virus

Seronegative bats were tested by a rabies tissue culture infection test (RTCIT) only, while seropositive bats were tested by both the RTCIT and the mouse inoculation test (MIT) as previously described (19,20). The RTCIT technique was conducted by using 100 μL of transport medium per well in duplicate wells on 96-well plates. For the MIT, 4-week-old outbred CD1 mice (Charles River, Margate, UK) were injected intracranially with 40-μL samples that were antibody positive. Two mice were used per sample. The MIT was conducted according to the Home Office guidelines (UK Project License PPL 70/4867), and mice were monitored for 41 days before being humanely killed.

Statistical Analysis

Prevalence was calculated for sites at which we did not expect to find seropositive bats. This prevalence includes bats chosen from all Scottish sites, and a separate prevalence was calculated for site 1 (Figure 2). We anticipated that site 1 would be a location where EBLV-2–positive bats might be found because this site was the geographic region in which the suspected human exposure to EBLV-2 was reported (Figure 2) (11). Confidence limits were calculated as follows. An initial estimate for the proportion of bats that were seropositive for EBLV-2 was calculated with a maximum likelihood function

$$L(p) = \prod_i \binom{x_i + y_i}{x_i} \left(1-(1-p)^i\right)^{x_i} \left((1-p)^i\right)^{y_i}$$

where p is the unknown probability of being seropositive, x_i is the number of positive sample of pools of size i, and y_i is the number of negative pools of size i. The maximum likelihood estimate ($p\hat{}$) was then used to generate the approximate 95% confidence limits by assuming 2 [ln L ($p\hat{}$) – ln L (p)] is approximately χ^2 distributed. All calculations were programmed in R (21).

Results

Blood was collected from 230 bats: 198 (85%) Daubenton's bats, 24 Natterer's bats (*Myotis natterii*), and 8 Pipistrelle's bats (*Pipistrellus* species). Of these, blood from 224 bats was subjected to the mFAVN test. Fifty-five (24.5%) blood samples were tested individually; the rest were combined into pools containing 2–9 samples, with most containing 3 samples. The distribution of these samples across different sites is shown in the Table. The effects of pooling samples on the performance of the mFAVN test have not been fully investigated, but no evidence suggests that a pool containing multiple seropositive bat samples shows test behavior quantitatively different from a pool containing 1 seropositive bat sample.

Calculations of prevalence were performed only for blood samples (or pools) in which a successful positive or negative result was obtained. Positive samples were obtained in 4 pools (containing serum from 9, 2, 2, and 3 bats) and 2 single samples, and were exclusively from Daubenton's bats caught at 2 sites (Figure 3). This finding represents 6–18 bats since a minimum of 1 bat from each pool may have been antibody-positive. Determining whether the high value of the reciprocal titer (243) produced by 1 pool of 3 bats (site 15) (Figure 2) represents >1 seropositive bat in this pool was not possible. All Natterer's bats (5 pools) and Pipistrelle's bats (1 pool) sampled were negative. The prevalence of EBLV-2 for the Natterer's and Pipistrelle's bats tested was not significant because of the limited number of each species sampled.

Host rRNA was detected in 218 (65%) of the samples, indicating that saliva, cells, or both were present on the swab. In the remaining 35%, RNA was absent or below the limit of detection. No difference was detected in the ability to detect RNA when wet and dry swabs were compared. None of the results of the first-round or heminested PCRs with any of the samples were positive for lyssaviruses. These data suggest that none of the bats tested were actively excreting virus.

Table. Number of samples analyzed, by bat species and location*

Site	Daubenton's	Natterer's	Pipistrelle's
1†	69 (21)		6 (1)
2	0		
3	10 (3)		
4	0	12 (3)	
5	3 (3)		
6	20 (20)		
7	2 (2)		
8	6 (6)		
9	1 (1)		
10	2 (1)		
11	2 (1)		
12	5 (2)	2 (1)	
13	20 (6)	2 (1)	
14	5 (4)		
15†	32 (11)		
16	8 (4)		
17	0	4 (0)	
18	4 (3)		
19	9 (0)		
Total	198 (88)	20 (5)	6 (1)

*Values in parentheses are the number of samples (pools or single) analyzed by a modified fluorescent antibody virus neutralization test.
†Sites that had positive results for antibodies to European bat lyssavirus type 2.

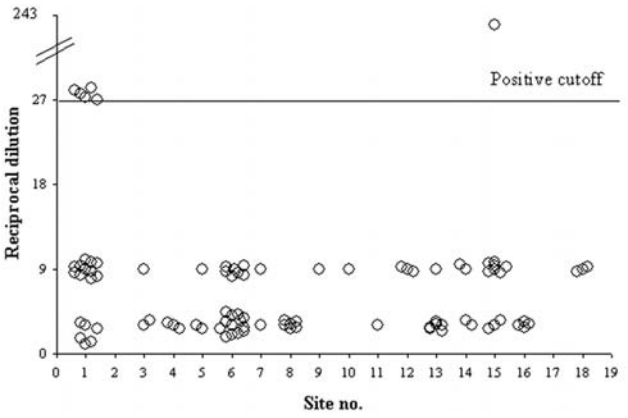

Figure 3. Antibody titers to European bat lyssavirus type 2 (EBLV-2) in bat sera from Scotland. An EBLV-2-specific modified fluorescent antibody virus neutralization (mFAVN) test was used to determine the level of circulating antibody in Daubenton's bats from 19 sites in Scotland. The test uses a 3-fold dilution series (9, 27, 81, 243, etc.) and the positive/negative cutoff is a titer (reciprocal dilution) of 27. Circles on the graph represent either single serum samples or pools of sera (88 for Daubenton's bats, 5 for Natterer's bats, and 1 from Pipistrelle's bats). All titers 27 are Daubenton's bats from 2 sites (5 from site 1 and 1 from site 15). No data were available for sites 2, 17, and 19.

Virus isolation tests were conducted using RTCIT (all bats swabs) and MIT (antibody-positive bat swabs only). All RTCIT samples were negative and at day 41 after injection, clinical signs of infection had not developed in any mouse. These data indicate that live virus was not detectable in the oral swab samples.

If the bats tested in this study (with the exception of those at site 1) were a truly random selection of Daubenton's bats across Scotland, the likely prevalence (95% CI) of bats testing seropositive for EBLV-2 would be 0.05%–3.80%. The 95% CI for the prevalence of EBLV-2–seropositive bats at site 1 was 2.9%–16.3%.

Discussion

The development of techniques to detect lyssavirus infection and previous exposure is fundamental to understanding both the risks to humans posed by EBLVs and in studying virus epidemiology. Results from Spanish (14–16) and North American (22) studies suggest that the relationship between lyssaviruses isolated from bats, the role of the immune system, and excretion of virus in the saliva are complex. Seroprevalence levels of EBLV-1 in individual and mixed-species bat colonies in Spain during 9 years of sampling increased from 3% in 1 year to 59% the next year and subsequently decreased to 10% by the end of the study (16). Other investigators in the United States have reported a high seroprevalence for rabies virus (15%–20% per colony, range <5%–34%) in big brown bats (*Eptesicus fuscus*) (22).

Bats in the United States and Spain may coexist with rabies virus and EBLV-1, respectively; some bats are healthy and breed successfully for a number of years (14–16,22). If this case also is true of EBLV-2, the virus would be difficult to detect directly, but a long-term observation of antibody-positive bats would be expected.

Our study shows that the seroprevalence of EBLV-2 is generally low (<4%) and that active virus excretion is below the limit of detection used. If this prevalence of EBLV-2 was distributed evenly throughout Scotland, samples from large numbers of bats would be needed to refine this estimate. The effect of using the same detection threshold in the mFAVN test for both single and pooled samples may result in an underestimate of the number of antibody-positive bats.

Antibodies to EBLV-2 in Daubenton's bats were found at only 2 of 19 sampled sites in Scotland. Whether the lower prevalence rates in Scotland will persist and are a function of either the bat or the virus being at the northerly edge of its range, or as suggested by a Spanish study (16), will change considerably over time, is not known.

EBLV-2 was not detected on oral swabs of the Daubenton's bats. These data suggest that virus was not excreted by the bats at the time of sampling and that an abortive peripheral infection with sterilizing immunity to EBLV-2 may have occurred. Thus, our findings are different from those in Spain and North America in which a low proportion of bats showed measurable levels of EBLV-1 or rabies virus in saliva associated with detectable antibody titers (14–16,22). These data also imply that differences in virulence exist between EBLV-2, EBLV-1, and rabies virus. An experimental study of EBLVs in ferrets demonstrated that EBLV-2 was rapidly cleared with the onset of a substantial neutralizing antibody titer (23).

Our understanding of the biology of bats and their interaction with EBLVs is limited, and it affects our ability to fully interpret prevalence rates. The principal tool used to determine the rates of disease prevalence, the mFAVN test, directly measures the EBLV-2 antigen-specific neutralizing antibody response. Changes affecting the immune state of the bat may have implications in our ability to detect an EBLV-2 infection. The gravid state and other physiologic stress scenarios in bats may change their immune response with respect to lyssaviruses (24). Unfortunately, bats, in particular myotid bats, are most accessible in their hibernacula, their maternity roosts (when most are pregnant or lactating), or at swarming sites (of which few are known and include mainly male bats).

Despite such problems, we have detected antibodies to EBLV-2 in the blood of Daubenton's bats, albeit at low levels and low rates of prevalence. Data were insufficient to determine the geographic extent of the study sites, but both sites with seropositive bats are in well-watered low-

land landscapes likely to support a high density of Daubenton's bats. Areas of higher bat density would normally be considered more likely to support endemic disease. The number of Daubenton's bats in the United Kingdom has been estimated at 150,000 (25), with ≈40,000 in Scotland (Scottish Natural Heritage, unpub. data), and colony sizes range from 10 to 200 with an average of 20 individuals (Bat Conservation Trust and Central Science Laboratory, unpub. data). The number of Daubenton's bats found across the United Kingdom has also been increasing by 4.4% per year since 1997 (Bat Conservation Trust, unpub. data). These data on the prevalence of EBLV-2 in Daubenton's bats, coupled with the first isolation of EBLV-2 in the United Kingdom in 1996 and the distribution of cases in the United Kingdom (Figure 1) (7–10), suggest that this zoonosis may be emerging in the United Kingdom and requires continuing surveillance and management (5).

Rabies virus can elicit a measurable antibody response after exposure, but not all exposures are lethal; some lead to an abortive infection (26). Although virus replication in the central nervous system was not measured in our study, virus replication can occur in this location without rabies developing in the host, mainly because of the susceptibility of the host to virus of low pathogenicity. Our data imply that bats in Scotland do not recover from infection after exposure to EBLV-2. Moreover, Daubenton's bats exhibit a low level of susceptibility to the virus and are subsequently developing an immune response after contact with EBLV-2 viral antigens.

In future studies, following up this research and successively resampling specific sites to establish disease profiles for ringed bats within this population will be important. To this end, blood samples of sufficient volume to permit individual tests must be obtained from seropositive bats over time at sites where positive cases have occurred (Figure 2) and at randomly selected locations.

The available evidence suggests that the prevalence of EBLV-2 in Daubenton's bats in Scotland is low and may be sporadic (27). These bats may roost less frequently in human dwellings than some other species; thus, the risk of human contact with infectious bats is low. Public health policies have been developed in the United Kingdom to further reduce exposure and potential for disease in those considered at risk. These measures include education, rabies vaccination for those working with bats, and postexposure treatment for people bitten or scratched by any bat species.

Acknowledgments

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Dr. Brookes is a veterinary virologist with 15 years of experience in the pathogenesis of exotic viruses, including 4 years of research focused on rabies and other lyssavirus pathogenesis studies. Her interests include virus replication, disease manifestation, immunoprotection, and microscopy.

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Influenza Outbreak Control in Confined Settings

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Influenza may rapidly disseminate within populations living in confined settings, causing considerable morbidity and disrupting daily activities. We describe an influenza A outbreak on a military base where 3,000 young adults, most of whom were unvaccinated, lived in close daily contact. Visits to the base clinic by 48 persons with acute respiratory illness within 2 days allowed early identification of this outbreak and prompted immediate epidemiologic investigation. Overall, 85 personnel (2.83% of base population) met the case definition for influenzalike illness. On-site laboratory confirmation with field detection kits, rapid implementation of respiratory illness control protocols, and a mass vaccination campaign were applied to limit disease dissemination. The outbreak was halted 14 days after the mass vaccination campaign was completed. We review the control measures available for controlling influenza outbreaks in confined settings and discuss the role of rapid mass vaccination within this context.

Influenza causes substantial illness and loss of work days among young adults, and outbreaks can affect the preparedness of military units (1). The generally recommended measures for controlling influenza outbreaks (e.g., isolation, quarantine, hygiene enhancement) (2) may not be sufficient to contain an outbreak in such confined settings, when attack rate may be as high as 45% (3). We describe an influenza outbreak in which rapid identification of the causative agent permitted mass vaccination to be used as a control measure, examine the effects of this intervention on disease dissemination, and discuss the potential role of this control measure in containing influenza epidemics.

Methods

Over a 2-day period during January 2002, 48 patients sought treatment at the clinic of a large military base in central Israel; their symptoms included fever, cough, and sore throat. This unusually high patient load prompted the clinic commander to notify headquarters, and a team from the Epidemiology Section of the Israel Defense Forces (IDF) Medical Corps arrived at the scene by the next morning (outbreak day 3) and initiated an epidemiologic investigation. The investigation team retrieved all patient visit records for the preceding 2 weeks from the clinic's computerized patient files and continued daily follow-up for 4 additional weeks. The case definitions defined acute respiratory illness (ARI) as cough, sore throat, or coryza. Influenzalike illness (ILI), a subset of ARI, was defined as ARI with temperature >37.8°C (>100.0°F). Cases were classified according to these case definitions, and epidemic curves were constructed. Demographic information, including sex, rank, unit, and influenza vaccination status, were retrieved from personnel records. Active surveillance for ARI was initiated among all post personnel.

Oropharyngeal and nasopharyngeal specimens were tested in the field by using rapid influenza tests (Influenza A/B Rapid Test, Roche Diagnostics, Basel, Switzerland), and these results were verified with immunofluorescence staining and viral culture. The IDF health corps at that time had >2,000 available doses of influenza vaccine. This vaccine was a subunit influenza preparation, containing influenza A and B strains equivalent to A/New Caledonia/20/99 (H1N1)-like, A/Moscow/10/99 (H3N2)-like, and B/Sichuan/379/99-like strains, in accordance with World Health Organizations recommendations for the Northern Hemisphere 2001–2002 winter season (4). Active surveillance was continued for 24 days to assess the effect of the employed control measures on the dissemination pattern of the outbreak.

Results

The base housed ≈3,000 men and women, of whom 136 (4.5%) had been vaccinated against influenza during the preceding 3 months as part of routine, seasonal preventive health measures. These 136 vaccinees made up 87.7% of the vaccine-eligible personnel in this base; 19 other personnel refused to receive the vaccine that winter. Initial

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investigation showed that ARI incidence rates during the 2-day period January 13–14, 2002 (outbreak days 1 and 2), were indeed significantly higher than the preceding daily average rates (24 vs. 4.1 patient visits/24 h, $p < 0.001$). During these 2 days, ILI was responsible for a significantly higher proportion of all clinic visits, compared with visits during the preceding 2 weeks (21% vs. 4.3%, $p < 0.001$).

The epidemic curves of ARI and ILI are shown in the Figure. During outbreak days 1 and 2, a total of 48 cases of ARI were recorded; 34 met the criteria for ILI. None of the patients had been vaccinated against influenza in the preceding 12 months. Cases were evenly distributed across all areas of the base and among all ranks, with no specific unit on post showing an exceptionally high attack rate. On weekends the base clinic treated emergency cases only, which explains the artifactual cyclic lacunae visible on the curve.

Ten oropharyngeal and nasopharyngeal specimens were tested on outbreak day 3 by using a rapid influenza kit; 1 was positive. Seven of these specimens were later found to be positive for influenza A virus by immunofluorescence staining and culture. This information was only available after the decision to undertake a mass vaccination campaign had been made and the campaign had been carried out. Typing the virus from culture showed that 6 of the 7 influenza-positive cultures were A/Moscow/10/99 (H3N2)-like, a strain included in the 2001–2002 vaccine composition. Specific typing of the seventh culture was unavailable.

Control measures immediately employed from outbreak day 3 and afterward included active case finding, strict isolation, and placing patients on sick leave, and infirmary records confirm that 82.4% of patients with physician-confirmed ILI were indeed sent for sick leave off post. Despite the introduction of the above measures, new ILI patients continued to appear, as shown in the Figure. Because of the potential for further dissemination of the disease among unvaccinated personnel and the operational implications of such disease spread on troop preparedness, additional steps directed towards active intervention were initiated. As sufficient quantities of antiviral drugs were not readily available at that time, >2,200 doses of influenza vaccine were rapidly transported on outbreak day 4 to the base for use in an immediate mass vaccination campaign. Within 2 days (outbreak days 4–5), 2,118 soldiers (>70% of the base population) were vaccinated.

The outbreak had a substantial impact on military activities. Key commanders and personnel were incapacitated by their illness for several days, thus disrupting the operational routine. Entry to and exit from the base were denied, except to those with documents showing they had been

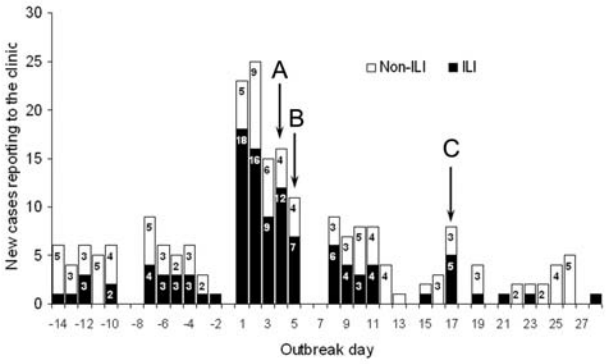

Figure. Daily incidence of acute respiratory illness (ARI) cases at base infirmary, by case definition. Shaded segment of bar indicates ARI cases that met the definition of influenzalike illness (ILI) (temperature >37.8°C). Nonshaded segment indicates ARI cases that did not meet the ILI criterion (non-ILI). Arrows: A) vaccination campaign initiation, B) vaccination campaign termination, C) day 14 after campaign initiation.

vaccinated, and training operations were suspended during the outbreak in an attempt to avoid noncrucial congregation and limit disease dissemination.

Over the next 2 weeks (outbreak days 4–17), patient visits to the clinic due to ARI and ILI remained high, despite the intervention measures taken; a mean daily rate of 3 cases of ILI was maintained. As shown in the Figure, illness remained high for 17 days, after which time illness rates returned to preepidemic levels. We defined outbreak termination as the day from which the daily onset of new ILI cases was ≤4 (the highest single daily ILI incidence observed during the preoutbreak period). Although the outbreak initially appeared to have ended on outbreak day 12, the 5 ILI patients seen in the cluster on outbreak day 17 exceeded this cutoff value so that the day of termination was set at outbreak day 18. After day 18, incidence declined to a mean daily rate of 1.9 cases for ARI and 0.4 for ILI. These rates were maintained for an additional 10 days, at which time active surveillance was stopped. Overall, 140 ARI cases were recorded within 17 days, 85 of which (60.7%) met the case definition for ILI. The ARI attack rate during this outbreak was 46.7/1,000, and the ILI attack rate was 28.3/1,000. None of the patients required hospitalization.

Discussion

An outbreak of influenza in a military base with a largely unvaccinated population was rapidly identified and was subsequently halted 14 days after a rapid mass vaccination program. Disease dissemination during influenza outbreaks in military bases can have reach attack rates as high as 37%–45% (3,5), and similar rates were noted during outbreaks on naval ships (6). With no control group in this

case, we cannot predict the exact attack rate that would have been reached, had this unusual intervention not been applied. However, several factors in the setting of this outbreak lead us to believe the expected attack rate would have been much higher than 2.83%. These factors include the initial high incidence rate; the even distribution of cases across all areas of the base and among all ranks; the large substrate for disease dissemination in a base of ≈3,000 young men, mostly unvaccinated, living in confined settings and in close everyday contact; and the continuous incidence of new cases, despite conservative measures employed, an incidence that had not completely subsided until 14 days after the mass vaccination campaign. Such a low attack rate probably cannot be attributed solely to the natural course of the outbreak and seems to indicate that control measures had a substantial beneficial effect.

Although this group of healthy, working adults is not at increased risk for serious complications, influenza is not a trivial illness in this group. Prominent manifestations include increased work absenteeism, impaired work productivity when ill, and a low yet clinically important incidence of serious complications (7,8). In the military, work absenteeism may hinder units' preparedness. In the case presented, the outbreak was contained in time to prevent the base strategic abilities from being compromised.

Seasonal vaccination of young adults, mainly in crowded settings, is both highly successful and cost-effective (9,10). Routine vaccination of similar population groups, including students and other persons in institutional settings, is currently encouraged by the Advisory Committee on Immunization Practices (ACIP) recommendations for prevention and control of influenza (2). IDF conducts an annual influenza vaccination campaign, directed at crucial fighting-unit personnel and personnel with chronic respiratory and cardiac disease. Most of the personnel of the base in question (>95%) did not fall under any of these categories and were not vaccinated for influenza. Influenza vaccination is not forced during the IDF annual vaccination campaigns, and each year a variable proportion of vaccine-eligible troops refuse to be vaccinated. On this base, 136 personnel, 87.7% of the base target population, were vaccinated during that year's vaccination campaign. Nineteen vaccine-eligible subjects (12.3%) refused to receive the vaccine that winter. This refusal rate is well within the expected range, according to previous years' experience (unpub. data).

The outbreak emphasizes the crucial role of continuous surveillance for respiratory disease in the military, as rapid detection is a major factor of successful intervention. The explosive pattern of this outbreak, as demonstrated by the sudden illness rate increase in the first 2 days of the outbreak, enabled rapid detection and initiation of prompt investigation. Illness caused by influenza virus is difficult to distinguish clinically from that of other respiratory pathogens on the basis of symptoms alone. Reported sensitivity of clinical definitions for ILI (defined as fever and cough) has ranged from 63% to 78%; reported specificity has ranged from 55% to 71%, respectively, when compared to diagnosis by viral culture (11,12). In this case, decisions concerning which control measures to implement, including mass vaccination, were made on outbreak day 3. The mass vaccination campaign was carried out during outbreak days 4 and 5, several days before culture-based influenza diagnosis was available. The diagnosis was therefore based on the combination of clinical signs, epidemiologic characteristics, and results of a rapid influenza identification kit. The kit is designed to detect both influenza A and B viruses (but not to distinguish between them), with a reported sensitivity of 77.4% and specificity as high as 93% (13). These characteristics render the kit inappropriate for the diagnosis of influenza in individual patients, but the kit remains a useful tool for implicating influenza as the causative agent in large outbreaks, even if only a few patients test positive (14).

Confirmation of influenza A with immunofluorescence staining and viral culture requires at least several days, a timeframe in which an influenza outbreak may grow out of control. Decision-making regarding control measures should not necessarily be delayed until such confirmation is achieved. In this case, decisions on intervention measures were made several days before culture results became available, and the diagnosis was based on clinical and epidemiologic characteristics and the results of the rapid influenza detection kit. Guidelines for outbreak control in young adults within confined settings (i.e., military bases, correctional facilities, or college dormitories) are scarce are probably similar to guidelines used for quelling nursing home outbreaks. In the latter case, recommended measures include active identification of patients with confirmed or suspected influenza, restriction of staff movement between wards or buildings, restriction of contact between ill staff or visitors and patients, influenza vaccination for staff and patients, and use of antiviral drugs (2). Some of these recommendations may not be applicable in an active military unit, most notably isolation and widespread restriction of movement. Antiviral agents in sufficient quantities may not always be available, as in our case, but when they are available, they may play an important role in stopping such outbreaks.

Mass vaccination as a means of controlling outbreaks has several limitations. The vaccine may reach its full potential effectiveness (70%–90% reduction in influenza illness) only when the vaccine and circulating viruses are antigenically similar (2). The incubation period of the influenza virus is 1–3 days, whereas the development of antibodies in adults after vaccination can take up to 2

weeks, depending on prior vaccination and sensitization. In this time, the virus can complete several infection cycles, therefore rendering mass vaccination inappropriate as a sole measure of intervention and only appropriate when the susceptible population size is large enough that the outbreak can be expected not to subside within 2 weeks. In instances when an influenza outbreak is not contained by using routine protocol and a large portion of the population is unvaccinated and remains at risk, mass vaccination can ensure the termination of the outbreak within ≈2 weeks. When available, prophylaxis with antiviral drugs must be considered an adjunct to other control measures such as isolation of patients, hygiene enhancement campaigns, and reduction of crowding to a necessary minimum in limiting disease dissemination to a minimum during this time frame. These control measures may confer important information for unit commanders, since the 2-week period provides a point of reference for projecting troop readiness. When the population at risk carries crucial deployment responsibilities, as was the case in our outbreak, mass vaccination may be imperative.

Following this outbreak, efforts have been directed at increasing the acceptance of seasonal influenza vaccination in the specific populations within the IDF. Brochures and lectures were used to deliver evidence-based information about the benefits of influenza vaccination to both the troops and the base physicians. Assessment of the effect of these measures on compliance with influenza vaccination is now underway.

When feasible and affordable, preventive seasonal influenza vaccination is preferable to rapid vaccination during an outbreak because of the above-described caveats of the latter strategy. For the time being, however, the IDF will continue to focus its annual influenza vaccination campaigns on specific groups, mainly because of the high costs associated with universal coverage. Under these circumstances, influenza outbreaks such as the one presented here can be expected to recur, and adequate quantities of antiviral agents and vaccines must be made readily available to control future outbreaks. Active surveillance for ILI, including continuous laboratory sampling, is now under way in specific field units within the IDF in an attempt to uncover the viral pathogens that account for ILI and estimate the true incidence of influenza in unvaccinated subpopulations. Since recent outbreaks of highly pathogenic avian influenza in East Asia may herald the next influenza pandemic, heed must be taken now to implement and evaluate an array of outbreak control measures during the interpandemic period.

Conclusions

Early reporting of a potential influenza outbreak among soldiers, on-site laboratory confirmation with field detection kits, and rapid implementation of mass vaccination combined with a respiratory illness control protocol likely limited the magnitude of this outbreak. In confined settings, when the threat of an acceleration of the outbreak is substantial, antiviral drugs are not available in abundant amounts, and sufficient vaccine doses are available, mass vaccination of the population at risk should be considered because it should ensure the termination of the outbreak within 10–14 days. Conservative control measures must serve as adjuncts to limit disease dissemination during this period until the vaccine takes effect.

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Buruli Ulcer Recurrence, Benin

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Buruli ulcer is a recognized public health problem in West Africa. In Benin, from 1989 to 2001, the Centre Sanitaire et Nutritionnel Gbemoten (CSNG) treated >2,500 Buruli ulcer patients. From March 2000 to February 2001, field trips were conducted in the Zou and Atlantique regions. The choice of the 2 regions was based on the distance from CSNG and on villages with the highest number of patients treated at CSNG. A total of 66 (44.0%) of 150 former patients treated at CSNG were located in the visited villages. The recurrence rate of CSNG-treated patients after a follow-up period of up to 7 years was low (6.1%, 95% confidence interval [CI] 2.0–15.6). We attribute this low rate to the high quality of Buruli ulcer treatment at an accessible regional center (CSNG). The World Health Organization definition of a Buruli ulcer recurrent case should be revised to include a follow-up period >1 year.

Mycobacterium ulcerans disease, also called Buruli ulcer, is a recognized public health problem in many countries, especially in West Africa, where prevalence has been increasing in recent years (1,2). Buruli ulcer–endemic foci are regularly associated with stagnant bodies of water (ponds, backwaters, and swamps). The disease takes various clinical forms, including ulcers, nodules, plaques, and edematous indurations. Surgical excision followed by skin grafting is the recommended treatment (3). Recent studies, however, suggest that an antimicrobial regimen of rifampicin (rifampin) plus streptomycin may be effective against early forms of Buruli ulcer (4). Follow-up data on the rate of recurrence in hospital-treated Buruli ulcer patients are rarely reported. In a study designed to assess the effectiveness of excising preulcerative Buruli lesions in field situations in Ghana, Amofah et al. found a local recurrence rate of 16% at the same site within a year of follow-up (5). Two more patients had a recurrence at a different site, for a total recurrence rate of 20%. In villages in Ghana, Teelken et al. compiled a group of 78 patients who had been treated in 2 different hospitals: 35% were not healed when followed up 3 years later. For 1 hospital, the rate of those not healed was 18%, and in the other, 47%. In their study, however, investigators were not able to differentiate between ulcers that had never healed and those that healed and then recurred (6).

Our village follow-up was organized and carried out by the Centre Sanitaire et Nutritionnel Gbemoten (CSNG), a rural health center in southern Benin that began surgically treating patients in 1989 (7). This study reports the recurrence rate of CSNG-treated patients after up to 7 years of follow-up.

CSNG, in the district of Zagnanado, is one of several reference centers for the treatment of Buruli ulcer in Benin. Situated in the Zou region, 1 of 4 Buruli ulcer–endemic regions of southern Benin, this center receives Buruli ulcer patients from Benin and neighboring countries. From 1989 to 2001, CSNG treated 2,564 Buruli ulcer patients (2): 1,801 were from Zou, 170 were from Atlantique, and 515 were from other regions of southern Benin. The origin of 78 patients was not recorded. From March 2000 to February 2001, field trips in villages of the Atlantique and Zou regions were organized to collect data on rates of recurrence of disease and inform the population of the Buruli ulcer public health problem. CNSG could not collect data before 2000 because of a lack of transportation. In a previous study, the origin and number of patients coming to CNSG for treatment were described (2). We showed that most Buruli ulcer patients admitted to CSNG from 1997 to 2001 were from Zou, and <10% were from the adjacent Atlantique region (2). For villagers living at a distance from CSNG, transportation and treatment costs represent a considerable concern, but the greatest expenditure for patients is the cost of living at CSNG, especially the cost of food (8).

Methods

From March 2000 to February 2001, 22 field trips were conducted in Zou and Atlantique. Eleven field trips were required to inform the local authorities about the objectives

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of the study, notify the population, and inform them of the time and date of the follow-up visit 15 days later. Detecting Buruli ulcer patients required 11 additional field trips.

Villages that were included in the study had to be in a region close to CSNG (Zou), where villagers were aware of the availability of a Buruli ulcer treatment center, or in a region far from CSNG (Atlantique), where distance to the center was a substantial problem. In each region, a list of patients from each region was compiled, and the districts and villages with the highest number of patients treated at CSNG were selected.

The field team included a staff physician of the CSNG, a microbiologist, and a driver. Publicity campaigns on Buruli ulcer were presented by CSNG in some villages in these regions. Before field trips, we compiled a list of Buruli ulcer patients from villages where publicity campaigns were organized. These patients were treated at CSNG at some point from 1989 to 2001. They were examined and interviewed with the cooperation, when necessary, of other persons, such as former patients treated at CSNG, teachers, parents, or for children, someone designated as representative of specific patients. These key persons helped us locate patients on our lists and sometimes find new patients and patients treated by traditional methods. All adolescent and adult participants were personally interviewed. For children, a competent adult who knew the study patient well enough to supply the requested information was interviewed. All patients, or parents of children included in the study, provided oral consent. Photographs were taken of some patients.

Posttreatment histories were taken in the language of each patient, either directly by the interviewer or through an interpreter, when necessary. Recurrences of Buruli ulcer symptoms were noted, specifically with respect to types and sites of new lesions and time between hospital discharge and follow-up. All patients with active Buruli ulcer were referred to CSNG.

Clinical diagnosis of 49 cases was confirmed by laboratory tests according to World Health Organization (WHO) guidelines (9). The remaining 17 cases were diagnosed clinically; all were typical Buruli ulcer and did not present reasonable differential diagnostic problems.

Results

Table 1 shows the total number of villages (173) in the 3 Buruli ulcer–endemic districts (28 in Ouinhi, 74 in Zogbodomey, and 71 in Zè). From 1989 to 2001, a total of 70 villages (40%) were Buruli ulcer–endemic in the Zou and Atlantique regions. Two districts from the Zou region, Ouinhi and Zogbodomey, had 21 and 24 Buruli ulcer–endemic villages, respectively, and in the Atlantique region, 1 district (Zè) had 25 Buruli ulcer–endemic villages. Of 70 Buruli ulcer–endemic villages, 24 (34.3%) could be visited from March 2000 to February 2001, 13 (28.9%) in Zou and 11 (44.0%) in Atlantique.

A total of 707 patients treated at CSNG originated in these districts: 419 from Ouinhi, 170 from Zogbodomey, and 118 from Zè. A total of 150 patients came from visited villages: 74 from Ouinhi, 39 from Zogbodomey, and 37 from Zè. A total of 66 (44.0%) of 150 Buruli ulcer patients formerly treated at CSNG were located, 41 (36.3 %) from Zou and 25 (67.6%) from Atlantique. The difference between the percentage of patients retrieved in Zou and Atlantique is significant ($p < 0.001$). We incidentally found 11 patients treated or under treatment by traditional healers and 28 new patients (data not shown).

The follow-up period (time between discharge and the follow-up visit) is indicated in Table 2. A total of 45 patients (73.8%) had at least 12 months of follow-up time (median 34 months), 12 patients (19.7%) had 6–11 months of follow-up time (median 9 months), and 4 patients (6.6%) had 2–5 months of follow-up time (median 4 months). The shortest period between hospital discharge and follow-up visit was 2 months, and the longest period was 7 years. For 5 patients, the exact date of discharge from the hospital was not recorded. All 5 patients had bone lesions, had been hospitalized several times, and had been discharged >1 year before the visit.

Of the 66 patients treated at CSNG, 4 (6.1%, 95% confidence interval [CI] 2.0–15.6) had a new Buruli lesion. One patient (4.0%) of 25 came from Atlantique and 3 (7.3%) of 41 from Zou (Table 3). The difference was not significant.

The location, type, and size of previous and new lesions are indicated in Table 4. Three patients had a cutaneous lesion at the previous site, and 1 had a large edematous

Table 1. Visited villages and patients retrieved in 2 districts of the Zou region and 1 district of the Atlantique region

	Atlantique	Zou		
Villages*	Zè	Ouinhi	Zogbodomey	Total
Total villages	71	28	74	173
Total BU-endemic villages, 1989–2001	25	21	24	70
BU-endemic villages visited (%)	11 (44.0)	7 (33.3)	6 (25.0)	24 (34.3)
Total BU patients treated at CSNG, 1989–2001	118	419	170	707
BU patients previously treated at CSNG, 1989–2001, in visited villages	37	74	39	150
BU patients followed-up in visited villages (% retrieved)	25 (67.6)	24 (32.4)	17 (43.5)	66 (44.0)

*BU, Buruli ulcer; CSNG, Centre Sanitaire et Nutritionnel Gbemoten.

Table 2. Follow-up period for Buruli ulcer patients in villages of the Atlantique and Zou regions, Benin

Period	No. patients (%)	Median follow-up period (Q1–Q3) (mo)*
2–5 mo	4 (6.6)	4 (2.5–4.0)
6–11 mo	12 (19.7)	9 (7.25–10.75)
12 mo–7 y	45 (73.8)	34 (19.0–44.5)
Total	61	
>1 year†	5	

*Q1, first quartile; Q3, third quartile.
†Exact date of discharge unknown.

lesion at a new site (right leg) with bone involvement (right tibia). The follow-up times of these 4 recurrent cases ranged from 12 to 30 months. At CSNG, 57 patients (3.4%, 95% CI 2.6–4.4) of the 1,687 admitted from 1997 to 2001 returned spontaneously to the center with a recurrent lesion (2). The follow-up times of these recurrent cases ranged from 1 to 68 months. Of the 4 recurrent cases, 2 developed within a year, decreasing the recurrence rate within 1 year to 3.0% (95% CI 0.5–11.5). The 2 other recurrent lesions had developed by follow-up visits at 17 and 30 months.

Of the 66 patients treated at CSNG and followed-up in the villages, 10 had received antimycobacterial drugs (streptomycin and rifampin) 1 or 2 days before surgical excision and a few days after surgery; 2 of them had recurrent cases.

Discussion

The importance of follow-up of Buruli ulcer patients is accepted but little studied. Recurrences of Buruli ulcer are not exceptional (10). Early follow-up is important to rapidly detect recurrent cases and refer patients to treatment. Delays in seeking medical advice can lead to severe complications, including dissemination of disease, especially the development of bone lesions (2,11).

Scheduled programs for repeated follow-up visits of all treated patients would be ideal but are rarely successful in most disease-endemic areas. In our study, only 66 (9.3%) of 707 patients from Ouinhi, Zogbodomey, and Zè treated at CSNG could be followed up. Not all Buruli ulcer–endemic villages could be visited, for financial and logistic reasons. In the 24 visited villages, 44% of former patients were located. If patients were not at home each time we visited them, friends or relatives were able locate them. The patients we could not find were those who lived outside the villages in dwellings that were distant and difficult to reach.

Buruli ulcer is well known to the villagers in disease-endemic areas. In Atlantique and Zou, the field officer, a resident villager, listed all Buruli ulcer patients in his village and guided the team to each patient's house. These guides (often teachers) offered their help for this spontaneously, without compensation, and were motivated by their concern for this health problem. All patients treated at CSNG (recurrent case or not) welcomed the survey team with enthusiasm. However, we cannot exclude the possibility that some patients were not located, especially patients who had been treated by traditional practitioners.

More patients were located in Atlantique (67.7%) than in Zou (36.3%). This finding may be partially due to the fact that most cases in patients from Atlantique were diagnosed from 1999 to 2001, while patients from Zou were treated before 1989. Therefore, locating recent patients from Atlantique was easier than finding those from Zou who were treated >10 years ago. In addition, all patients from Atlantique came from 1 district and were concentrated in geographically restricted areas that were easy to survey. Patients from Zou were more geographically dispersed. Access to some villages and to some houses outside the villages was in general more difficult in Zou districts than in the Atlantique district.

The Buruli ulcer recurrence rate in CSNG-treated patients was low (6.1%) in comparison with those usually reported. Lunn found recurrence rates from <20% to >50% (12). According to WHO, these rates vary from 16% for patients whose conditions are diagnosed early to 28% for patients who seek treatment late (13). In the 2 field studies in Ghana, recurrence rates were 16%–47% (5,6). Even though some rates reported from Ghana are somewhat higher than our highest rate, we do not consider the differences meaningful.

Recurrence rates are directly proportional to the length of follow-up. Muelder and Nourou followed up 28 patients from Sagon (Benin) for up to 42 months and found that the longer the follow-up periods, the higher the accumulative recurrence rate (14). In our study with a follow-up period of up to 7 years, most patients treated at CSNG were in good health.

Two of our recurrent cases did not fit into the definition of recurrences established by WHO, namely, that the recurrence should appear within 1 year of completing

Table 3. Buruli ulcer patient follow-up in villages of the Atlantique and Zou regions, Benin*

Patients treated by surgery at CSNG†	Region		Total, n (%)
	Atlantique, n (%)	Zou, n (%)	
Cured	24 (96.0)	38 (92.7)	62 (93.9)
Recurred	1 (4.0)	3 (7.3)	4 (6.1)
Total	25	41	66

*Pearson p value for the table nonsignificant.
†CSNG, Centre Sanitaire et Nutritionnel Gbemoten.

Table 4. Location, type, and size of previous and new lesions in 4 Buruli ulcer recurrent cases

Patient no.	Size, type, and location of previous lesion*	Type, size, and location of new lesion*	Time since discharge from hospital (mo)
97-294	Small ulcer, right foot	Small ulcer, same site	30
99-280	Large plaque + edema, left leg	Large plaque + edema, same site	12
–	Small ulcer, left foot	Small ulcer, same site	12
99-227	Large edema, left leg + bone lesion, left tibia	Large edema right leg + bone lesion right tibia	17

*Small, <5 cm diameter; large, >5 cm diameter.

treatment (1). In a previous study (11), we noted that some patients came to CSNG with a recurrence >1 year after discharge from the center, most of them with bone lesions. Among the 4 patients who had a recurrence of the disease, a new lesion developed in l patient at a site distant from the initial lesion. This patient had a bone lesion at the time of his first disease episode. Patients with bone lesions are prone to have disseminated lesions at multiple sites (2,11) and to have recurrences >1 year after discharge from the hospital (11). Detecting such disseminated lesions should also imply a follow-up period longer than 1 year. According to our results and those of previous studies, the WHO definition of recurrence should be revised to include a follow-up period longer than 1 year ("delayed recurrence").

As for other disease such as tuberculosis (15) or urinary tract infections (16), distinguishing recurrence or relapse (endogenous recurrent infection) from reinfection (exogenous recurrent infection) is important in Buruli ulcer. This distinction cannot yet be rigorously made for Buruli ulcer, but considering a second lesion that appears next to or within the first lesion as relapse seems reasonable. In the case of bone lesions, however, we propose the term relapse can also be applied even if the second lesion is situated far from the first lesion (11). Recurrence at a different site may result from hematogenous or lymphatic spread of the etiologic agent from earlier *M. ulcerans* disease at a different site. In this case, bone is almost always associated with new lesions; however, they may result from reinfections. The development of fingerprinting molecular biology tools, for example, restriction fragment length polymorphism (17) and mycobacterial interspersed repetitive unit–variable-number tandem repeat (18), seems promising for the resolution of this problem.

Reinfection seems to be infrequent. Gooding et al. demonstrated that when Buruli ulcer does not develop in persons who have been exposed to *M. ulcerans*, they have probably developed an immune response to *M. ulcerans* (19). This finding confirms the earlier hypothesis that disease develops in only a portion of exposed persons in Buruli ulcer–endemic areas (20). In addition, small, self-healing minor ulcers may go undetected or dismissed by the patient (21).

Treatments other than surgery also led to recurrences. Meyers et al. (22) reported 2 recurrent cases among 6 Buruli ulcer patients after heat treatment. The follow-up periods of the 6 patients ranged from 3 to 22 months. In both patients, the recurrence was at a site distant from the heat-treated lesion, and no evidence showed that the initial lesion had reactivated or that the new lesions represented extension of the heat-treated ulcers. In fact, 1 of the 2 patients had 2 previous recurrences, and the other returned 19 months after hospital discharge with new, large Buruli lesions on ankles and lower legs. The authors suggested a probable reinfection from the environment.

Some antimycobaterial drugs are effective against *M. ulcerans* in vitro (23,24) and in vivo in animals (25,26), but the effect of antibacterial treatment in humans remains obscure (27,28). Recently, Grosset (4) demonstrated that early forms of Buruli ulcer may be treated by a combination of streptomycin and rifampicin. In our study, the number of patients who received antimycobacterial treatment was small (10), and the duration of treatment was too short (1–2 weeks) to expect any noticeable effect. Moreover, 2 of our recurrent case-patients had received antimycobacterial treatment. Therefore antimycobacterial use would not be expected to explain, even in part, the low rates of recurrence observed in the present study. Antibiotherapy is considered an adjuvant or complementary treatment to surgery. Effective bactericidal drugs for humans remain a research priority and may play a role in reducing recurrences.

Few health professionals are knowledgeable about Buruli ulcer or have worked in Buruli ulcer–endemic areas. Buruli ulcer does not yet appear in health statistics, and few physicians or surgeons are trained to treat this disease. Recurrences of Buruli ulcer could likely be reduced by improving training of doctors in correct excision procedures (6) and by following up patients regularly.

Not all villages of the Buruli ulcer–endemic districts chosen in the present study were known to be Buruli ulcer–endemic. As shown in Table 1, Buruli ulcer was endemic in 70 of 173 villages (40.5%). Although not the object of the present study, Buruli ulcer–nonendemic villages should be studied further to determine their true level of Buruli ulcer endemicity. Further research should also compare Buruli ulcer–endemic and Buruli ulcer–nonendemic villages from the same district to determine which factors may play a role in the prevalence of Buruli ulcer (29).

In conclusion, our study showed that the recurrence rate after surgery at CSNG after a follow-up period ranging from 2 months to 7 years is low. Creating regional centers that allow patients easy access to treatment with short travel distances and low treatment costs, coupled with educational sessions, could help other centers attract and treat most Buruli ulcer patients in their region. This proximity would render the follow-up of patients easier and be a source of new information on the disease for the population. This process would lessen the stigma of Buruli ulcer by considering it a disease and limiting the number of Buruli ulcer patients who attend traditional healers. Research to develop an effective antimycobacterial treatment remains a priority, and progress in this area may alleviate the problem of recurrences. New molecular tools may help differentiate recurrence and reinfection and clarify the definition of a recurrent case.

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Childhood Hemolytic Uremic Syndrome, United Kingdom and Ireland

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We conducted prospective surveillance of childhood hemolytic uremic syndrome (HUS) from 1997 to 2001 to describe disease incidence and clinical, epidemiologic and microbiologic characteristics. We compared our findings, where possible, with those of a previous study conducted from 1985 to 1988. The average annual incidence of HUS for the United Kingdom and Ireland (0.71/100,000) was unchanged from 1985 to 1988. The overall early mortality had halved, but the reduction in mortality was almost entirely accounted for by improved outcome in patients with diarrhea-associated HUS. The principal infective cause of diarrhea-associated HUS was Shiga toxin–producing *Escherichia coli* O157 (STEC O157), although in the 1997–2001 survey STEC O157 phage type (PT) 21/28 had replaced STEC O157 PT2 as the predominant PT. The risk of developing diarrhea-associated HUS was significantly higher in children infected with STEC O157 PT 2 and PT 21/28 compared with other PTs. Hypertension as a complication of HUS was greatly reduced in patients with diarrhea-associated HUS.

The most serious manifestation of infection with Shiga toxin–producing *Escherichia coli* (STEC) in humans is hemolytic uremic syndrome (HUS). This syndrome comprises a triad of microangiopathic hemolytic anemia, thrombocytopenia, and acute renal failure, usually after a prodromal illness of acute gastroenteritis (1). At least 80% of childhood HUS is attributable to infection with STEC (2), mainly serogroup O157, although other serogroups are implicated (2–10). The peak incidence of HUS is in children <5 years of age (1). Surveillance of pediatric HUS provides valuable information on human infection with STEC.

In a prospective survey of pediatric HUS from 1985 to 1988 in the British Isles, the average annual incidence was ≈0.79 per 100,000 children <16 years of age (3). In the intervening years, the number of laboratory-confirmed cases of STEC O157 in England and Wales increased from 50 in 1985 (11) to 1,087 in 1997 (12). Similar increases were seen in Scotland and Ireland (Figure). Some of the increase in STEC O157 might reflect improved laboratory techniques, improved detection or reporting of milder cases, and a greater awareness of the need to investigate diarrheal disease for STEC. If the increase in STEC O157 was real, however, then the incidence of childhood HUS should have also increased. Therefore, we conducted prospective surveillance of childhood HUS in the United Kingdom and Ireland from 1997 to 2001 to describe the impact of disease and clinical, epidemiologic, and microbiologic characteristics of the patients. We also compared our findings, where possible, with those of a previous study (3,4).

Patients and Methods

Definition of HUS

HUS was defined as 1) acute renal failure, including oligoanuria and elevated creatinine level for age, 2) microangiopathic hemolytic anemia (hemoglobin level <10 g/L with fragmented erythrocytes), and 3) thrombocy-

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topenia (platelet count $<130{,}000 \times 10^9$/L) in the absence of septicemia, malignant hypertension, chronic uremia, or primary vascular disease. Diarrhea-associated HUS was defined as disease that immediately followed diarrhea or bloody diarrhea. Nondiarrhea-associated HUS was defined as disease in which ≥1 episodes did not follow an episode of diarrhea.

Case Ascertainment

Prospective, active surveillance of children <16 years of age was undertaken from February 1, 1997, to January 31, 2001, in the United Kingdom and Ireland. Pediatricians actively reported new cases of HUS in children to the British Paediatric Surveillance Unit or the Scottish Centre for Infection and Environmental Health, by using a card mailed to them each month (13,14). This card allowed them to indicate how many new cases of HUS they had diagnosed. Physicians were also encouraged to telephone the Public Health Laboratory Service Communicable Disease Surveillance Centre or Scottish Centre for Infection and Environmental Health to expedite the identification and investigation of localized outbreaks.

Clinical and Epidemiologic Data Collection

After a report was submitted to the British Paediatric Surveillance Unit, a short, standard, structured questionnaire was mailed to the reporting clinician to collect basic epidemiologic data that included family history, clinical features, laboratory investigations, and initial outcome. Outcome information was requested both on the initial questionnaire, and if unclear, on a follow-up questionnaire ≈1 year from onset. Full renal recovery was defined as normal blood pressure and creatinine level for age and the absence of proteinuria on strip reagent urinalysis. Reminders were sent at the end of the study period to those clinicians who did not return the questionnaire within a month and to any nonresponders.

Microbiologic Investigations

Clinicians were asked to obtain fecal and serum samples from all patients with HUS (both with and without diarrhea) according to a standard clinical protocol. Fecal samples were examined for *E. coli* O157 at the local microbiology laboratory. Presumptive *E. coli* O157 isolates were referred to the Laboratory of Enteric Pathogens at Colindale or the Scottish *E. coli* O157 Reference Laboratory in Edinburgh (in Aberdeen until April 1999). Fecal specimens were obtained from some patients from whom *E. coli* O157 had not been isolated. Serum samples were also referred to the reference laboratories.

Presumptive *E. coli* O157 isolates were confirmed biochemically as *E. coli* and as O157 by serotyping. Confirmed *E. coli* O157 isolates were phage typed and tested for *stx* genes by DNA hybridization or polymerase chain reaction (PCR). Fecal specimens referred to the reference laboratories were examined for *E. coli* O157 by using immunomagnetic separation and also for all STEC by PCR and DNA hybridization (12). Serum samples were tested for antibodies to *E. coli* O157 lipopolysaccharide (15,16). Methods for examination of isolates and fecal samples sent to the reference laboratories from other cases of STEC infection during the study period have been described previously (12).

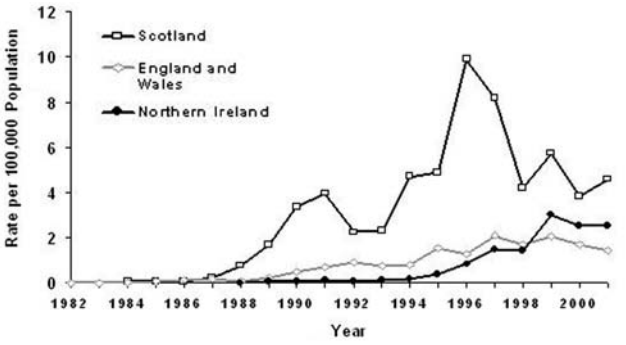

Figure. Laboratory-confirmed infection with Shiga toxin-producing *Escherichia coli* O157 in the United Kingdom, 1982–2001. Data sources: Public Health Laboratory Service and Scottish Center for Infection and Environmental Health.

The distribution of phage types (PTs) of STEC O157 in patients with HUS was compared with that of all cases of infection with STEC O157 in children <16 years of age in England, Wales, and Scotland (comparable data for Northern Ireland and Ireland were not available).

Data Validation and Removal of Duplicate Data

Duplicate reports for the same patient were possible since cases might be reported both from district general hospitals and from specialist pediatric nephrology units. Duplicates were removed by using full name and date of birth. Where duplicates were identified, reports from a specialist in pediatric nephrology were used in preference to those from district general hospitals. The microbiologic and questionnaire data were entered into a Microsoft (Redmond, WA, USA) Access database and linked by using full name and date of birth.

Ethical Approval

This study was reviewed and approved by the Ethics Committee of the Public Health Laboratory Service and the Ethics Committee of the South Birmingham Health Authority.

Data Analysis

Incidence rates and 95% confidence intervals (CIs) were calculated by using population denominators

obtained from the National Statistics Office (for the United Kingdom) and the Central Statistics Office (for Ireland). Descriptive and statistical analyses were performed in Microsoft Access and Excel and in STATA version 7 (Stata Corp., College Station, TX, USA). Differences in proportions were assessed with the chi-square test, and means were compared with the Z test.

Results

Response Rate

During the 4-year period of surveillance, 92% of pediatricians responded to the active reporting system. Four hundred thirteen cases of HUS were confirmed, of which 218 occurred in girls. Three hundred ninety-five patients had diarrhea-associated HUS, and 18 had nondiarrhea-associated HUS.

Incidence

Although most of the HUS cases were reported from England, the highest incidence rates were in Scotland (Table 1). The average annual incidence for those <5 years of age in Scotland was significantly higher than that in England (risk ratio 2.28, 95% CI 1.63–2.30, $p < 0.001$) (Table 1). The age and sex distributions are also shown in Table 1.

Clinical Features and Complications

The clinical features and complications for children from the 1997–2001 survey are summarized in Table 2 and compared with those of the HUS cases from the 1985–1988 survey (3,4). The proportions of HUS patients with and without a diarrheal prodome were very similar in the 2 surveys. The mean duration of illness from symptom onset to diagnosis of HUS decreased, and smaller proportions of children with diarrhea-associated HUS in the 1997–2001 survey showed severe acute abdominal symptoms or hypertension. HUS was reported in 20 pairs of siblings and, in 4 instances, between cousins, with the index patient in each incident having had a diarrheal prodrome.

Of the 18 children with nondiarrhea-associated HUS, 1 developed HUS during treatment for acute lymphoblastic leukemia. The clinical course was complicated by pancreatitis, diabetes mellitus, and seizures. Six children had recurrent HUS, although only 3 had recurrences during the surveillance period. Five of these children had 1 episode of diarrhea-associated HUS (bloody diarrhea in 3 cases). However, all 6 children had relapses without diarrhea and were considered cases of nondiarrhea-associated HUS. All 6 children were negative for STEC.

Treatment

Sixty-three patients with diarrhea-associated HUS received antimicrobial agents (penicillin, metronidazole, or a second- or third-generation cephalosporin) before admission. Eight patients received ciprofloxacin. No statistically significant difference in outcome was seen for those receiving antimicrobials agents (data not shown).

Clinical Outcome

Eighty-two percent of the patients were treated in specialist pediatric nephrology centers. Outcome data were available for 389 (98%) with diarrhea-associated HUS and 18 (100%) with nondiarrhea-associated HUS. Seven (1.8%) children with diarrhea-associated HUS died during acute illness compared with 14 (5.6%) of 252 ($\chi^2 = 6.8$, $p = 0.009$) in the earlier survey. The early death rate for diarrhea-associated HUS was 22% (4/18) from 1997 to 2001 compared with 21% (4/14) from 1985 to 1988 ($p = 0.7$, Fisher exact test). The overall early death rate for the combined group was 2.5% compared with 5% in the 1985–1988 survey ($\chi^2 = 6.8$, $p = 0.01$). Eight of the 11 deaths in the 1997–2001 survey were in children <5 years of age. In the group treated with antimicrobial agents, 3 deaths (5%) occurred compared with 4 deaths (1.2%) in the group that was not treated. This difference was not significant, and no significant relationship was seen when the outcomes of death and renal impairment were analyzed together (data not shown).

Renal recovery was reported in 342 (88%) of those with diarrhea-associated HUS compared with 10 (56%) of those

Table 1. Number, sex distribution, and incidence of childhood cases of hemolytic uremic syndrome, United Kingdom and Ireland, 1997–2001

	Year							Incidence per 10^5/y (all cases)	95% CI*	Patient, <5 y of age	Incidence per 10^5/y (patients <5 y)	95% CI*
	1	2	3	4	Males	Females	Total					
England	83	66	65	73	139	148	287	0.71	0.56–0.89	185	1.54	1.12–2.0
Scotland	12	19	20	12	28	35	63	1.56	0.9–2.43	41	3.4	1.84–6.17
Wales	3	5	4	5	5	12	17	0.71	0.27–1.70	10	1.49	0.36–4.18
Northern Ireland	2	6	4	4	9	7	16	0.97	0.27–1.70	7	1.45	0.2–4.6
Ireland	3	7	10	10	14	16	30	0.83	0.38–1.6	24	2.33	0.85–5.1
Total	103	103	103	104	195	218	413	0.71	0.56–0.89	267	1.54	1.12–2.0

*CI, confidence interval.

Table 2. Clinical features and acute complications of diarrhea-associated hemolytic uremic syndrome (HUS) in children from the 1997–2001 British Paediatric Surveillance Unit survey compared with children from the 1985–1988 survey

	1985–1988 survey	1997–2001 survey	p value
No. of cases	288	413	
Cases with a diarrheal prodrome	273 (95%)	395 (96%)	0.6
Mean (range) time from onset of diarrhea to diagnosis of HUS	8 days (1–34)	6 days (range 1–35)	<0.001
Severe acute abdominal symptoms	40 (15%)	36 (9%)	0.03
Seizures or other neurologic complications	51(19%)	52 (13%)	0.06
Hypertension	86 (32%)	92 (23%)	0.02
Cardiomyopathy	4 (1%)	7 (2%)	1.0
Diabetes mellitus	4 (1%)	8 (2%)	0.77

with nondiarrhea-associated HUS (p = 0.001, Fisher exact test). Hypertension was more likely to develop in patients with diarrhea-associated HUS than those with nondiarrhea-associated HUS (p = 0.05). Seizures or other neurologic complications (p = 0.008), and death (p = 0.0007) were also more likely to occur in those with diarrhea-associated HUS.

Microbiologic Findings for Fecal Samples and Sera

Stools and or sera were available for testing from 393 of 395 patients with diarrhea-associated HUS. Only 25 fecal samples from which presumptive *E. coli* O157 was not isolated locally were examined for the presence of STEC and non-O157 STEC by the reference laboratories. Shiga toxin–producing *E. coli* O157 was confirmed in 10, and 1 was positive for STEC O26.

The proportion of cases confirmed through fecal sampling or serology was much greater (84%) than in 1985–1988, when only 31% of cases were laboratory confirmed (p < 0.001). In the 1997–2001 survey, 161 (60%) of 270 serum samples from patients with diarrhea-associated HUS were positive for antibodies to *E. coli* O157 lipopolysaccharide. Combining results from fecal sampling and serology showed that 329 (83%) of 395 patients with diarrhea-associated HUS were infected with STEC O157; 1 was infected with STEC O26. A total of 330 (84%) patients with diarrhea-associated HUS were infected with STEC.

In the remaining 65 patients with diarrhea-associated HUS, no infective agents were found in 59, *Campylobacter* was found in 2, *Shigella sonnei* PT12 in 1, *Streptococcus pneumoniae* in 1, group C *Streptococcus* in 1, and *Staphylococcus aureus* in 1. In the STEC-positive patients, coinfections with *Campylobacter* (3 patients), group B *Streptococcus* (1 patient), *Cryptosporidium* (3 patients), *Salmonella* (3 patients), and rotavirus (1 patient) were identified.

Eight patients had evidence of infection with *S. pneumoniae*, without other infections including STEC; 7 did not have diarrhea. Two patients were girls and 6 were boys. Three patients had hypertension, 2 showed signs of hemorrhage, and 4 had seizures. Five of the 8 patients made a full renal recovery, but 1 has permanently impaired renal function, and 2 died.

Properties of STEC O157 Strains

The properties of STEC strains belonging to serogroup O157 are summarized in Table 3. Phage typing results were available for 220 (84%) of 261 STEC O157 isolates from patients with diarrhea-associated HUS. The risk of developing this type of HUS was significantly higher for children infected with STEC O157 PT21/28 and STEC O157 PT2.

Overall the STEC O157 PTs that predominated were PT21/28 and PT2. However, geographic differences were striking: PT2 was dominant in England and Wales, PT21/28 in Scotland, and PT32 (6/10) in Ireland. Patients in Scotland with diarrhea-associated HUS were less likely to be infected with STEC O157 PT2 than those in England and Wales ($\chi^2 = 10.62$, p = 0.001) but were more likely to be infected with PT21/28 ($\chi^2 = 14.72$, p = 0.0001). In the 1985–1988 survey, PT2 was dominant (25/38, 66%) and PT21/28 was not found.

Data on Shiga toxin typing were available for 220 strains. Most (213/220, 97%) had only *stx* 2 genes and 7 (3%) had *stx* 1 + 2 genes.

Discussion

In this survey, the average annual incidence of HUS for the United Kingdom and Ireland from 1997 to 2001 was unchanged from the incidence from 1985 to 1988, despite large increases in laboratory-confirmed cases of STEC O157 infection. This finding probably reflects underdiagnosis of STEC infection. Similar proportions of cases with diarrhea-associated and nondiarrhea-associated HUS were found, but the proportion with a confirmed diagnosis had increased. As in the 1985–1988 survey, the principal cause of HUS was STEC O157, but in the 1997–2001 study STEC O157 PT21/28 had replaced STEC O157 PT2 as the predominant PT. This finding reflected the emergence of PT21/28 first in Scotland and subsequently in England and Wales (17,18). However, despite the overall dominance of

Table 3. Properties of STEC strains of *Escherichia coli* serogroup O157 from patients <16 years of age with HUS with and without diarrhea compared with STEC O157 strains from all infected children <16 years of age, 1997–2001*

Phage type	STEC-infected cases with HUS with diarrhea	STEC-infected cases with HUS without diarrhea	All STEC-infected cases	%	Relative risk versus all other phage types (95% CI)
England, Wales, and Scotland					
2	86	537	623	14	1.59 (1.23–2.05)
21/28	101	592	693	15	1.80 (1.41–2.31)
8	1	236	237	0.4	0.04 (0.005–0.26)
32	4	159	163	2	0.22 (0.09–0.6)
4	9	126	135	7	0.64 (0.34–1.22)
Others	19	296	315	6	–
Total	220	1,946	2,166	10	–
England and Wales					
2	77	475	552	14	1.72 (1.3–2.28)
21/28	67	357	424	16	1.96 (1.47–2.6)
8	1	209	210	0.5	0.04 (0.006–0.3)
32	3	145	148	2	0.19 (0.06–0.58)
4	8	118	126	6	0.62 (0.31–1.23)
Others	16	248	264	6	–
Total	172	1,552	1,724	10	–
Scotland					
2	9	62	71	13	1.21 (0.61–2.38)
21/28	34	235	269	13	1.56 (0.86–2.82)
8	0	27	27	0	–
32	1	14	15	7	0.61 (0.09–4.1)
4	1	8	9	11	1.02 (0.16-6.62)
Others	3	48	51	6	–
Total	48	394	442	11	–

*STEC, Shiga toxin–producing *Escherichia coli*; HUS, hemolytic uremic syndrome; CI, confidence interval.

PT21/28, geographic differences in PT distribution occurred across the United Kingdom and Ireland.

The illness and death rate of patients with diarrhea-associated HUS remained high. These children were younger than those with nondiarrhea-associated HUS caused by STEC and had significantly poorer outcomes. Hypertension as a complication of HUS was greatly reduced among patients with diarrhea-associated HUS. The overall death rate had halved, but the reduction in deaths was almost entirely accounted for by improved outcomes in these cases.

The 1985–1988 and 1997–2001 surveys were similar in most regards. Acquisition of data was identical, and the same diagnostic criteria for HUS were used. However, a difference in microbiologic investigation of stool samples was seen in the 2 surveys. In the 1985–1988 survey, fresh fecal samples were sent directly to the Public Health Laboratory Service Division of Enteric Pathogens for culturing and complete strain identification directly from clinical samples. In the 1997–2001 survey, the initial diagnostic work was undertaken in local microbiology departments, and presumptive isolates were forwarded to the appropriate reference laboratories for confirmation and complete identification. The only clinical samples forwarded to the reference laboratories were some of those from HUS patients who were negative on examination at a local laboratory. Although all facilities in the Public Health Laboratory Service had been using a standard protocol since 1995, following a recommendation of the Advisory Committee on the Microbiological Safety of Food that all diarrheal samples be tested for STEC O157 (11,19), this testing was not done at all National Health Service laboratories. A potential criticism of this study is that non-O157 STEC strains might have been missed. This omission is unlikely because 83% of cases had evidence of *E. coli* O157 infection. Little testing for non-O157 STEC occurs in primary diagnostic laboratories in the United Kingdom. However, this situation needs to be balanced against the fact that involvement of local laboratories in assisting clinical investigations expedited fecal sample testing, as shown by a marked increase in sampling, coupled with an improved diagnostic yield. The proportion of culture-positive specimens, which was more than twice as high as in the previous survey, was also higher than proportions reported by other investigators (2,6,9,10). In the 1997–2001 survey, the additional benefit of serologic testing for antibodies to *E. coli* O157 as a diagnostic tool was evident.

A combination of increasing diagnostic yield and shortening time between onset of diarrhea and diagnosis of infection with STEC O157 should allow clinicians to monitor patients and intervene earlier should signs of renal

involvement occur (20,21). Presumably, the significant reduction in time to diagnosis is a function of increasing awareness of STEC O157 infection and its complications among clinicians and microbiologists. This finding might explain the reduction in hypertension and deaths in patients with diarrhea-associated HUS through earlier intervention, including management of dehydration in children before onset of HUS, which has been shown to improve outcome (20). Although the reduction in neurologic complications of diarrhea-associated HUS was not significant, it might be clinically important.

STEC O157 remains the dominant cause of HUS in the United Kingdom and Ireland, with other serotypes contributing little to the overall impact of disease. This scenario is similar to the situation in the United States (2), but contrasts sharply with that in Australia, where infection with STEC O157 is rare (7). In mainland Europe, STEC O157 is the most common cause of HUS, but the contribution of other serotypes is also important (5,6,8–10). The number of cases of HUS not caused by STEC O157 in the 1997–2001 survey was small and much lower than in the previous survey. Eighty-five percent of all O157 strains isolated from children with HUS had either of 2 PTs: PT2 and PT21/28. STEC O157 PT 21/28 emerged during this study and was first seen in Scotland, appearing in England and Wales 2 years later (17,18). Compared with all the STEC O157 strains in children <16 years of age typed by the reference laboratories, PT2 and PT21/28 strains were overrepresented in the patients with HUS, suggesting that these strains might have specific virulence in the children. Most of the PT2 and PT21/28 strains produced *stx*2 either alone or in combination with *stx*1. Shiga toxin type 2 is generally considered an important virulence factor in the pathogenesis of HUS (22,23).

HUS is the most important clinical effect of STEC infection in humans, and young children are more vulnerable than any other age group. It follows that surveillance of childhood HUS is a valuable tool for monitoring the effect of STEC in a population and provides early warning of change. The diagnosis of HUS is obvious and unambiguous, and changes in the incidence of the condition are readily detected and meaningful. Moreover, by focusing on a small indicator population, we observed that this method of surveillance is relatively inexpensive and efficient.

The incidence of HUS in the 1997–2001 survey was similar to that found in 1985–1988 (3). This finding suggests that the incidence of clinically relevant STEC infection has remained constant, at least in children. Therefore, the increase in laboratory reporting over the same time implies increased awareness and readiness to investigate diarrhea or illness by using appropriate microbiologic techniques. More laboratory testing and improved reporting might indicate that milder cases of disease are recognized, so that the proportion of cases of infection with STEC leading to HUS has decreased. Alternatively, improving the management of diarrhea-associated HUS through an earlier diagnosis might allow clinicians to intervene earlier in the disease process, as demonstrated by reductions in hypertension and deaths. This means that the course of the disease might have been altered, explaining, at least in part, the unchanged overall incidence.

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Tuberculosis Elimination in the Netherlands

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This study assessed progress towards tuberculosis (TB) elimination in the Netherlands by using DNA fingerprinting. *Mycobacterium tuberculosis* strains were defined as new if the IS*6110* restriction fragment length polymorphism pattern had not been observed in any other patient during the previous 2 years. Other cases were defined as clustered and attributed to recent transmission. In the period 1995–2002, the incidence of TB with new strains was stable among non-Dutch residents and declined among the Dutch. However, the decline among the Dutch was restricted to those >65 years of age. Moreover, the average number of secondary cases per new strain did not change significantly over time. We conclude that the decline of TB in the Netherlands over the past decade was mainly the result of a cohort effect: older birth cohorts with high infection prevalence were replaced by those with lower infection prevalence. Under current epidemiologic conditions and control efforts, TB may not be eliminated.

Reported rates of tuberculosis (TB) in the Netherlands in 2003 were 3.5 per 100,000 among Dutch residents and 125 per 100,000 among the non-Dutch. The non-Dutch are formally defined as those without a Dutch passport, but in practice they include mostly foreign-born persons. During the past 10 years, TB reports in the Netherlands declined among the Dutch (from 693 in 1993 to 531 in 2002) and remained approximately stable among the non-Dutch (at an average of 892 per year). To what extent these patterns were attributable to changes in TB transmission and to what extent to changes in the introduction of new strains from abroad or from reactivation of latent infection are unclear.

According to mathematical models, eliminating TB as a public health problem, defined as a prevalence of *Mycobacterium tuberculosis* infection of <1%, has been predicted to occur among the Dutch by 2030 if no further changes in transmission occur (1,2). However, if elimination is defined as an incidence of smear-positive disease of <1 case per million population per year (1), TB is not expected to be eliminated by that date because of transmission from foreign-source cases (2). The proportion of all Dutch TB cases in the period 1993–1998 attributable to recent transmission from a non-Dutch source case has been estimated at 12% to 20% with DNA-fingerprinting data (3). With time, an increasing proportion of TB cases among the Dutch were expected to be attributable to recent transmission from non-Dutch source cases (2). However, direct observations with fingerprinting results have not yet been used to evaluate these model predictions.

Immigration patterns in the Netherlands have varied in the past decade: large numbers of persons from countries with high TB endemicity have sought asylum in the early 1990s. In recent years, these numbers became smaller after stricter immigration laws were passed. Shifts in countries of origin of immigrants have also occurred, and some of these countries had much higher TB rates than others. Therefore, the introduction of new strains from abroad may be expected to have varied over time. Control measures, in contrast, have shown little change over the study period. TB screening is obligatory at entry and is offered every 6 months for 2 years on a voluntary basis. No routine screening for and treatment of latent infection exist for immigrants.

This study attempted to determine, by DNA-fingerprinting of *M. tuberculosis* isolates, to what extent TB trends from 1995 to 2002 were determined by changes in the introduction of new strains and by changes in ongoing transmission. We also describe the trend of TB transmission from non-Dutch source patients to the Dutch population. The combined evidence is used to assess the prospects for eliminating TB in the Netherlands.

Methods

Patient and treatment data since 1993 were available in the Netherlands Tuberculosis Register, an anonymous case

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register maintained by the KNCV Tuberculosis Foundation. Reporting to the register is voluntary, but cross-matching with mandatory reports to the ministry of health on all patients who have started TB treatment suggests >99% completeness. The register includes data on demographic characteristics, clinical features, risk groups, treatment given, and treatment outcome. From January 1993 to December 2002, a total of 15,331 TB patients were registered, including those without bacteriologic confirmation.

Over the same period, 10,356 first *M. tuberculosis* isolates of TB patients were subjected to standard IS*6110* restriction fragment length polymorphism (RFLP) analysis (4). Subtyping with the polymorphic GC-rich sequence probe was carried out for strains with <5 IS*6110* copies. IS*6110* RFLP patterns were analyzed by using the Bionumerics software, version 3.5 for Windows (Applied Maths, Sint-Martens-Latem, Belgium).

Information from the 2 databases was combined; sex, date of birth, postal area code, and year of diagnosis were used as identifiers. A perfect match was obtained for 7,529 (73%) isolates and a near-perfect match for 981 (9%). Both groups were included, yielding a total study size of 8,510 (82%) culture-positive patients. Mismatches may be due to administrative errors in a database, unreliable date of birth (e.g., for some immigrant groups), or postal area code (e.g., homeless), and the exclusion of persons with identical identifiers.

TB can occur soon after primary infection or reinfection (recent transmission) or as the result of endogenous reactivation of latent infection (5). The cut-off point for separating recent from remote transmission is arbitrary: some researchers used 5 years (5–7), others 2 years (8), and others 1 year (9). We estimated the percentage of cases with identical RFLP patterns occurring within a given period after each culture-positive case with Kaplan-Meier survival analysis, as suggested by Jasmer et al. (9). The Kaplan-Meier estimate of the probability that a patient was followed by another with an identical fingerprint was 46.2% for the total study period and 33.7% for a 2-year period. Thus, of all cases followed by a patient with an identical fingerprint within 10 years, 73% were followed within 2 years. Using this information, we defined strains as new if the RFLP pattern had not been observed in another patient during the previous 2 years. Other strains were attributed to ongoing transmission. During the first 2 study years (1993–1994), judging whether strains were new was not possible; therefore, data from these 2 years were used to define new strains from 1995 onwards but were otherwise excluded from the analysis.

The observation period in which secondary cases can be observed is longer for strains introduced earlier in the study period than for those introduced later. To obtain an unbiased estimate of the trend of the number of secondary cases generated by source cases, secondary cases arising >2 years after a new strain was introduced were excluded. Thus, all patients were assigned to 1 of the following 3 mutually exclusive categories: case with a new strain, secondary case within 2 years of the introduction of a new strain, and secondary case >2 years after the introduction of a new strain. To assess the trend of transmission between Dutch and non-Dutch persons, secondary cases were attributed to a source case-patient, defined as the patient from whom the new strain was first isolated (10).

Population data by year, age group, sex, and (Dutch/non-Dutch) nationality were obtained from Statistics Netherlands (available from http://statline.cbs.nl/StatWeb) and used as denominators for incidence rates. Relative risks of TB by year of diagnosis, age, sex, and Dutch or non-Dutch nationality were determined separately for new strains and secondary cases with Poisson regression. Risk factors for the average number of secondary cases per new strain were also identified with Poisson regression (3).

Results

Of the 8,510 TB patients with known RFLP results in the period 1993–2002, 1,580 were found in 1993 to 1994, and 6,930 in 1995 to 2002. Of the latter, 4,594 (66%) had new strains, 1,198 (17%) had secondary cases within 2 years of the introduction of a new strain, and 1,138 (16%) had secondary cases >2 years after a new strain was introduced.

The incidence of TB with new strains was on average 52 per 100,000 among the non-Dutch and 1.4 per 100,000 among the Dutch. The incidence declined over the study period among the Dutch (rate ratio per year 0.96, 95% confidence interval [CI] 0.94–0.98) and was stable among the non-Dutch (rate ratio per year 1.02, 95% CI 1.00–1.03, p = 0.06) (Figure 1). The incidence of all cases attributed to recent transmission, regardless of the duration of the cluster, was 23 per 100,000 among the non-Dutch and 0.9 per 100,000 among the Dutch. The incidence declined among the Dutch (rate ratio per year 0.97, 95% CI 0.95–1.00, p = 0.03) but not among the non-Dutch (rate ratio per year 0.99, 95% CI 0.97–1.02) (Figure 1).

Reduction of TB incidence with new strains among the Dutch was restricted to those ≥65 years of age (Figure 2). Incidence was stable at 0.85/100,000 in the age group <65 years (rate ratio per year 1.0, 95% CI 0.97–1.03), declined from 3.5 to 2.2 in those 65–74 years of age (rate ratio per year 0.91, 95% CI 0.86–0.95), and declined from 9.4 to 4.8 in those ≥75 years of age (rate ratio per year 0.92, 95% CI 0.87–0.95). The incidence rate in the ≥75-year age group declined more rapidly than the number of cases in that age group, since the population in that age group increased from 847,000 in 1995 to 979,000 in 2002.

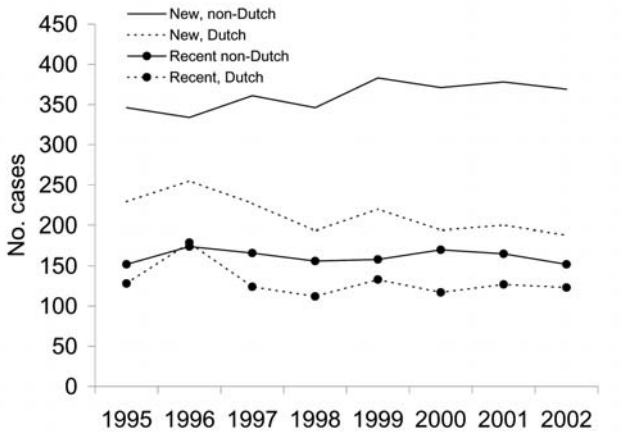

Figure 1. Tuberculosis incidence (new strains and strains attributed to recent transmission) among Dutch and non-Dutch in the Netherlands, 1995–2002.

Of the 4,594 patients with new strains in the period 1995–2002, a total of 3,459 were found in the period 1995–2000 and could be followed up for 2 years. Of the 1,318 Dutch patients with new strains, 182 (14%) generated secondary cases at an average of 1.7 cases per cluster (1.2 Dutch and 0.5 non-Dutch) (Table 1). The average number of secondary cases generated was 0.23 per new strain and declined steeply with the age of the source case-patient (rate ratio per age group 0.74, 95% CI 0.70–0.78) (Table 1). The average number of secondary cases generated did not depend on the sex of the source patient ($p > 0.5$). The average number of secondary case-patients per new strain did not differ significantly over time (rate ratio per year 0.96, 95% CI 0.89–1.02).

Of the 2,141 non-Dutch patients with new strains, 283 (13%) generated secondary cases at an average of 1.9 cases per cluster (0.5 Dutch and 1.4 non-Dutch) (Table 2). The average number of secondary cases generated was 0.25 overall, declined with age of the source patient (rate ratio per age group 0.86, 95% CI 0.80–0.92), and was lower for female than male source patients (rate ratio 0.68, 95% CI 0.57–0.81) (Table 2). The average number of secondary cases per new strain over time did not change (rate ratio per year 0.97, 95% CI 0.93–1.02).

In clusters starting in the period 1995–2000, an increasing proportion of Dutch secondary TB cases was attributable to a non-Dutch source case as time progressed (χ^2_{trend} 4.49, $p = 0.03$) (Table 3). This trend was observed not only among those cases arising within 2 years of the start of the cluster (Table 3) but also among all Dutch secondary cases, regardless of cluster duration (χ^2_{trend} 42, $p < 0.001$, data not shown). The proportion of Dutch secondary cases attributable to a non-Dutch source case declined steeply with age, both among all Dutch secondary case-patients (χ^2_{trend} 41, $p < 0.001$) and among those arising within 2 years of the start of the cluster (χ^2_{trend} 27, $p < 0.001$) (Table 3). The proportion of cases attributed to a non-Dutch source patient was not associated with sex of the Dutch secondary case-patient (Table 3).

Discussion

This study suggests that the declining TB incidence among the Dutch in the Netherlands during the past decade has been achieved under stable control conditions. Among the Dutch, the incidence of TB attributable to new strains declined, particularly among the elderly. The incidence of TB cases due to recent transmission declined as well, a result of fewer new strains being introduced. The average number of secondary cases per new strain did not change significantly. An overall reduction in incidence of clustered cases among the U.S.-born population was also observed in San Francisco (9) and New York (11) and was attributed to improved control. In the Netherlands, we do not attribute the decline to improved control but to a cohort effect. Research may determine to what extent the reported declines in San Francisco and New York could be explained by a reduction in number of secondary cases per newly introduced strain and whether a cohort effect played a role in those settings.

In industrialized countries the annual risk for *M. tuberculosis* infection has declined steeply over the past century (12); as a result, compared to younger persons, older persons were exposed to much higher risks for infection in their youth (13). Thus, the prevalence of infection increases sharply with age. The risk for TB due to reactivation of latent infection therefore increases with age as well. Within the older age groups, this risk is now declining with each calendar year as earlier birth cohorts leave and more recent birth cohorts with lower infection prevalence enter the age group.

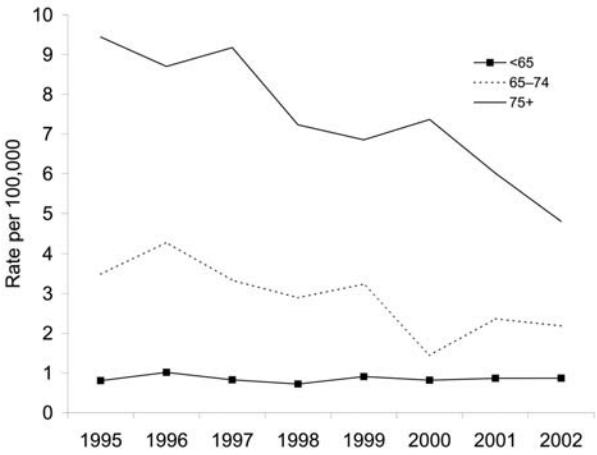

Figure 2. Incidence rate of tuberculosis (new strains) by age group among the Dutch, 1995–2002.

Table 1. Tuberculosis cases with new strains among the Dutch, 1995–2000, and their secondary cases within 2 years

	Cases with new strains	Cases with new strains being first of cluster, n (%)	Other cases in these clusters within 2 years of first case	
			Dutch	Non-Dutch
Year				
1995	229	34 (15)	52	17
1996	255	36 (14)	40	19
1997	227	29 (13)	32	8
1998	193	31 (16)	34	16
1999	220	29 (13)	31	10
2000	194	23 (12)	27	17
Age group				
<25	102	32 (31)	43	16
25–34	154	41 (27)	43	30
35–44	140	28 (20)	37	10
45–54	115	11 (10)	14	6
55–64	150	19 (13)	22	9
65–74	219	22 (10)	28	8
≥75	438	29 (7)	29	8
Sex				
Male	740	103 (14)	121	44
Female	578	79 (14)	95	43
Total	1,318	182 (14)	216	87

The incidence of culture-positive TB with new strains among Dutch persons <65 years of age was stable in the past decade, at 0.85 per 100,000 population in our matched dataset, and thus ≈1/100,000 or 10 per million if failure to match is taken into account. Of these new case-patients with a known sputum smear result, 63% had smear-positive TB. If elimination of TB as a public health problem is defined as achieving an incidence of new smear-positive TB cases of <1 per million (1), elimination is unlikely to be achieved under current epidemiologic conditions and control efforts.

Our study confirms previous predictions from a mathematical model about the increasing importance of transmission from immigrants to the Dutch population (2). The number of cases observed among the Dutch is best explained by immigrant scenarios 1 and 2 in the modeling study (2), which assume that a Dutch TB patient is 8 times more likely than a non-Dutch TB patient to infect a Dutch person.

Over time, the decline of TB incidence among elderly Dutch will become less important as the birth cohorts with a high prevalence of infection are replaced with cohorts

Table 2. Tuberculosis cases with new strains among the non-Dutch, 1995–2000, and their secondary cases within 2 years

	Cases with new strains	Cases with new strains being first of cluster, n (%)	Other cases in these clusters within 2 years of first case	
			Dutch	Non-Dutch
Year				
1995	346	39 (11)	19	68
1996	334	46 (14)	21	70
1997	361	46 (13)	19	52
1998	346	43 (12)	20	72
1999	383	63 (16)	29	77
2000	371	46 (12)	19	61
Age group				
<25	580	101 (17)	51	151
25–34	783	98 (13)	24	124
35–44	370	42 (11)	33	78
45–54	171	18 (11)	5	22
55–64	116	13 (11)	9	15
65–74	88	6 (7)	2	4
≥75	33	5 (15)	3	6
Sex				
Male	1,226	171 (14)	82	264
Female	915	112 (12)	45	136
Total	2,141	283 (13)	127	400

Table 3. Dutch tuberculosis cases attributed to recent transmission and diagnosed within 2 years of the start of clusters

	First case of cluster	
	Dutch	Non-Dutch (% non-Dutch first case)
Year		
1995	16	3 (16)
1996	40	19 (32)
1997	42	22 (34)
1998	32	18 (36)
1999	34	27 (44)
2000	27	23 (46)
2001	19	9 (32)
2002	6	6 (50)
Age group		
<25	29	43 (60)
25–34	46	32 (41)
35–44	32	16 (33)
45–54	27	12 (31)
55–64	24	9 (27)
65–74	34	10 (23)
≥75	24	5 (17)
Sex		
Male	124	80 (39)
Female	92	47 (34)
Total	216	127 (37)

with much lower infection rates. Contact with highly TB-endemic countries through immigrants and international travel, on the other hand, is becoming increasingly important as a determinant of TB trends in the Netherlands. This finding was shown in this study by the increasing proportion over time of Dutch patients with secondary cases attributed to a non-Dutch source patient. This finding suggests the need for further reorientation of the focus of TB control within the Netherlands towards immigrants and their contacts and reemphasizes the importance of global TB control for achieving TB elimination in countries with low incidence of this disease (14).

The separation of TB patients into those with new strains, attributed to reactivation or acquisition abroad, and secondary cases attributed to recent transmission is likely to be imperfect for the following reasons. Some strains identified as new may have represented ongoing transmission in the presence of strain evolution. Some strains attributed to ongoing transmission may represent remote transmission, particularly among the elderly (15). In this national database, epidemiologic confirmation of linkage between patients was far from complete (16). However, in a recent, more detailed study in Amsterdam, most clustered patients were found to have epidemiologic links (17). Missing data as a result of incomplete matching may have contributed to a slight underestimate of the observed clustering percentage and of the number of secondary cases per source case (18,19). However, since the matching percentage was not associated with calender year (data not shown), this underestimate should not affect the trend estimates. Some source cases may have been misclassified, in particular in large clusters. These sources of misclassification are expected to reduce the observed difference between cases with new strains and those attributed to recent transmission but do not invalidate the main conclusion that TB incidence among the Dutch was reduced mainly because of fewer reactivation cases among persons ≥65 years of age.

We conclude that the decline of TB in the Netherlands during the past decade was mainly the result of a cohort effect: older birth cohorts with high infection prevalence were replaced by those with lower infection prevalence. Contact through immigrants and international travel with countries with high TB incidence increasingly determines TB trends in the Netherlands and will prevent achieving TB elimination under current conditions. Global TB control is required to achieve TB elimination in countries with a low incidence of this disease.

Dr. Borgdorff is professor of International Health at the University of Amsterdam and executive director of the KNCV Tuberculosis Foundation. His areas of interest include the molecular epidemiology of TB and other communicable diseases.

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Epidemiology of *Escherichia coli* O157:H7 Outbreaks, United States, 1982–2002

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Escherichia coli O157:H7 causes 73,000 illnesses in the United States annually. We reviewed *E. coli* O157 outbreaks reported to Centers for Disease Control and Prevention (CDC) to better understand the epidemiology of *E. coli* O157. *E. coli* O157 outbreaks (≥2 cases of *E. coli* O157 infection with a common epidemiologic exposure) reported to CDC from 1982 to 2002 were reviewed. In that period, 49 states reported 350 outbreaks, representing 8,598 cases, 1,493 (17%) hospitalizations, 354 (4%) hemolytic uremic syndrome cases, and 40 (0.5%) deaths. Transmission route for 183 (52%) was foodborne, 74 (21%) unknown, 50 (14%) person-to-person, 31 (9%) waterborne, 11 (3%) animal contact, and 1 (0.3%) laboratory-related. The food vehicle for 75 (41%) foodborne outbreaks was ground beef, and for 38 (21%) outbreaks, produce.

Escherichia coli O157:H7 was first recognized as a pathogen in 1982 during an outbreak investigation of hemorrhagic colitis (1). *E. coli* O157 infection can lead to hemolytic uremic syndrome (HUS), characterized by hemolytic anemia, thrombocytopenia, and renal injury (2). Still, it was not until 1993, after a large multistate *E. coli* O157 outbreak linked to undercooked ground beef patties sold from a fast-food restaurant chain (3), that *E. coli* O157 became broadly recognized as an important and threatening pathogen. Clinical laboratories began examining more stool specimens for *E. coli* O157 (4). In 1994, *E. coli* O157 became a nationally notifiable infection, and by 2000, reporting was mandatory in 48 states. An estimated 73,480 illnesses due to *E. coli* O157 infection occur each year in the United States, leading to an estimated 2,168 hospitalizations and 61 deaths annually (5), and it is an important cause of acute renal failure in children (6,7).

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Although reported outbreaks account for only a minority of *E. coli* O157 cases, outbreak investigations contribute greatly to understanding *E. coli* O157 epidemiology by identifying transmission routes, vehicles, and mechanisms of contamination (8). Outbreak findings oblige regulatory and public health agencies and industry to evaluate prevention and control measures so similar outbreaks can be prevented. Knowledge of transmission routes and vehicles allows consumers to be educated on reducing risky behavior that can decrease their risk for infection. We report here surveillance results for *E. coli* O157 outbreaks reported to the Centers for Disease Control and Prevention (CDC) from 1982 through 2002, to highlight the epidemiology of this emerging pathogen.

Methods

Outbreaks of *E. coli* O157:H7 and Shiga toxin–producing *E. coli* O157:NM (subsequently referred to as *E. coli* O157) investigated by state and local health departments were reported to CDC by telephone, outbreak report, or through the routine foodborne disease outbreak surveillance system (9). In preparation for this summary, an epidemiologist reviewed all reports including published outbreaks not otherwise reported. Information collected from each outbreak report included city, setting, and suspected transmission route and vehicle. The date of first illness, hospitalizations, number of ill persons, bloody diarrhea, culture-confirmed illness, HUS, and deaths were also obtained. We defined an outbreak as ≥2 cases of *E. coli* O157 infection (at least 1 culture-confirmed) with a common epidemiologic exposure. For purposes of defining an outbreak, we considered a case as a stool culture yielding *E. coli* O157, or bloody diarrhea, or HUS. Each investigator reported the total number of outbreak-related cases, often including those with compatible clinical illness but without culture confirmation of illness.

Infections acquired outside the United States were not included.

We defined outbreak onset as month and year first illness onset was reported, and outbreak setting as place where exposure occurred. Outbreaks due to a distributed food item and not isolated to a single venue or event were classified as communitywide. Fast-food settings were defined as establishments where payment is made before receiving food. Outbreaks were classified into 1 of 6 transmission routes on the basis of how most patients acquired the infection (foodborne, person-to-person, recreational water, drinking water, animal exposure, or laboratory). Outbreaks with a common exposure but in which a major transmission route was not identified were classified as unknown transmission route. Median outbreak sizes were compared by using the Kruskal-Wallis test. Outbreak-related HUS and death rates were compared by using a chi-square test.

Foodborne outbreaks were defined as the occurrence of ≥2 cases of *E. coli* O157 infection resulting from ingestion of a common food, or if food vehicle was undetermined, sharing a common meal or food facility. Food vehicles were grouped into the following categories: ground beef, other beef, produce, dairy, other, or unknown. Food vehicles were implicated statistically in case-control studies ($p \leq 0.05$), by isolation of *E. coli* O157 from a suspect item, or by being the only common food item consumed by cases. A multistate outbreak was defined as exposure to a common vehicle occurring in >1 state. HUS cases were classified by individual investigators and included those cases diagnosed as thrombotic thromobocytopenic purpura following *E. coli* O157 infection.

Results

From 1982 to 2002, a total of 350 outbreaks were reported from 49 states, accounting for 8,598 cases of *E. coli* O157 infection. Among cases, there were 1,493 (17.4%) hospitalizations, 354 (4.1%) cases of HUS, and 40 (0.5%) deaths. The number of reported outbreaks began rising in 1993, and peaked in 2000 with 46 (Figure 1). Outbreak size ranged from 2 to 781 cases, with a median of 8 cases. Median outbreak size appears to have declined from 1982 to 2002 (Figure 2). Most outbreaks (89%) occurred from May to November. Of the 326 outbreaks reported from a single state, Minnesota reported the most (43 outbreaks), followed by Washington (27 outbreaks), New York (22 outbreaks), California (18 outbreaks), and Oregon (18 outbreaks). Among the 350 outbreaks, transmission routes for 183 (52%) were foodborne, 74 (21%) unknown, 50 (14%) person-to-person, 21 (6%) recreational water, 11 (3%) animal contact, 10 (3%) drinking water, and 1 (0.3%) laboratory-related transmission route (Table).

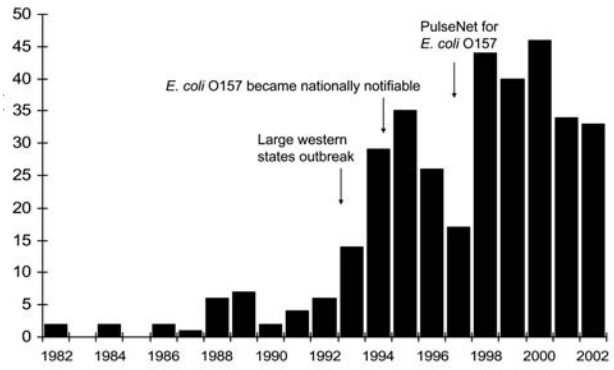

Figure 1. *Escherichia coli* O157 outbreaks by year, 1982–2002 (N = 350).

Foodborne Outbreaks

Food remained the predominant transmission route from 1982 to 2002 (Figure 3), accounting for 52% of 350 outbreaks and 61% of 8,598 outbreak-related cases. Foodborne outbreaks most frequently occurred in communities (53 [29%] of 183), restaurants/food facilities (51 [28%]), and schools (16 [9%]). Median size of foodborne outbreaks varied by setting: the smallest occurred in individual residences (3 cases), and the largest outbreaks in residential facilities (44 cases), followed by camps (36 cases). Among 51 restaurant and food facility outbreaks, 22 were in chain establishments (including 12 fast-food establishments) and 29 in single establishments. The median number of cases per restaurant/food facility outbreak was larger in chain than single establishments (21 vs. 8, $p < 0.001$). Among the 183 foodborne outbreaks, the food vehicle in 75 (41%) was ground beef, in 42 (23%) was unknown, in 38 (21%) was produce, in 11 (6%) was other beef, in 10 (5%) was other foods, and in 7 (4%) was dairy products.

Ground Beef

The first *E. coli* O157 outbreak was reported in 1982 and linked to ground beef, which remains the most common vehicle among foodborne outbreaks (75 [41%] of

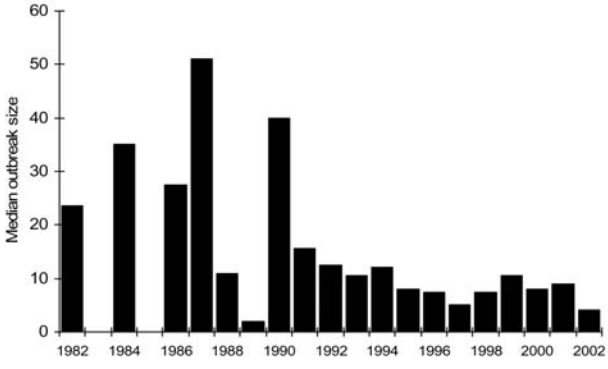

Figure 2. Median size of *Escherichia coli* O157 outbreaks by year.

Table. Outbreaks and cases of *Escherichia coli* O157 infection by transmission route, 1982–2002

	Outbreaks			Outbreak size	Cases		
Transmission route	n	Total %	Foodborne %	Median (range)	n	Total %	Foodborne %
Ground beef	75	21	41	8 (2–732)	1,760	20	33
Unknown food vehicle	42	12	23	8 (2–86)	646	8	12
Produce	38	11	21	20 (2–736)	1,794	21	34
Other beef	11	3	6	17 (2–323)	563	7	11
Other food vehicle	10	3	5	15 (2–47)	206	2	4
Dairy product	7	2	4	8 (2–202)	300	3	6
Subtotal, foodborne	183	52		11 (2–736)	5,269	61	
Unknown transmission route	74	21		4 (2–140)	812	9	
Person-to-person	50	14		7 (2–63)	651	8	
Recreational water	21	6		8 (2–45)	280	3	
Animal contact	11	3		5 (2–111)	319	4	
Drinking water	10	3		26 (2–781)	1,265	15	
Laboratory-related	1	<1		2	2	<1	
Subtotal, other routes	167	48		5 (2–781)	3,329	39	
Total	350				8,598		

183) (Figure 4), although it accounts for only 33% of 5,269 foodborne-related cases. Outbreaks involving ground beef peaked in summer months: 71% occurred from May to August. Of the 40 outbreaks for which ground beef preparation style was reported, 27 (68%) were linked to hamburgers and 5 (13%) to meat sauce. Ground beef–associated outbreaks occurred most frequently at the communitywide level (36 of 75 [48%]), followed by 11 (15%) at picnics or camps, 8 (11%) at individual residences, 7 (9%) at restaurants, and 4 (5%) at schools. Of the 7 ground beef–associated restaurant outbreaks, 5 occurred in fast-food restaurants in 1982 (2 outbreaks), 1992–1993 (1 outbreak), 1995 (1 outbreak), and 1999 (1 outbreak). The last hamburger-associated fast-food restaurant outbreak was reported in 1995.

Other Beef

Types of beef other than ground beef were implicated in 11 outbreaks. Five outbreaks were associated with consumption of roast beef, 2 with steak, 1 with sirloin tips, and 1 with salami. The other 2 outbreaks were identified only as "beef" and "raw roast beef."

Produce

Produce-associated outbreaks were first reported in 1991 and have remained a prominent food vehicle (Figure 4), accounting for 38 (21%) of 183 foodborne outbreaks and 34% of 5,269 foodborne outbreak-related cases. Produce-associated outbreaks peaked in summer and fall; 74% occurred from July to October. Thirteen (34%) produce-associated outbreaks were from lettuce, 7 (18%) from apple cider or apple juice, 6 (16%) from salad, 4 (11%) from coleslaw, 4 (11%) from melons, 3 (8%) from sprouts, and 1 (3%) from grapes. Produce-associated outbreaks most commonly occurred in restaurants (15 [39%]), and 7 (47%) of these were reported to be due to cross-contamination during food preparation. Twenty (53%) produce-associated outbreaks did not involve kitchen-level cross-contamination, including the 7 outbreaks associated with apple cider or apple juice, 7 of 10 lettuce-associated outbreaks, 3 of 4 coleslaw-associated outbreaks, and the 3 alfalfa-associated or clover sprout-associated outbreaks. None were reported to be due to imported produce. The median number of cases in produce-associated outbreaks was significantly larger than that of ground beef-associated outbreaks, 20 vs. 8, ($p < 0.001$).

Dairy Products

Seven outbreaks were associated with dairy products, including 4 from consuming raw milk. The others were due to cheese curds made from raw milk, from butter made from raw milk, and from commercial ice cream bars (possibly due to cross-contamination).

Person-to-Person Outbreaks

Fifty outbreaks were spread by the fecal-oral route. Outbreak settings included 40 (80%) child daycare centers; 5 (10%) individual residences; 3 (6%) communities, 1

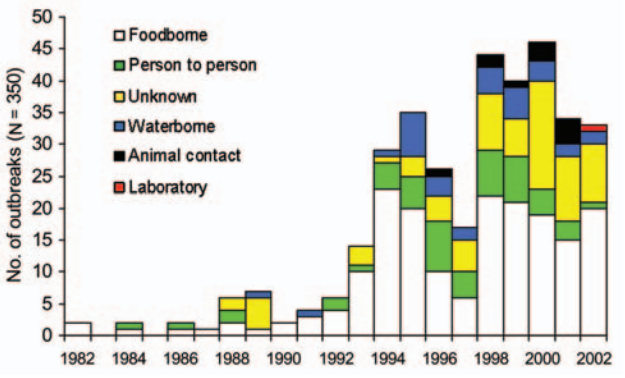

Figure 3. Transmission routes of *Escherichia coli* O157 outbreaks by year.

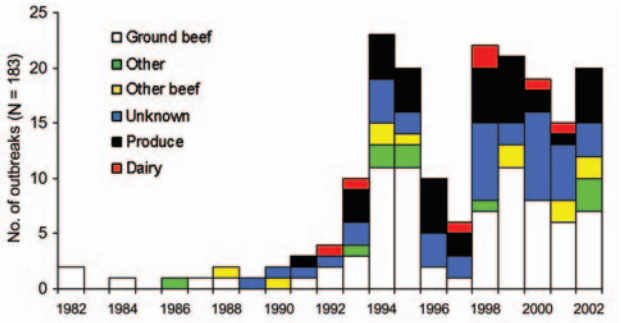

Figure 4. Vehicles of foodborne *Escherichia coli* O157 outbreaks by year.

(2%) school, and 1 (2%) residential facility. Outbreak size ranged from 2 to 63 cases (median 7). Person-to-person outbreaks peaked during summer; 70% occurred from June to August.

Waterborne Outbreaks

Thirty-one outbreaks were waterborne: 21 from recreational water and 10 from drinking water. Recreational water-associated outbreaks were first reported in 1991; 14 (67%) occurred in lakes or ponds, and 7 (33%) in swimming pools. Outbreak size ranged from 2 to 45 cases (median 8 cases). Outbreaks occurred from June to September.

Outbreaks due to contaminated drinking water tended to be much larger than all other outbreaks, with a median size of 26 vs. 8 cases, ($p = 0.08$) and occurred from May to December. Drinking water outbreaks accounted for 3% of all outbreaks, but 15% of all outbreak-related cases. Four of the outbreaks were attributed to local well water systems, 3 involved municipal water supply systems, and 1 each was due to spring water, residential faucet water, and ice thought to be cross-contaminated. Two of the 3 municipal water suppliers did not use chlorination, and the other had a malfunctioning chlorinator.

Animal Contact Outbreaks

First reported in the United States in 1996, outbreaks due to animal contact are 1 of the newest recognized transmission routes. Direct or indirect cow or calf exposure was noted in all 11 outbreaks: 5 on farms, 2 at county fairs, 2 at petting zoos, 1 at a barn dance, and 1 at a camp. Nine of the outbreaks occurred from July to November. Outbreak size ranged from 2 to 111 cases and accounted for 4% of the 8,598 outbreak-related cases.

Laboratory-related Outbreak

One outbreak was reported in 2002 from a laboratory. It involved 2 culture-confirmed cases. Two technicians were infected while validating an *E. coli* O157 sterilization technique.

Outbreaks with Unknown Transmission Route

Outbreaks reported as unknown transmission route accounted for 21% of outbreaks and 9% of all outbreak-related cases. Most (92%) occurred from May to November. Median size was 4 cases (range 2–140).

Multistate Outbreaks

Twenty-four multistate *E. coli* O157 outbreaks were reported since 1992; they ranged from 1 to 3 per year, except in 1999, when 6 were reported. The number of states involved ranged from 2 to 8 with a median of 3. All were due to foodborne transmission. Sixteen (67%) were from ground beef and 6 (25%) from produce.

HUS Cases

Among 346 outbreaks that reported HUS cases, 132 (38%) reported at least 1 case of HUS (range 1–55 cases, median 2 cases), for a total of 354 HUS cases. The HUS rate (number of cases per 100 outbreak-related illnesses) was 4.1. From 1982 to 2002, the HUS rate appeared to decline overall (Figure 5). The HUS rate differed significantly by transmission route ($p < 0.001$) and was highest among swimming outbreaks (10.7), followed by person-to-person (6.8), unknown (6.7), animal contact (5.6), foodborne (3.5), and drinking water (2.1) related–outbreaks. Among foodborne outbreaks, the HUS case rate was significantly higher among ground beef–associated outbreaks compared with all other foodborne outbreaks (5.5 vs. 2.5, $p < 0.001$).

Deaths

Among 325 outbreaks that reported number of deaths, 25 (8%) reported at least 1 (range 1–4), for a total of 40 deaths. Twenty-five (63%) deaths were in persons with HUS; 15 (38%) were due to other causes. Among 12 outbreaks reporting age at death, age ranges were 1–4 years and 61–91 years. Case-fatality rate (number of deaths per 100 outbreak-related illnesses) was 0.5 and appeared to decrease from 1982 to 2002 (Figure 5). The case-fatality rate did not vary significantly by transmission route; however, the rate was significantly higher among outbreaks in residential facilities than in other settings (6.6 vs. 0.4, $p < 0.001$). Residential facilities where deaths occurred included a nursing home, a custodial institution, and an acute-care facility.

Discussion

From 1982 to 2002, a total of 350 *E. coli* O157 outbreaks were reported in the United States from 49 states. Despite regulatory efforts to improve the safety of the U.S. food supply, foodborne *E. coli* O157 outbreaks remain common. Ground beef remains the most frequently identified vehicle, and produce-associated outbreaks

are commonly reported. In addition, nonfoodborne transmission routes remain prominent. Person-to-person outbreaks occur most frequently in child daycare centers. Waterborne outbreaks caused by both drinking and recreational water continue to be reported, and outbreaks due to animal contact are increasingly reported.

In January 1993, the largest *E. coli* O157 outbreak from ground beef was reported in 4 western states, involving >700 ill persons, mostly children; more than one quarter were hospitalized, HUS developed in 7.5%, and 4 children died (3,10). Illness was linked to eating undercooked hamburgers at a chain fast-food restaurant, prompting a recall of >250,000 hamburgers, which likely prevented many additional illnesses and deaths.

Outbreak investigations that implicated fast-food hamburgers have led to major improvements in meat safety in the U.S fast-food industry. In 1993, the U.S. Food and Drug Administration revised the Model Food Code for restaurants, with new temperature guidelines for ground beef (11). In 1994, the National Livestock and Meat Board's Blue Ribbon Task Force developed objective measures of meat "doneness" and encouraged use of automated cooking systems (12). No fast-food hamburger-associated outbreaks have been reported since 1995, demonstrating that changes in the fast-food industry, such as carefully regulating cooking temperature of hamburgers, are both possible and effective.

In addition, outbreak investigations coupled with traceback investigations of implicated meat have identified contaminated beef lots, leading to large recalls of potentially contaminated beef (3). These recalls of up to 25 million pounds of beef (13) likely prevented many additional infections. Despite these improvements, ground beef continues to be frequently implicated in *E. coli* O157 outbreaks. Raw beef, especially ground beef, can be contaminated with *E. coli* O157 and should be cooked thoroughly to kill pathogens and handled carefully to avoid cross-contamination of other food items. As ground beef outbreaks are commonly reported from home-prepared ground beef, educational efforts should be focused on teaching consumers safer handling and cooking practices.

Outbreaks provide information about inadequacy of processing methods. For example, in 1994, an *E. coli* O157 outbreak due to eating commercially distributed dry-cured salami product involved 23 persons; HUS developed in 13% (14). This outbreak prompted U. S. Department of Agriculture officials to develop regulations to ensure the safety of shelf-stable fermented sausages (15); no further *E. coli* O157 outbreaks due to U.S.–manufactured salami have been reported since.

E. coli O157 outbreaks due to produce have become increasingly common. While half of produce-associated outbreaks were due to kitchen-level cross-contamination,

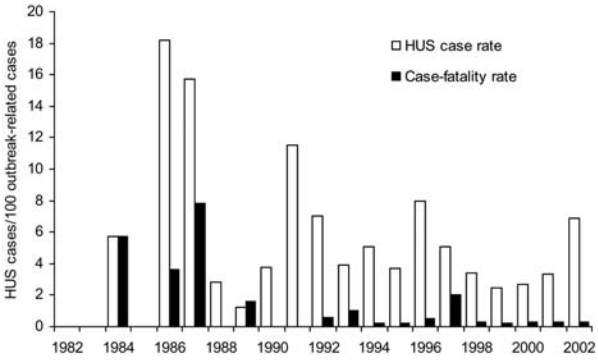

Figure 5. Hemolytic uremic syndrome (HUS) and case-fatality rate per 100 outbreak-related illnesses.

which calls for further prevention efforts targeting food preparers, the other half were due to produce already contaminated with *E. coli* O157 before purchase, including lettuce, sprouts, cabbage, apple cider, and apple juice (16–20). These produce items could have become contaminated in the field from manure or contaminated irrigation water; during processing due to contaminated equipment, wash water, or ice or poor handling practices; during transport; or through contaminated storage equipment. Washing produce with water or a chlorine-based solution reduces *E. coli* O157 counts only modestly (21,22); therefore, once consumers obtain contaminated produce intended for raw consumption, little can be done to prevent illness. Efforts by industry to decrease contamination of sprouts have had limited success (23,24). Until effective measures for preventing *E. coli* O157 contamination of produce items such as lettuce, cabbage, and sprouts can be implemented, consumers should be educated about potential risk of consuming these items raw. Further regulatory and educational efforts are needed to improve the safety of produce items.

In 1996, a large *E. coli* O157 outbreak occurred in 3 western states and British Columbia, involving 70 illnesses, mostly children; more than one third of patients were hospitalized, HUS developed in 20%, and 1 child died (20). Illness was attributable to drinking commercial unpasteurized apple juice. However, as a result of this outbreak investigation, apple cider and apple juice that are shipped interstate in the United States since 1998 are either pasteurized or, if sold raw, carry a warning label advising consumers of potential harmful bacteria in the product (25). Since 1998, only 2 outbreaks due to unpasteurized apple cider have been reported, 1 at a local fair and 1 from locally produced cider that carried a warning label.

Prevention efforts focused on hygiene are needed to reduce transmission in daycare settings. In outbreaks of other primary transmission routes, secondary cases occur, which emphasizes the importance of educating caretakers

to avoid direct contact with fecal matter and to apply stringent handwashing rules.

Drinking and recreational water have the potential to infect many persons. The largest U.S. *E. coli* O157 outbreak occurred in 1999 at a county fair due to contaminated drinking water and involved 781 ill persons; 9% were hospitalized, HUS developed in 2%, and 2 died (26). The implicated water was from a temporary unregulated well at the fairground. Properly functioning water systems with adequate chlorine levels should protect against *E. coli* O157 contamination. Many U.S. households, however, receive municipal water that is not chlorinated. Further safeguards are therefore needed to ensure the safety of unchlorinated water systems and to ensure that chlorinated water systems are properly functioning. Educational efforts targeted at caretakers of young children should continue to help reduce contamination of recreational water areas by fecal matter (27,28).

Outbreaks associated with animal contact represent a newly recognized transmission route for *E. coli* O157 in the United States. Cattle hides may become contaminated from fecal matter. Persons touching cattle or surfaces in the cattle's environment may contaminate their hands with *E. coli* O157. If hands are not washed thoroughly after contact with cattle or their environments, the bacteria can infect these persons through a hand-to-mouth route. Recent strategies published to help reduce transmission of enteric pathogens from farm animals to children include informing the public about risk for transmission of enteric pathogens from farm animals to humans, separating eating facilities from animal contact areas, and providing adequate handwashing facilities (29).

The overall decreased HUS and case-fatality rates in the last 2 decades likely represent increased reporting of less clinically severe outbreaks, especially after *E. coli* O157 became a reportable disease. The high HUS rate found in swimming-associated outbreaks may be due partly to the higher proportion of young children involved and their vulnerability to development of HUS. The reason for the higher HUS rate found among ground beef–related outbreaks is unclear and may reflect reporting bias. Outbreaks occurring in residential facilities such as nursing homes had a particularly high case-fatality rate, which emphasizes the need for prevention efforts, both educational and regulatory, to lower the incidence of *E. coli* O157 infections in such facilities.

Since 1992, molecular subtyping of *E. coli* O157 by pulsed-field gel electrophoresis has improved early outbreak detection. PulseNet (30), the national network for comparing molecular subtypes of common foodborne bacterial pathogens, including *E. coli* O157 since 1997, has greatly assisted in both identifying outbreaks and linking apparently unrelated outbreaks. Continued molecular subtyping of *E. coli* O157 strains from both humans and the environment will assist in detecting outbreaks and allow for identification of multistate, geographically dispersed outbreaks due to contaminated commercial products (30).

Outbreak surveillance has several limitations. *E. coli* O157 outbreaks captured by CDC's surveillance system likely represent only a small proportion of outbreaks that occur. Many outbreaks go unrecognized, are classified as outbreaks of unknown etiology, and are not reported to local public health officials or CDC (31). Smaller outbreaks and outbreaks with unknown transmission routes and vehicles are less likely to be reported, and this summary likely under represents such outbreaks. Including patients with compatible clinical illness without culture confirmation is another limitation of outbreak surveillance. However, given the broad clinical spectrum of *E. coli* O157 infection, and the limited number of infected persons with culture-confirmed illness (5), such inclusion allows us to better assess the true public health impact of *E. coli* O157. In addition, outbreak reporting may not be uniform across time periods or states. Therefore, trends should be interpreted carefully, given the changing factors that may impact outbreak detection and reporting. The increased numbers of outbreaks reported since 1993 but with smaller sizes are likely due to increased awareness of disease, improved diagnostics, increased *E. coli* O157 testing, and improved outbreak detection through molecular subtyping.

Outbreak investigations, especially for emerging pathogens such as *E. coli* O157, are critical for better understanding these pathogens' epidemiology, which affect policy and behavior changes. While a summary of outbreaks cannot draw firm conclusions on disease trends, illustration of transmission routes, food vehicles, outbreak size, and clinical outcomes over time empowers public health officials, regulatory agencies, and health educators to target appropriate interventions and reevaluate current prevention strategies.

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Web-based Investigation of Multistate Salmonellosis Outbreak

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We investigated a large outbreak of *Salmonella enterica* serotype Javiana among attendees of the 2002 U.S. Transplant Games, including 1,500 organ transplant recipients. Web-based survey methods identified pre-diced tomatoes as the source of this outbreak, which highlights the utility of such investigative tools to cope with the changing epidemiology of foodborne diseases.

The epidemiology of foodborne illnesses is influenced by a variety of factors, some of which have changed dramatically in recent years. The increased availability of preprocessed foods and the improved survival of persons with immune defects have affected the sources and nature of foodborne illness (1–4). Increased mobility of Americans through interstate travel has complicated the identification and investigation of outbreaks. New technologies for outbreak investigation have the potential to greatly assist public health officials in successfully managing these changing factors. We describe an outbreak of *Salmonella enterica* serotype Javiana infections affecting a large group of geographically dispersed organ transplant recipients. The prompt and successful investigation of this outbreak was facilitated by the use of Web-based surveys.

The Study

On July 16, 2002, the Minnesota Department of Health identified 2 cases of *S.* Javiana infection among persons who attended the 2002 U.S. Transplant Games, an Olympics-style athletic competition among recipients of solid organ and bone marrow transplants that was held June 25–29 at theme park A in Orlando, Florida. Isolates from the 2 patients were indistinguishable when subtyped by pulsed-field gel electrophoresis (PFGE). Approximately 6,000 people, including 1,500 transplant recipient athletes, attended the 2002 Transplant Games.

To identify additional cases, state health departments were asked to report any *S.* Javiana isolates with a PFGE pattern indistinguishable from the outbreak strain. To develop hypotheses about potential sources of infection, we conducted in-depth telephone interviews with several persons who were identified with culture-confirmed illness.

On the basis of interview results, we conducted a Web-based cohort study among Transplant Games attendees to identify risk factors for infection by using eQuest, a software package developed by the Centers for Disease Control and Prevention (CDC) that allows rapid development of Web-based surveys (5). Using email addresses provided to us by the Transplant Games organizers, we electronically distributed a message on July 20 to attendees (including athletes and spectators), requesting that they complete an outbreak survey. We included information about salmonellosis and its treatment and provided a link in the email to the secure Web site containing the outbreak survey. In each survey respondent's household, we collected information for a single person visiting Orlando, regardless of whether he or she had been ill. A case was defined as fever or diarrhea with onset between June 25 and July 7 in a person who visited Orlando. Submitted answers were automatically stored in a secure electronic database and linked only to a random survey number.

To identify the specific food item responsible for illness, we performed a Web-based case-control study. On July 31, we distributed a survey containing detailed questions about specific food items available in theme park A to persons who had responded to the first survey. Case-patients were questioned about food items eaten in the 3 days before illness onset. Controls were defined as well survey respondents, and all were questioned about the middle 3 days of the Transplant Games (June 26–28).

Plant X, the processing plant that supplied tomatoes to theme park A, was inspected on August 13. Molecular subtyping of confirmed *S.* Javiana isolates was performed at state public health laboratories (6). Diced Roma tomatoes from unopened boxes that had been stored frozen at theme park A were cultured at the Florida State Public Health Laboratory.

Statistical analyses were conducted by using SAS software version 8.2 (SAS Institute Inc., Cary, NC, USA) to calculate odds ratios (OR) and 95% confidence intervals (CI). Multivariable logistic regression analyses were conducted for variables that were significantly associated with illness.

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Through laboratory surveillance, 21 additional *S.* Javiana infections with indistinguishable PFGE patterns were identified in 10 states, for a total of 23 identified culture-confirmed cases. Dates of illness onset were from June 24 to July 8. Of 22 patients for whom travel information was collected, 19 reported visiting theme park A in the last week of June; 16 visited theme park A but did not report any contact with the Transplant Games, which suggests a true outbreak of *S.* Javiana infections among visitors to the theme park.

An electronic link to the Web-based cohort study survey was distributed on July 20, 2002, to 1,100 Transplant Games attendees. Among these 1,100, we received survey responses from 369 persons (34%) in 42 states; 80% responded within 48 hours. Of the 369, a total of 82 (22%) reported illness and 41 (53%) were female. The median age of ill respondents was 47 years (range 4–71 years); 48 (59%) were transplant recipients. Dates of symptom onset were June 26–July 7 (Figure). Predominant symptoms included diarrhea (93%), abdominal pain (79%), and fever (51%). Three respondents (4%) had been hospitalized. No deaths were reported to the organizers of the Transplant Games or CDC. Among ill respondents, 75 (91%) reported eating food items at specific food courts in theme park A.

The Web-based case-control study was distributed on July 31 to the 369 persons who responded to the first survey. By August 2, a total of 222 persons (60%) responded. Of 217 valid responses, 41(19%) were ill persons who met the case definition; the remaining 176 were healthy controls. Ill persons were significantly more likely to report eating dishes containing diced Roma tomatoes than were well persons (44% of ill vs. 15% of well, OR = 4.3, 95% CI 2.1–9.1). Other food items that were significantly associated with illness on univariate analysis were dishes containing shredded iceberg lettuce (OR = 3.7, 95% CI 1.8–7.4), pre-shredded cheddar cheese (OR = 2.9, 95% CI 1.5–5.9), fresh ground beef (OR = 3.0, 95% CI 1.4–6.4), and pre-sliced beefsteak tomatoes (OR = 4.6, 95% CI 1.1–19.4) (Table). In multivariable logistic regression modeling, only diced Roma tomatoes remained independently associated with illness at the 0.05 significance level.

Diced Roma tomatoes were supplied to theme park A from plant X, where whole Roma tomatoes were mechanically diced and washed in a manually chlorinated recycled water tank. Levels of chlorine in the tank were variable (≈1.5–3.5 ppm free chlorine), providing potential opportunity for the amplification of any existent microbial contamination. Review of invoices showed that diced Roma tomatoes used in food courts patronized by persons with outbreak-related illness were processed at plant X from June 20 through July 3. No diced tomatoes from the implicated lots were available for testing. Microbiologic evaluation of an unopened box of plant X diced Roma tomatoes processed on July 12 indicated the presence of fecal coliforms (150–1,000 CFU/g).

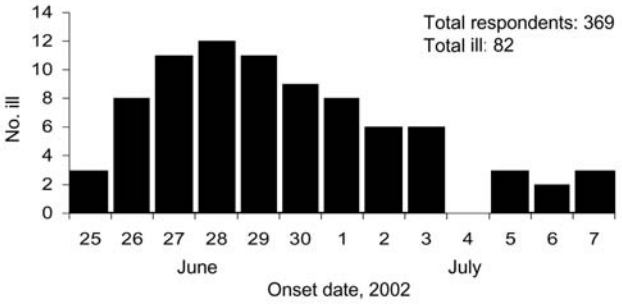

Figure. Diarrheal illness among attendees of the 2002 U.S. Transplant Games in Orlando, Florida.

Conclusions

The nature of this outbreak highlights several changing features of foodborne disease epidemiology, including the enhanced mobility of persons through air travel, an increasing reliance on pre-processed foods, and an expanding immunocompromised population at risk. Through a Web-based investigation, we were able to rapidly identify the source of this outbreak and inform an immunocompromised population of its potential risk for illness. Our approach allowed us to contact and question several hundred geographically dispersed persons in a matter of days. Survey respondents' answers were automatically stored in a secure electronic database, eliminating the need for data entry. With the development of questionnaire templates, a public health official could select sets of questions and precoded answers from pull-down menus and modify them to design an outbreak-specific, Web-based questionnaire that is automatically linked to an electronic database (5). These methods make it increasingly possible to design and post a Web-based investigative tool for wide distribution within hours.

Our investigation has several limitations. Use of a Web-based investigation tool limited responses to only those Transplant Games attendees with known email addresses and Internet access. The initial response rate to our survey was only 34%; households with ill persons may have been more likely to respond to our Web-based survey. However, most (>75%) respondents to both surveys were from households in which no one had experienced illness, which provided us with a sufficient number of responses from both well and ill persons to identify the source of the outbreak. Hospitalized and severely ill persons may have been too sick to respond to the survey or may have been unable to access the Internet, which limited our ability to calculate accurate hospitalization or attack rates among persons attending the Transplant Games. Although isolation of *S.* Javiana from plant X diced tomatoes would have

Table. Univariate analysis of food exposures among ill and well persons who attended the Transplant Games in Orlando, Florida*

Exposure	Ill (N = 41), n (%)	Well (N = 176), n (%)	OR	95% CI	p value
Shredded iceberg lettuce	24 (59)	49 (28)	3.7	1.8–7.4	0.0002
Shredded cheddar cheese	22 (54)	50 (28)	2.9	1.5–59	0.002
Diced Roma tomatoes	18 (44)	27 (15)	4.3	2.1–9.1	<0.0001
Fresh ground beef	14 (34)	26 (15)	3.0	1.4–6.4	0.004
Pre-sliced beefsteak tomatoes	4 (10)	4 (2)	4.6	1.1–19.4	0.02
Frozen ground beef	12 (29)	31 (18)	1.9	0.9–4.2	0.09

*OR, odds ratio; CI, confidence interval.

strengthened our findings, the tomatoes tested were processed well after the outbreak period, when levels of contamination may have differed considerably.

As use of the Internet becomes more widespread for participation in regional, national, and international conferences, groups, and listservs, electronic mail cohorts are becoming more commonplace. The development of Web-based public health investigative tools can facilitate future investigations of outbreaks affecting geographically dispersed persons who may be part of an electronic mail cohort. A Web-based approach to data collection can also play a critical role in rapidly sharing data in outbreaks involving multiple jurisdictions (7). The growing use of Web-based technologies in public health investigations will have to be balanced with the need to protect the privacy of personal information in the online environment (8,9). The careful application of emerging technologies and conventional epidemiologic techniques can help public health officials effectively cope with the multitude of changing factors that shape public health in the United States.

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Dr. Srikantiah completed this work while she was an Epidemic Intelligence Service Officer assigned to the Foodborne and Diarrheal Diseases Branch at CDC. She is currently a clinical fellow in the Division of Infectious Diseases and a postdoctoral fellow at the Center for AIDS Prevention Studies at the University of California, San Francisco. Her primary research interest is in the epidemiology of HIV and tuberculosis coinfection in the developing world.

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Ross River Virus Disease Reemergence, Fiji, 2003–2004

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We report 2 clinically characteristic and serologically positive cases of Ross River virus infection in Canadian tourists who visited Fiji in late 2003 and early 2004. This report suggests that Ross River virus is once again circulating in Fiji, where it apparently disappeared after causing an epidemic in 1979 to 1980.

The growing appreciation of travelers as sentinels for the emergence of infectious diseases is based on the immunologic naiveté of travelers, their defined exposure in time and space, and sufficient diagnostic resources after their return to an industrialized country. Reports of infectious diseases in travelers in unusual numbers or from new geographic locations can inform a public health response. We report 2 recent apparent cases of Ross River virus disease ("epidemic polyarthritis") in Canadian travelers to Fiji, ≈1,000 miles from the region (Australia, New Guinea, and the Solomon Islands) where the virus is endemic, enzootic, and often epidemic (1).

Ross River virus, a mosquitoborne alphavirus in the family *Togaviridae*, is a single-stranded, enveloped RNA virus. Other viruses in this family include Chikungunya, o'nyong-nyong, Sindbis, and eastern and western equine encephalitis. In Australia, the major vectors of Ross River virus to humans are various *Culex* and *Aedes* mosquitoes. Marsupials (especially kangaroos and wallabies) are the most important vertebrate amplifying hosts (1). Several thousand cases of epidemic polyarthritis are reported annually in Australia, making Ross River virus the most important arboviral pathogen in that country (2,3). In 1979, Ross River virus spread dramatically to the South Pacific islands (probably imported by a viremic person arriving from Australia), including Fiji, American Samoa, Wallis and the Cook Islands, causing the largest Ross River virus epidemic ever recorded (4–8). In Fiji alone, ≈500,000 persons were infected, and nearly 50,000 of them became ill (4,7). The evidence suggests that *Aedes polynesiensis* was the primary vector and that human-mosquito-human transmission predominated without substantial involvement of nonhuman vertebrates in virus amplification (7). Once the epidemic ended, Ross River virus evidently disappeared from the region, possibly because of the lack of suitable marsupial reservoir hosts (7,8). In 1999, a suspected case of Ross River virus disease was reported in a German traveler returning from Fiji and Rarotonga in the Cook Islands (9). We present evidence for 2 cases of Ross River virus infection acquired in Fiji in late 2003 and early 2004.

Case 1

A 39-year-old Canadian man flew to Fiji on October 28, 2003, and returned to Canada on November 10, 2003. Immediately on arrival in Canada, he started experiencing generalized body aches, which lasted until November 17. On November 15, he noticed an erythematous maculopapular rash over his whole body, as well as inguinal lymphadenopathy. The rash and swollen nodes subsided on November 18 and were replaced with the sudden onset of pain and swelling in his right ankle joint and pain without swelling in his right knee and right elbow. Two days later, barely able to walk, the patient sought medical attention at the McGill Centre for Tropical Diseases. He denied any fever or chills and had no history of joint disease. An examination found substantial periarticular tenderness, warmth, erythema, and swelling of his right ankle with essentially full range of motion (Figure). His travel history included an uneventful 4-day trip to Melbourne, Australia, in 1999, involving a trip to the countryside, followed by a week in Bali. In view of his clinical symptoms and recent travel history, a preliminary diagnosis of Ross River virus disease was considered. Laboratory investigations included a complete blood count (CBC); urinalysis; and measurement of levels of liver enzymes, serum creatinine, uric acid, rheumatoid factor, antistreptolysin O, and anti-DNase B, all of which were normal. The erythrocyte sedimentation rate (ESR) was 23 mm/h, and the antinuclear antibody (ANA) test was positive with a speckled pattern. Serum specimens were collected from the patient on days 10, 21, and 141 after the onset of illness; they were screened for elevated immunoglobulin (Ig) M antibody against geographically relevant arboviral antigens by enzyme immunoassays (EIA), when EIA was available for a particular arbovirus. Positive Ross River virus IgM results were then confirmed by plaque reduction neutralization tests. All serologic tests were performed at the arboviral diseases laboratory of the Centers for Disease Control and Prevention (CDC) in Fort Collins, Colorado,

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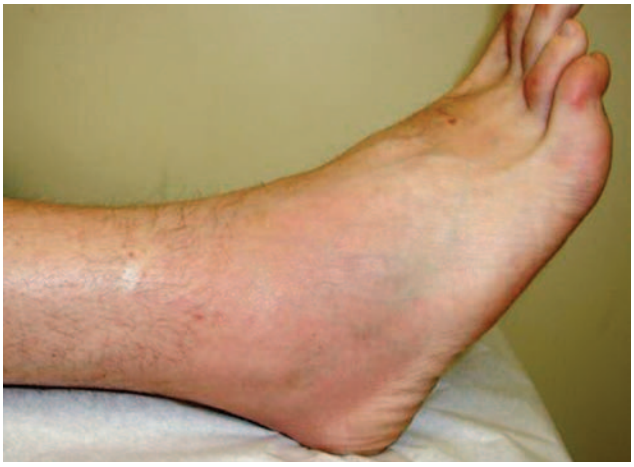

Figure. Patient 1: ankle swelling, pain, tenderness, erythema, and warmth on day 10 of illness.

as previously described (10,11). Results of tests for alphavirus antibodies are shown in the Table. Serologic evidence of a dengue infection was absent in both this patient and the patient described in the next section.

Case 2

On March 5, 2004, a 44-year-old Canadian woman returned to Canada after visiting New Zealand for 2 months and Fiji for 1 month; she did not travel through Australia or another known Ross River virus–endemic area. She had previously visited Fiji uneventfully in 1995. On March 14, she experienced the abrupt onset of fatigue; the next day she was feverish, nauseated, and anorexic and had severe arthralgia in her ankles and feet. For several days, she experienced extreme hypersensitivity to touch, particularly of her soles, severe enough to prevent weight bearing, and had mild ankle swelling. Her joint pains worsened over several days and spread to her knees, hips, and upper extremities. On March 16, she noticed a nonpruritic, erythematous, maculopapular rash, with small vesicular lesions on the palms, which involved the extremities and face but not the trunk; the rash resolved after 4 days. On March 17, she had normal CBC results and serum creatinine kinase level, mildly elevated liver enzymes, and an ESR of 62 mm/h. By day 10 of illness, she was able to resume limited sedentary work. One month after illness onset, fatigue and joint pain persisted, but physical examination results were normal, apart from difficulties in ambulation due to pain; tests for ANA and rheumatoid factor were negative, C-reactive protein level was normal, and ESR was 30 mm/h. Four months after illness onset, she continued to have gradually resolving arthralgia and fatigue that limited daily activities. At CDC, serologic tests were performed on serum specimens obtained on days 16 and 33 of illness (Table).

Conclusions

The clinical features and serologic results in these 2 cases provide strong circumstantial evidence for Ross River virus transmission in Fiji during late 2003 and early 2004, which suggests that heightened surveillance is needed as well as epidemiologic and ecologic studies in that region. While both cases were highly clinically compatible with epidemic polyarthritis, and tests for Ross River virus–specific serum IgM antibody were positive in both, the first case is the most convincing serologically because seroconversion (i.e., a 4-fold titer change) in neutralization tests was also observed. The subsequent decrease in this patient's Ross River virus–specific IgM reactivity and neutralizing antibody titer within a few months also argues for a recent Ross River virus infection. In the second case, the high but stable anti-Ross River virus neutralizing antibody titer may reflect the fact that the earliest sample available for testing was obtained >2 weeks after illness onset when the patient's anti–Ross River virus neutralizing antibody titer may have already peaked.

If Ross River virus was circulating in Fiji in 2003 and 2004, at least 2 basic hypotheses may explain its reemergence there. The first of these, which seems the most plausible, involves occasional reintroduction of this virus from the known disease-endemic region (e.g., by viremic persons arriving from Australia), sometimes resulting in local transmission, ultimately followed by local extinction. Circumstantial evidence to support this hypothesis includes the fact that, during the same period that the 2 patients described here traveled to Fiji, Australia was experiencing its usual summer surge in Ross River virus incidence (3). The second hypothesis, considered unlikely (7), is that Ross River virus became established in Fiji after the 1979–1980 epidemic but remained undetected while causing sporadic and largely unrecognized human cases. No recent serosurveys or other data are available to address this question.

The ability of arboviruses to be moved from one region to another, even from one continent or hemisphere to another, has long been appreciated (12). This occurrence may be more frequent than is apparent. Fortunately, the conditions for local transmission and long-term survival of an arbovirus in a new area are often highly complex, so that most such introductions are probably abortive. The recent introduction of West Nile virus to North America and its permanent establishment there, however, is a sobering demonstration that newly introduced arboviruses sometimes achieve long-term survival in new areas where preadapted vectors and suitable vertebrate amplifying hosts are available (13). Ross River virus is almost certainly imported into North America fairly frequently because this virus is endemic and often epidemic in Australia, human travel between Australia and North America is

Table. Results of tests of patients' serum for antibodies to selected alphaviruses*

Patient	Interval (d)§	IgM results†		PRNT titers‡		
		RRV	BFV	RRV	BFV	SINV
1	10	Positive	Negative	320	<10	<10
	21	Positive	Negative	1,280	<10	<10
	141	Equivocal	Negative	160	ND	ND
2	16	Positive	Negative	5,120	<10	ND
	33	Positive	Negative	5,120	<10	ND

*Ig, immunoglobulin; PRNT, plaque-reduction neutralization test; RRV, Ross River virus; BFV, Barmah Forest virus; SINV, Sindbis virus; ND, not done.
†IgM-capture enzyme immunoassay; samples tested at 1:400 dilution; positive samples had a positive-to-negative (P/N) absorbance ratio >3.0; equivocal samples had a P/N ratio 2.0–3.0 (10); no test for anti-SINV IgM was available.
‡90% plaque-reduction endpoints; >10 is considered positive (11).
§From onset of illness to serum collection.

frequent, high levels of viremia lasting several days often develop in Ross River virus–infected persons, and cases of Ross River virus disease among visitors to Australia are commonly reported (14,15). Notably, ≈100 viremic travelers enter New Zealand every year from Queensland alone (16). Fortunately, however, to date all such importations into North America evidently have been abortive, and if an introduction of Ross River virus to North America should ever result in local amplification and transmission by preadapted vectors (e.g., *Ae. aegypti* or *Ae. albopictus* [8], activity would probably be short-lived and remain localized, and a lack of optimal vertebrate reservoirs would probably keep the virus from becoming established.

The recent North American experience with West Nile virus, however, emphasizes how uncertain such predictions can be. Therefore, travel medicine specialists and other healthcare providers in North America (and other disease-nonendemic areas) should be familiar with the clinical features of Ross River virus disease, as well as its potential public health importance, and realize that diagnostic tests for this infection currently are available at only a few public health reference laboratories (e.g., CDC).

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Dr. Klapsing is an internal medicine resident at the Klinikum Stuttgart, Stuttgart, Germany. He was on rotation with the Rheumatology Department of the McGill University Hospital Centre, Montreal, at the time of this investigation. His interests are in outcome research.

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Mycobacteria in Nail Salon Whirlpool Footbaths, California

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In 2000, an outbreak of *Mycobacterium fortuitum* furunculosis affected customers using whirlpool footbaths at a nail salon. We swabbed 30 footbaths in 18 nail salons from 5 California counties and found mycobacteria in 29 (97%); *M. fortuitum* was the most common. Mycobacteria may pose an infectious risk for pedicure customers.

In October 2000, we investigated the first known outbreak of *Mycobacterium fortuitum* cutaneous infections acquired from whirlpool footbaths, also called footspas, at a nail salon in northern California (1). Over 100 pedicure customers had prolonged boils on the lower legs that left scars when healed (1,2). In the investigation, we swabbed the area behind the screen of the recirculation inlet in each of 10 footspas at the nail salon and recovered strains of *M. fortuitum* from all 10. Isolates from 3 footbaths and 14 patients were indistinguishable by pulsed-field gel electrophoresis and by multilocus enzyme electrophoresis (1).

Before this outbreak, *M. fortuitum* and other rapidly growing mycobacteria (RGM) caused localized cutaneous infections but usually in a healthcare-associated setting with surgical or clinical devices contaminated with water from the hospital or from the municipal water system (3). In the nail salon outbreak, we suspected that the mycobacteria entered the footspas through the municipal tap water and thrived in the large amount of organic debris accumulated behind the footspa recirculation screens. However, cultures of tap water at that nail salon later in the investigation yielded RGM in the *M. chelonae-abscessus* group but not *M. fortuitum* (1).

Since RGM are commonly found in municipal water systems (4–6), and since the nail care business is a $6 billion and growing industry in this country (7), we hypothesized that similar whirlpool footbath–associated RGM infections occurred sporadically but went unnoticed. Soon after we alerted the health communities to this outbreak, 3 cases of lower extremity RGM infections associated with 2 different nail salons were documented from southern California (8).

No study has been published on the prevalence of mycobacteria in whirlpool footbaths. To determine the prevalence of nontuberculous mycobacteria in this common nail salon equipment, we undertook a mycobacteriologic survey of footspas in nail salons in California from November to December 2000.

The Study

Five large counties from different parts of California (Alameda, Sacramento, Orange, Riverside, and San Diego) participated in the survey. Counties chosen served large populations and had multiple nail salons with whirlpool footbaths. In each county, a team including the regional investigator of the California Bureau of Barbering and Cosmetology and a local public health professional visited selected nail salons. They assessed footspa equipment, cleaning solutions, and cleaning techniques and frequencies. Swab samples were also collected.

In each participating county, a convenience sample of ≥3 different nail salons equipped with whirlpool footbaths located in the town's main business section was randomly selected for the survey. Salon managers were questioned about cleaning and disinfection regimens of their footspas. Pedicure equipment time in service within the salon and make and model numbers of whirlpool pedicure equipment were noted. For each salon, 2 separate footspas were sampled, unless that salon only had 1 footspa, in which case only 1 swab was collected. Using a screwdriver, investigators removed the grate or filter screen covering the recirculation port in each footspa basin and inspected the area behind the screen for debris. A sterile, cotton-tipped culturette was used to swab this area and placed in standard transport medium.

At the California Microbial Disease Laboratory, each swab was removed from the transport medium, placed into a 50-mL tube containing 5 mL of sterile water, and the contents vortexed. The swab was then removed from the tube, and the remaining suspension was decontaminated with an equal volume of N-acetyl-L-cysteine-sodium hydroxide for 15 minutes, followed by neutralization with phosphate buffer and concentration by centrifugation (9). The sediment was spread onto Middlebrook 7H10 and Middlebrook 7H11/Mitchison 7H11 selective agar plates, Lowenstein-Jensen slants, Bactec 12B, and Bactec Mycobacteria Growth Indicator Tube 960 system (Becton-Dickinson, Sparks, MD, USA) liquid media.

The mycobacteria isolated were identified by ≥1 of the following methods: rapid DNA probes using nucleic acid hybridization (10), high performance liquid chromatography that produces mycolic acid patterns (11), and biochemical tests (9). *M. simiae* and *M. lentiflavum* were

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differentiated by urease activity and photochomogenicity; for both of these, *M. simiae* is positive and *M. lentiflavum* is negative (12). *M. smegmatis* group organisms were not differentiated to the species level. *M. mageritense* was identified by polymerase chain reaction restriction analysis at a Mayo Clinic laboratory and by DNA sequencing at the University of Texas Health Center in Tyler.

Thirty-one swabs were collected from 30 whirlpool footbaths in 18 nail salons from the 5 California counties. Twelve salons had ≥2 footspas; 6 had only 1 footspa. Of these 30 footspas, nontuberculous mycobacteria were cultured from 29 (97%). From 15 (50%), >1 mycobacterium species were isolated. No mycobacteria or other acid-fast organisms were isolated from 1 footspa that had only been in use for 11 days, whereas the positive footbaths had been in use for an average of 22 months (range 3–84 months).

Isolated from the whirlpool footbaths were 10 species of mycobacteria, 6 of which were RGM: *M. fortuitum*, *M. mucogenicum*, *M. smegmatis* group, *M. mageritense*, *M. neoaurum*-like RGM, and a pigmented unidentified nontuberculous mycobacterium (Table). *M. fortuitum* was the most frequently isolated mycobacterium, found in 14 (47%) of the 30 footspas surveyed and from all 5 counties. Rapid growers, including *M. fortuitum*, were found in 23 (76%) of the footspas. Slow-growing mycobacteria species were also recovered, including *M. avium* complex, *M. gordonae*, *M. simiae*, and *M. lentiflavum*. These species were less frequent than the rapid growers, except for *M. avium* complex, which was found in 5 (17%) of the footspas.

Mycobacterial species vary in their ability to survive the selective NaOH decontamination step that was used in this study (13). Some solid media cultures that grew *M. fortuitum* had only a few colonies of this species; others had nearly confluent growth; and still other cultures grew in broth only, not solid media, making it impossible to determine the quantity of growth. For these reasons, quantitative information about the number of colonies present on solid media is not reported.

The whirlpool footbaths sampled came from 3 manufacturers. Disinfectants reportedly used included a variety of brand name products and chlorine bleach, used at intervals of 1 to 14 days. Five (17%) footspas reportedly did not go through any disinfectant process. Twenty-five (83%) of the surveyed footbaths had collected visible debris or slime behind the recirculation screen cover, either on the screen itself, on the tub surface, or both. Fifteen (50%) of footspa operators reported never having cleaned behind this screen. One footspa had no screen or visible debris; nevertheless, it tested positive for mycobacteria.

Table. Mycobacteria, by species, isolated from 30 whirlpool footbaths in 18 nail salons, California, 2000

Mycobacteria	N (%) of spas
*Mycobacterium fortuitum**	14 (47)
*M. mucogenicum**	7 (23)
*M. mageritense**	6 (20)
M. avium complex	5 (17)
M. smegmatis group*	4 (13)
UNTM*	3 (10)
M. simiae	3 (10)
M. gordonae	2 (7)
M. neoaurum-like*	2 (7)
M. lentiflavum	2 (7)

*Rapid growers. UNTM, unidentified nontuberculosis mycobacteria.

Conclusions

Mycobacteria were isolated from virtually all pedicure spas surveyed, the sole exception being the footspa that had only been in service for 11 days. Mycobacteria were recovered whether or not disinfectants were reportedly used and whether or not debris was visible behind the recirculation screen.

RGM, *M. fortuitum* in particular, were the most frequently isolated mycobacteria. Our survey suggests that potentially pathogenic mycobacteria are widespread in these footspas across California. These organisms most likely were introduced into the footspas through the municipal water supply, where they colonized parts of the spas and probably the plumbing. Given that these whirlpool footbaths are widespread in California but similar infections known to date are rare, the presence of such mycobacteria alone may not be sufficient to cause pedicure customers to get cutaneous infections from using these spas. Our 2000 outbreak investigation noted an unusually large amount of debris behind the footspa recirculation screens, which might have provided a niche for mycobacteria to colonize and proliferate to large numbers. In that outbreak, customers who shaved their legs before using these implicated footspas were at higher risk for furunculosis than those who did not (1). However, some customers in that outbreak were infected even though they reportedly did not shave their legs before using the pedicure spas. Thus, while we documented the widespread presence of potentially pathogenic mycobacteria in footspas, the risk for infection remains unclear.

A limitation of this study is our inability to quantify the risk for cutaneous infection to pedicure customers despite finding widespread presence of RGM. We could not quantify reliably the amount of mycobacteria in each footspa with a positive culture. Furthermore, what we found in these footbaths may not be representative of other California counties or other states.

Nonetheless, our findings document the ubiquitous presence of potentially pathogenic mycobacteria among footspas of nail salons in California. The 2000 outbreak might have been a warning of what can happen again if this emerging infection is not adequately addressed. In 2004, a case report documented 2 cases of *M. mageritense*

furunculosis associated with using footbaths at a nail salon in Georgia (14).

The California Board of Barbering and Cosmetology adopted new regulations in May 2001 requiring nail salons to follow specific cleaning and disinfection procedures to ensure that their footspa equipment is properly cleaned and maintained (15). Since our survey was conducted before these new regulations were implemented, further monitoring and research are needed to determine whether complying with the regulations will decrease the potential risk for mycobacterial cutaneous infections among pedicure customers.

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Coxiella burnetii in Bulk Tank Milk Samples, United States

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Dairy cattle are a primary reservoir of *Coxiella burnetii*, which causes Q fever. However, no recent nationwide studies have assessed the prevalence and risks of Q fever in dairy cattle. We report ≥94% prevalence in samples of bulk tank milk from U.S. dairy herds tested during the past 3 years.

Q fever is a ubiquitous zoonosis caused by *Coxiella burnetii*, an obligate intracellular rickettsial organism. Since the first independent reports by Australian and American investigators in 1935, Q fever has been found throughout the world, except New Zealand (1). *C. burnetii* infections have been reported in humans, farm animals, pet animals, wild animals, and arthropods (2). Among farm animals, dairy cattle, sheep, and goats are the major reservoirs of *C. burnetii*. Animals are often naturally infected but usually do not show typical symptoms of *C. burnetii* infection. Clinical signs of *C. burnetii* infection are abortion in sheep and goats and reproductive disorders in cattle (1,3). *C. burnetii* can be isolated from the blood, lungs, spleen, and liver of infected animals in the acute phase of the disease. The uterus and mammary glands are primary sites of infection in the chronic phase of *C. burnetii*. Shedding of *C. burnetii* into the environment occurs mainly during parturition by birth products, particularly the placenta of sheep. Also, shedding of *C. burnetii* in milk by infected dairy cattle is well documented (1,3).

Previous studies on the prevalence of *C. burnetii* in dairy cattle were based mainly on serologic tests, including complement fixation, indirect immunofluorescent assay (IFA), and enzyme-linked immunosorbent assay (ELISA). However, the seroprevalence of *C. burnetii* infection in cattle varies widely from 1 country to another and from 1 state to another in the United States. In Japan, a prevalence of 1.1% to 3.9% of *C. burnetii* infection in cattle was reported in the 1950s. However, a 1992 survey reported that 29.5% of healthy cattle and 84.3% of cattle with reproductive disorders in Japan had antibodies to *C. burnetii* shown by using IFA (4). In Canada, 67% of the 200 dairy herds were ELISA-positive for antibodies to *C. burnetii* (5). The reported seroprevalence of Q fever in the United States varies from 1% to 73%. Reports from the same state show wide differences depending on testing methods and the year of surveys; for example, the seroprevalence in Wisconsin was 33% in 1957 but 73% in 1962 (6). Seroepidemiologic studies have indicated that *C. burnetii* antibody seroprevalence in cattle has increased from the prevalence 20 or 30 years ago (7). However, the real prevalence of *C. burnetii* infection in cattle is not available, due in part to the lack of surveillance (8). Shedding of *C. burnetii* in milk by infected cattle was shown in studies conducted during the 1940s and 1950s.

Isolation of the Q fever agent by laboratory workers is difficult because the agent has a high infectivity rate, it is cumbersome in in vitro culture conditions, and handling it requires rigorous compliance requirements. Q fever is considered a "select agent" because it can potentially be used in bioterrorism and its handling is federally regulated. Recently, polymerase chain reaction (PCR) assays have been used to detect *C. burnetii* (9). A trans-PCR assay was implemented to detect *C. burnetii* in milk by targeting a transposon-like sequence found only in *C. burnetii* (10). The trans-PCR assay detects *C. burnetii* in samples immediately, unlike serologic assays that detect antibodies that could have been introduced months earlier.

A real-time PCR assay targeting IS*1111* was developed in this study to measure amounts of *C. burnetii* shed in milk. Our study was to assess the prevalence of *C. burnetii* in bulk milk samples from dairy herds in the United States by using PCR.

The Study

The samples in this study were somatic cells extracted from bulk tank milk aliquots submitted to the New York State Animal Health Diagnostic Laboratory to detect bovine viral diarrhea that persistently infected lactating dairy cattle. The samples tested do not represent a random sampling, as tests were done only on samples available. The samples are heavily weighted to the Northeast, but some are from the Midwest and West. We tested 316 bulk tank milk samples from dairy herds in the United States during a 3-year period from January 2001 to December 2003 by using trans-PCR (Figure). Positive results were confirmed by nested PCR and DNA sequencing. The sequencing results of the 687-bp PCR product were consistent with the published sequence of IS*1111* with 100% homology. A summary of the PCR test results of the bulk tank samples is shown in Table 1. The overall prevalence of *C. burnetii* in the tested samples was 94.3% with little variation (93.2% to 94.7%) from year to year. Samples from New York State did not show significant variation from other states, which indicates that *C. burnetii* infection in the dairy herds was persistent or steady, with little tem-

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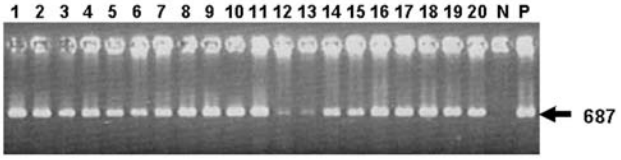

Figure. Agarose gel electrophoresis of *Coxiella burnetii* Trans-polymerase chain reaction products amplified from total DNA of bulk tank milk samples. Lanes 1 to 20, bulk tank milk samples; N, water negative control; P, positive control (DNA of Nine Mile strain). The arrow indicates the amplification of a 687-bp fragment in the IS*1111* sequence of *C. burnetii*.

poral or regional variations. Milk samples were collected 6 times from 2002 to 2004 from specific cattle in a small, *C. burnetii*–positive dairy to monitor the infection in specific cattle. A summary of the results of tests on milk samples from specific cattle is presented in Table 2. While 28 (52.8%) of 53 cattle were *C. burnetii* positive in 2002, 23.5% and 31.3% of the cattle were positive in 2003 and 2004, respectively. Daily and weekly shedding levels of 5 *C. burnetii*– positive cattle were assayed by real-time PCR. Real-time PCR was conducted by using the primers and probe designed by the Primer Express program (Applied Biosystems, Foster City, CA). The primer set consisted of primers trans-f (5′-GGGTAAAACGGTGGAACA ACA-3′) and trans-r (5′-ACAACCCCCGAATCTCATTG-3′). The internal probe trans-p (5′-AACGATCGCGTATCTT-TAACAGCGCTTG-3′) was labeled with the reporter dye 5-carboxyfluoroscein (FAM) on the 5′ end and the quencher dye N′, N′, N′, N′-tetramethyl-6-carboxyrhodamine (TAMRA) at the 3′ end. The reactions and assay conditions were according to the manufacturer's instructions (Applied Biosystems). Each cattle shed a similar amount of *C. burnetii* daily over 7 days; weekly shedding over 4 weeks was also similar. Shedding levels of positive cattle were estimated to be 10^1–10^4 cells/mL each. The bulk tank milk samples of the herd stayed at a level of 10^2 cells/mL over 3 years.

Conclusions

Bulk tank milk has been used for surveillance samples in dairy herds for several bovine diseases including bovine viral diarrhea. More than 90% of U.S. dairy herds sampled were infected with *C. burnetii* based on bulk tank milk testing over a 3-year period. This high prevalence did not show temporal or regional variation, suggesting that *C. burnetii* infections in dairy herds are common throughout the United States. Our report of *C. burnetii* in dairy herds is not surprising if earlier reports regarding an increase of bovine infection in North America are considered. An early investigator concluded that *C. burnetii* was endemic throughout the United States in the 1950s and predicted that a high bovine infection rate could occur in other parts of the country, as it did in southern California where a 98% infection rate was reported (11). A California survey reported that 20 (100%) of 20 herds in 17 counties throughout the state contained seropositive cattle, and 82% of 1,052 specific cattle from the herds were seropositive (12). An increase in the prevalence of Q fever from 2.3% in 1964 to 66.8% in 1984 was reported in Ontario dairy herds (13). Our study found a notable decrease in shedding of *C. burnetii* in milk by specific cattle from 52.8% to 23.5% over 3 years (Table 2). This decrease was not because animals stopped shedding the organism but because uninfected animals replaced shedding animals with an average annual replacement rate of 30%. With the exception of the first year of our study, the shedding rate appeared to be steady at 20% to 30% over 2 years. A similar shedding rate was found in a California study; 23% of 840 cattle were shedding *C. burnetii* in their milk (13). Continual daily and weekly shedding in the milk by the infected cattle suggests chronic infection by *C. burnetii*. Chronic *C. burnetii* infection of dairy cattle could be the most important source of human infection simply based on sheer numbers (1). Extrapolation of our data to the national dairy herd suggests that nearly 3 million lactating cattle are shedding *C. burnetti* daily. Though the mode and extent of transmission from bovine to human has not been determined, epidemiologic studies indicate that Q fever

Table 1. Prevalence of Q fever in bulk tank milk of U.S. dairy herds (2001–2003)

Year	State	No. of samples	Positive (%)	Negative (%)
2001	New York State	20	18 (90.0)	2 (10.0)
	Other states*	24	23 (95.8)	1 (4.2)
	Subtotal	44	41 (93.2)	3 (6.8)
2002	New York State	61	58 (95.1)	3 (4.9)
	Other states*	60	56 (93.3)	4 (6.7)
	Subtotal	121	114 (94.2)	7 (5.8)
2003	New York State	43	40 (93.0)	3 (7.0)
	Other states*	108	103 (95.4)	5 (4.6)
	Subtotal	151	143 (94.7)	8 (5.3)
	Total	316	298 (94.3)	18 (5.7)

*CA, CT, CO, IL, IN, MA, MD, MI, MN, MO, NC, OH, PA, UT, VA, VT, WA, WI.

Table 2. Q fever infection rate of specific cows in a bulk tank–positive dairy herd based on *Coxiella burnetii* shedding in their milk over 3 years (2002–2004)

Month/year collected	No. of samples	Positive (%)	Negative (%)
Jul 2002	53	28 (52.8)	25 (52.8)
Aug 2002	53	28 (52.8)	25 (52.8)
Sep 2003	51	16 (31.3)	35 (68.6)
Dec 2003	48	14 (29.2)	34 (70.8)
Feb 2004	52	13 (25.0)	39 (75.0)
Jul 2004	34	8 (23.5)	26 (76.5)

develops in farmers, veterinarians, and slaughterhouse workers who are in contact with domestic animals (14). While infection from commercial milk is unlikely because of the pasteurization process, ingestion of raw milk has been linked to higher seroprevalence rates.

In the aftermath of September 11, 2001, and the anthrax incidents, the use of biologic warfare is no longer a distant possibility. *C. burnetii* is considered a potential bioterrorism agent because of its high infectivity (a single organism may cause disease in human), its ease of dispersion in aerosols (because of its small, sporelike structure), and its resistance to extreme environmental conditions and chemicals (15). Therefore, further investigations are needed to determine the implications of the high prevalence of *C. burnetii* in dairy herds, to address the potential risk to public health, and to be prepared for outbreaks and bioterrorism events. Currently, no commercial vaccines are available for cattle, and no effective treatment protocol exists for infected animals.

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Human Spotted Fever Rickettsial Infections

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Serum specimens from patients at 4 sites in Peru were tested for evidence of spotted fever group rickettsial infection. Results showed that 30 (18%) of 170 patients had spotted fever group rickettsial infections, which likely caused their illnesses. These findings document laboratory-confirmed spotted fever from diverse areas of Peru.

Rickettsial spotted fever was first described in South America in 1931 in Sao Paulo, Brazil (1). The etiologic agent, *Rickettsia rickettsii*, and the tick vector, *Amblyomma cajenennse* (the Cayenne tick), were subsequently identified. Serologic evidence of *R. rickettsii* infections has been documented in several countries in South and central America, including Argentina (2), Brazil and Uruguay (3), Colombia (4), Costa Rica (5), Panama (6), and Mexico (7). A recent study documented for the first time serologic evidence for spotted fever group (SFG) *Rickettsia* infections in 1 region of northern Peru (8). We describe serologic evidence of SFG rickettsial infections in diverse areas of Peru, including laboratory-confirmed infections among patients with clinical febrile disease.

The Study

Serum samples were obtained from 4 areas in Peru: Chiclayito and Salitral (Piura Department); La Merced (Junin Department); and Cusco (Cusco Department) (Figure). Chiclayito is a small village (population 6,133) ≈30 m above sea level on the outskirts of the city of Piura in the northern coastal desert. Salitral is a small rural village (population 1,503) ≈162 m above sea level in a more temperate region of the Salitral District (Morropon Province, Piura Department) ≈3 h by car from Chiclayito. La Merced is the capital of the Chanchamayo District (Chanchamayo Province, Junín Department) and located ≈751 m above sea level ≈350 km east of the Peruvian capital city of Lima, on the eastern side of the Andes. The district has a population of 31,000; approximately half live in La Merced. Cusco (population 260,000) is located ≈3,350 m above sea level in the southern Peruvian Andes 1,089 km southeast of Lima.

Sera from patients representing the 4 surveillance sites were tested for antibodies against SFG rickettsiae after written informed consent was provided by each patient (Department of Defense Institutional Review Board No. 31535). Patients enrolled had a fever ≥38ºC and at least 2 other signs or symptoms including headache, myalgia, arthralgia, rash, and bleeding. Patients with a positive blood film for malarial parasites or obvious disease such as diarrhea or upper respiratory illness were excluded.

Paired (acute- and convalescent-phase) patient serum samples were evaluated for immunoglobulin (Ig) G antibodies reactive with *R. rickettsii* antigen by either an indirect immunofluorescence assay (IFA) or enzyme immunoassay (EIA). Serum specimens were also tested by IFA for typhus group rickettsial antibodies and were uniformly negative. IFA analysis was conducted according to directions provided by the manufacturer (PanBio, INDX, Inc., Baltimore, MD, USA). Endpoint titers were recorded as the reciprocal of the last dilution exhibiting specific fluorescence. Titers ≥1:64 were considered positive. Patients with confirmed spotted fever were those who showed a ≥4-fold increase in *R. rickettsii* IgG titer from acute phase to convalescent phase of illness.

The EIA was conducted by using a 4-step indirect immunoassay to detect *R. rickettsii* IgG, as described (8). A positive serum dilution exceeded the mean plus 3 standard deviations between the absorbance of *R. rickettsii* antigen and the negative control antigen of 5 control serum specimens. Serum samples were titrated to endpoint and the highest dilution found positive was recoded as the *R. rickettsii* IgG titer. Serum from a serologically confirmed case-patient showed a ≥4-fold increase in antibody titer from the acute to the convalescent phase.

A total of 170 patients, 50 from Chiclayito and the Salitral Health Centers (Piura Department), 67 from Cusco Hospital (Cusco Department), and 53 from La Merced Hospital (Junin Department), were tested for antibodies to SFG rickettsiae. IFA testing was done at the Peruvian National Institute of Health, while EIAs were conducted at Naval Medical Research Center Detachment. Not all patients were tested by both assays (Table 1). Of the 170 patients tested, 30 (18%) yielded results that suggested that SFG rickettsial infections were the most likely cause of their illnesses (Table 1). Patients from all 4 study sites in 3 departments of Peru had evidence of SFG rickettsiae infections as the cause of illness. Frequencies of confirmed patients in the 3 departments did not differ significantly

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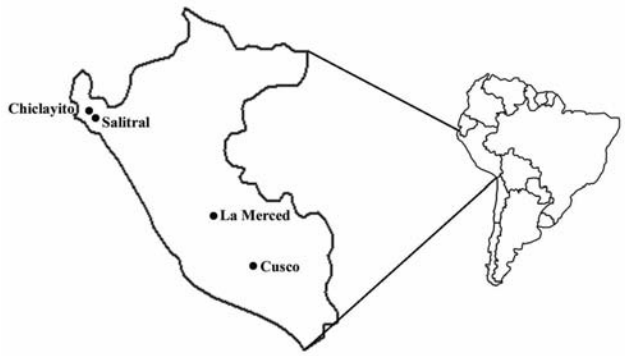

Figure. Four study sites in Peru surveyed for human spotted fever rickettsial infections.

($p > 0.52$). Table 2 shows the frequencies of spotted fever by age and sex for the 164 patients for whom data were recorded. Age groups did not differ significantly ($p > 0.5$). The frequency of spotted fever was 27% in female patients and 10% in male patients ($p < 0.005$).

The signs and symptoms of patients with confirmed spotted fever who came to the treatment facility included fever and malaise (100%), chills (94%), weakness (94%), shortness of breath (94%), prostration (81%), arthralgia (62%), abdominal pain (62%), cough (56%), nausea (56%), and runny nose (56%). None of the patients died, and most patients had a relatively mild febrile illness. There were no clear clinical differences in patients with confirmed cases of spotted fever compared with febrile patients who did not have spotted fever.

Evidence of SFG rickettsial infection was observed in samples taken from febrile patients in Cusco, Junin, and Piura departments. The etiologic agent or agents responsible for the spotted fever illnesses remain unknown. Appropriate samples from these patients were not available for isolation or molecular identification by a polymerase chain reaction.

Conclusions

Host inflammation may partly contribute to the pathogenic sequelae with intra-endothelial cell infection in more severe SFG infection (9). Patients infected with *R. akari* typically experience a mild and or asymptomatic disease characterized by low-grade fever, sweats, headache, and a vesicular eruption over the trunk and extremities (10). *R. akari* is maintained transovarially in the mite vector and transmitted to humans by the house mouse mite (*Liponyssoides sanguineus*). Infections have generally been reported among higher risk populations such as intravenous drug users (11), or within the densely populated inner city (12). Less is known about the susceptibility of rural agrarian populations. The concentration of humans in close proximity to house mice and their mites are factors that could contribute to an increase in rickettsialpox in the region. Sporadic cases of rickettsialpox may be confused with chickenpox, a common illness associated vesicular rash. However, none of the confirmed SFG rickettsia-infected patients had vesicular rashes typical of rickettsialpox.

Cat flea typhus, caused by *R. felis*, is a mild disease similar to murine typhus (13). Typical clinical findings include fever, headache, and occasional rash. The clinical manifestations of patients infected with SFG rickettsiae are similar to those described for cat flea typhus. However, recent discoveries of novel rickettsioses caused by distinct SFG rickettsiae in Europe, Africa, Australia, Asia, and North America during the last 25 years (14,15) suggest that the infections reported in this study may be the results of a novel SFG rickettsial agent. Future work is needed to identify the agent involved and to clearly link clinical signs and symptoms with diagnoses.

The higher frequency of cases in women suggests occupational exposure since in these areas of Peru women are generally more involved with domestic activities near the home. Possibilities for increased exposure of women may include more frequent work in the fields, thus exposing them to arthropod vectors; closer contact with domestic animals that may be involved in maintaining the SFG rickettsial agent (although no evidence was collected to support this); or exposure to house mouse mites in the home. Serologic evidence suggests that SFG rickettsiae were responsible for causing febrile illnesses in these 4 study sites of Peru, which demonstrates that SFG rickettsia result in human disease in Peru. Further studies are needed to document the species of SFG rickettsiae and to determine the vectors of these rickettsial infections. In addition, epi-

Table 1. EIA and IFA test results for antibodies to *Rickettsia rickettsii* among patients from 4 areas of Peru*

	EIA		IFA		EIA and/or IFA	
Department	No. tested†	No. positive (%)‡	No. tested†	No. positive (%)§	No. tested†	No. positive (%)
Cusco	36	6 (17)	56	11 (20)	67	16 (24)
Junin	42	8 (19)	19	2 (11)	53	10 (19)
Piura	50	4 (8)	0		50	4 (8)
Total	128	18 (14)	75	13 (17)	170	30 (18)

*By enzyme immunoassay (EIA) or indirect immunofluorescence antibody assay (IFA).
†Febrile patients from whom acute- and convalescent-phase serum specimens were available for testing.
‡Criterion for confirmation was a ≥4-fold increase in *R. rickettsii* immunoglobulin (Ig) G antibody titer from acute to convalescent phase of illness by EIA.
§Criterion for confirmation was a ≥4-fold increase in *R. rickettsii* IgG antibody titer from acute to convalescent phase of illness by IFA.

Table 2. Spotted fever frequency by age and sex

	Male patients			Female patients		
Age (y)	No. positive	No. tested	% positive	No. positive	No. tested	% positive
5–10	2	21	11	3	14	21
11–15	2	16	14	3	12	25
16–20	2	17	13	3	10	30
21–30	1	17	6	7	23	30
>30	2	19	12	4	15	27
Total	9	90	10	20	74	27

demiologic studies are needed to identify the risk factors, document the clinical spectrum, and suggest public health recommendations for prevention.

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Patient Contact Recall after SARS Exposure

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We reinterviewed healthcare workers who had been exposed to a patient with severe acute respiratory syndrome (SARS) in an intensive care unit to evaluate the effect of time on recall reliability and willingness to report contact activities and infection control precautions. Healthcare workers reliably recalled events 6 months after exposure.

Severe acute respiratory syndrome (SARS) quickly spread within hospitals after it was first identified in Toronto, Canada, in March 2003. Healthcare workers who cared for severely ill patients with SARS were at high risk of acquiring an infection (1).

Risk factors associated with SARS transmission have been assessed by using retrospective data from medical chart reviews and healthcare worker interviews (2–4). Infection control practitioners routinely use this method to determine the degree of exposure to communicable diseases in hospitals, but its reliability and validity are unknown. To better understand the impact of time on recall reliability and healthcare workers' willingness to report infection control breaches, we reinterviewed a cohort of healthcare workers who had been exposed to a patient with SARS and who had previously been studied (3).

The Study

During the first Toronto SARS outbreak in March 2003, 69 healthcare workers at risk for SARS were interviewed a median of 1.2 months (range 1 to 1.5 months) after exposure (3). Five months (range 4.8 to 5.3 months) after participating in this initial study, 30 of these healthcare workers were asked to participate in another study. These workers were eligible for participation in this second investigation because they had entered the index patient's room from 24 hours before intubation to 4 hours after intubation. Both investigations involved telephone or face-to-face interviews to determine the amount of time the worker had spent in contact with the patient, the activities that had occurred while the worker was in the patient's room, and the personal protective equipment used by the worker. The second questionnaire was more detailed than the first but contained a substantial number of questions that were identical to those in the first questionnaire.

Responses to identical questions in the initial and follow-up interviews were compared and expressed as proportions. Responses obtained during the initial interview were considered the reference standard for comparison with follow-up interview responses. Agreement between the initial and follow-up responses was quantified by using the kappa statistic and confidence intervals. The kappa statistic (κ) is a commonly used measurement of agreement or repeatability in epidemiologic studies. Kappa values from 0.20 to 0.39 indicated fair agreement, values from 0.40 to 0.59 indicated moderate agreement, values from 0.60 to 0.79 indicated good agreement, and values >0.80 indicated excellent agreement (5).

Twenty-seven of the 30 eligible healthcare workers agreed to the second interview (Table 1). The proportion of healthcare workers who reported the same exposure in the follow-up interview as during the initial interview was >80% for most respiratory and airway management activities and >90% for procedures such as vascular catheter insertion. However, the proportion of similar responses was lower for routine patient care activities such as bedding change (67%) and nebulizer treatments (70%) (Table 2).

Agreement between initial and follow-up responses was high for most respiratory and airway management activities, including suctioning after intubation ($\kappa = 0.63$), manipulation of oxygen face mask or tubing ($\kappa = 0.70$), manual ventilation ($\kappa = 0.63$), and mechanical ventilation ($\kappa = 0.70$). Agreement was fair to moderate for the following respiratory procedures: intubation ($\kappa = 0.46$), suctioning before intubation ($\kappa = 0.34$), and patient coughing while the healthcare worker was in the room ($\kappa = 0.38$). Agreement was high for routine patient care activities, including emptying urinary catheter collection bag or collecting urine sample ($\kappa = 0.63$), bathing the patient ($\kappa = 0.87$), and performing oral care or obtaining nasal swabs ($\kappa = 0.71$). Agreement was also high for inserting an arterial line ($\kappa = 0.75$) and for cleaning the patient's room ($\kappa = 0.65$).

Healthcare workers were asked during both interviews to estimate whether they had spent >10 minutes, >30 minutes, or >4 hours in the patient's room. Twenty-two (88%) of the 25 healthcare workers that participated in both interviews provided the same estimates of exposure duration. Two healthcare workers overestimated and 1 underestimated the

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Table 1. Characteristics of healthcare workers and severe acute respiratory syndrome (SARS) index patient contact

Characteristics*	No. (%) of healthcare workers (N = 27)
Demographic characteristics	
Age group (y)	
20–29	7 (26)
30–39	9 (33)
40–49	8 (30)
≥50	3 (11)
Sex: female	18 (67)
Occupation	
Nurse	10 (37)
Respiratory therapist	8 (30)
Other†	9 (33)
No. with laboratory-confirmed SARS	3 (11)
Contact characteristics	
Cumulative time spent in patient's room‡	
≤10 min	9 (35)
11–30 min	8 (31)
31 min–4 h	6 (23)
>4 h	3 (11)
Touched patient	16 (59)
Face ≤3 feet of patient	18 (67)
Contact with mucous membranes or respiratory secretions	7 (26)
Patient coughing or spitting while healthcare worker was present	11 (41)
Performed or assisted with intubation	2 (7)

*Characteristics ascertained from initial interview.
†Other occupation categories include service assistants, residents, 1 physician, and 1 pharmacist.
‡Based on 26 healthcare worker responses.

time spent in the patient's room. Kappa values ($\kappa = 0.52$) did not vary according to the duration of exposure.

Relative to their initial responses, on follow-up, healthcare workers tended to overestimate their presence in the patient's room during respiratory and airway management activities, particularly nebulization therapy. However, during the second interview, they were less likely to report being in the room while a bi-level positive air pressure unit was being used or while bedding was being changed. The rates of overestimated responses versus underestimated responses for other patient care activities were similar (Table 2). Healthcare workers who subsequently developed cases of laboratory–confirmed SARS were not more or less likely to remember their presence or absence during patient care activities (data not shown).

In the hospital, use of additional precautions (gown, gloves, and surgical masks for room entry) for methicillin-resistant *Staphylococcus aureus* was practiced by the healthcare workers (6). Compliance varied among healthcare workers, but the proportion of workers with the same response during the follow-up interview was >80% for all infection control precautions, except wearing a gown (76%, data not shown). In general, responses in the 2 interviews showed little variation in infection control precautions.

Conclusions

Our results indicate that healthcare workers in this study reliably recalled contact practices, patient care activities, and infection control precautions 5 months after their initial interview and 6 months after exposure to a patient with SARS. The proportion of identical follow-up responses averaged >85% for contact practices, patient care activities, and infection control precautions. Agreement between initial and follow-up responses was good to excellent for most respiratory practices and airway management activities, routine patient care activities, and other medical procedures.

The lowest proportion of identical responses observed on the initial and follow-up interview was for being in the patient's room while the patient was coughing or spitting (59%), with a kappa value (0.38) indicating fair agreement. The risk of droplet and airborne spread of communicable diseases is assumed to be greater if a patient is frequently coughing. Hence, different infection control precautions have been recommended when caring for patients who are coughing (7). However, our results suggest that recollection of contact during this activity may not be reliable. Whether this poor reliability is related to the effect of time on memory or the intermittent nature of coughing is unclear.

The inferences that can be drawn from this study are limited by the relatively small size of our cohort. Caring for patients with SARS can be a memorable and frightening event (8,9), and recall reliability in our study may not be generalized to other clinical situations. Furthermore, the similarities among questions during the 2 interviews may

Table 2. Reliability of healthcare worker recalling presence in a SARS patient's room during patient care activities*

Activity	No. (%) affirmative during initial interview	No. (%) same response on follow-up interview	No. (%) of responses overestimated	No. (%) of responses underestimated	No. (%) missing	Kappa	95% CI
Respiratory characteristics and airway management							
Intubation	2 (7)	25 (93)	1 (4)	1 (4)	0	0.46	0.00–1.00
Suctioning before intubation	2 (7)	24 (89)	2 (7)	1 (4)	0	0.34	0.23–0.91
Suctioning after intubation	2 (7)	25 (93)	2 (7)	0	0	0.63	0.17–1.00
Manipulation of oxygen face mask/tubing	13 (48)	22 (82)	3 (11)	2 (7)	0	0.70	0.44–0.97
Manual ventilation	2 (7)	25 (93)	2 (7)	0	0	0.63	0.17–1.00
Mechanical ventilation	6 (22)	24 (89)	2 (7)	1 (4)	0	0.70	0.38–1.00
Patient coughing/spitting while healthcare worker present	11 (41)	16 (59)	8 (30)	0	3 (11)	0.38	0.11–0.66
Routine patient care							
Changing bedding	11 (41)	18 (67)	2 (7)	7 (26)	0	0.26	0.09–0.60
Bathing patient	4 (15)	26 (96)	1 (4)	0	0	0.87	0.61–1.00
Emptying urinary catheter collection bag or collecting urine sample	4 (15)	25 (93)	0	2 (7)	0	0.63	0.17–1.00
Oral care or nasal swab	4 (15)	25 (93)	1 (4)	1 (4)	0	0.71	0.32–1.00
Stool sample or rectal swab	0	25 (93)	1 (4)	0	1 (4)	–	–
Nebulizer treatments	0	19 (70)	6 (22)	0	2 (7)	–	–
Procedures							
BiPAP	19 (70)	18 (67)	2 (7)	6 (22)	1 (4)	0.32	0.01–0.66
Electrocardiogram	3 (11)	23 (85)	3 (11)	1 (4)	0	0.42	0.04–0.88
Insertion of central venous catheter	0	27 (100)	0	0 (0)	0	–	–
Insertion of peripheral intravenous catheter or arterial catheter	5 (18)	25 (93)	1 (4)	1 (4)	0	0.75	0.43–1.00
Insertion of nasogastric tube	0	26 (96)	1 (4)	0	0	–	–
Insertion of urinary (Foley) catheter	0	27 (100)	0	0	0	–	–
Chest physiotherapy	0	26 (96)	1 (4)	0	0	–	–
Other							
Cleaning room/furniture	2 (7)	26 (96)	0	1 (4)	0	0.65	0.02–1.00
Cleaning medical equipment	3 (11)	22 (82)	2 (7)	1 (4)	2 (7)	0.51	0.03–0.99

*SARS, severe acute respiratory syndrome; CI, confidence interval; BiPAP, bi-level positive air pressure.

have resulted in the potential for recall bias, causing an overestimation of reliability within respondents (10). Finally, our study measured the reliability rather than the validity of healthcare worker recall for determining exposure risk. Nonetheless, our findings that healthcare workers reliably recalled exposure after several months following the event should be reassuring to investigators studying risk factors for SARS transmission in hospitals and to infection control practitioners assessing exposure to communicable diseases.

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Molecular Mechanisms of West Nile Virus Pathogenesis in Brain Cells

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We analyzed the response of human glioma cells to West Nile virus infection by investigating host transcriptional changes. Changes in expression of 23 genes showed similarities to those in other neurodegenerative diseases. These changes may be useful as potential biomarkers and elucidate novel mechanisms behind the neuropathology of infection with this virus.

West Nile virus (WNV), a member of the family *Flaviviridae*, is the etiologic agent of West Nile fever. Since WNV is neurotropic, severe human meningoencephalitis is a common complication of infection and results in a considerable number of deaths. The medulla of the brainstem in the central nervous system (CNS) is the primary target of WNV (1).

WNV replicates in a wide variety of cell types, and studies have traditionally been carried out in Vero (green monkey kidney) and C6 (mosquito) cells. However, little work has been done with CNS cells. We conducted a global transcriptional analysis of human glioblastoma cell response to infection with WNV during peak virus production to determine the crucial virus-host interactions that take place during a severe neuroinvasive attack and identify putative mechanisms involved in WNV pathogenesis. The factors governing the development of neurologic disease, host immune response, patterns of clinical features, and outcomes are poorly understood in those infected with neurotropic flaviviruses (2).

A total of 173 genes were differentially expressed, many of which were not found in previous transcriptional studies of other flaviviruses (3). From these, 23 genes were identified that may play a role in cellular neurodegeneration. These novel changes induced by WNV may serve as biomarkers and help explain the neuropathologic features observed.

The Study

Most laboratory studies of WNV infections have been carried out in animal cell lines or human cell lines of non-CNS origins. In this study, human glioblastoma (A172) cells were found to be a useful laboratory model for investigating WNV infections. A172 (human glioblastoma) cells were maintained at 37°C in Dulbecco modified Eagle medium (Sigma, St. Louis, MO, USA) supplemented with 10% fetal calf serum. Confluent monolayers of A172 cells were infected with the Sarafend strain of WNV at a multiplicity of infection of 1. Twenty-four hours after infection, cells showed signs of cytopathic effects (cell-rounding) and produced high virus titer (10^8 PFU/mL). This demonstrated the highly susceptible nature of the neuroglial cells to WNV infection. Batches of cells were infected for microarray experiments, and a quantitative polymerase chain reaction was used to verify the reproducibility of the changes in gene expression.

Total cellular RNA was extracted from mock-infected and infected cells by using the RNeasy Mini kit and QIAshredder (Qiagen, Hilden, Germany). The CyScribe first-strand cDNA labeling kit (Amersham Biosciences, Piscataway, NJ, USA) was used to incorporate fluorescent Cy3-dCTP or Cy5-dCTP (Amersham Biosciences) into cDNA probes. The probes were subsequently purified by using CyScribe GFX purification columns (Amersham Biosciences). Equal amounts of labeled cDNA probes (≈25 pmol) were combined for microarray hybridizations. Human 1A microarrays (Agilent Technologies, Palo Alto, CA, USA) were used, and hybridizations were performed on a Lucidea SlidePro Hybridizer (Amersham Biosciences). The microarray experiment was carried out in triplicate: 1 of the microarrays was with a dye-swap labeling to prevent skew in the results due to bias in CyDye incorporation.

Analyses of the scanned microarray images were performed with BRB ArrayTools version 3.1 (developed by R. Simon and A.P. Lam, National Cancer Institute, Bethesda, MD, USA, and available at http://linus.nci.nih.gov/BRB-ArrayTools.html), and normalized by using the Lowess method. A stringent lower limit threshold was set at 3 standard deviations of the pixel intensities of the negative control spots, and images were screened for changes in expression values of at least 2-fold. The differentially regulated genes were separately uploaded into EASE (4) to determine the biologic themes that were significantly overrepresented (Fisher exact test with p values < 0.01). A total of 173 cellular genes were identified by ArrayTools to be differentially expressed in the WNV-infected A172 cells. EASE clustered 39 of the upregulated genes and 41 of the downregulated genes into specific functional groups (available at http://sps.nus.edu.sg/~kohweele/awn_genes.htm).

Functional classes that were found to be enriched in the upregulated genes encompassed those related to immunity, responses to external stimulus and pathogens, and apoptosis. Genes relating to the ubiquitin cycle, transcription

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regulation, and other physiologic processes were also identified by EASE. Functional classes that were downregulated were not commonly observed in a virus infection system. For instance, genes relating to the mitochondria, ribosomes, and protein biosynthesis were highly overrepresented in down regulation (available at http://sps.nus.edu.sg/~kohweele/awn_genes.htm). From this set of genes, a group of 23 genes that may provide the molecular basis for the observed pathogenesis in the A172 cells was identified (Table 1).

A quantitative reverse transcription–polymerase chain reaction (qRT-PCR) was carried out to ensure an independent assessment of the microarray results. Genes for the qRT-PCR were selected to represent the broad spectrum of identified functional classes from the microarrays. The hypoxanthine guanine phosphoribosyltransferase gene was used as an internal control (primers for the PCR can be found at http://sps.nus.edu.sg/~kohweele/awn_genes.htm). RNA was reverse-transcribed by using SuperScript III (Invitrogen, Carlsbad, CA, USA), and a real-time PCR was carried out with Platinum SYBR Green (Invitrogen). A negative template control that contained all SYBR green reagents except DNA was performed in parallel on the iCycler iQ (Bio-Rad Laboratories, Hercules, CA, USA). The results corroborated the microarray data, thereby verifying the accuracy of the statistical analysis (Table 2). However, the qRT-PCR showed greater dynamism in fold changes than the microarray results because of the greater sensitivity of PCR compared with fluorescent detection.

Conclusions

In this study, WNV infection of human brain glioma cells showed advanced cytopathic effects within 24 h after infection and produced high virus yields. This demonstrated that human glioma cells from CNS are susceptible to WNV infection and are suitable for the study of viral pathogenesis.

The activation of the innate antiviral immune response pathways is often the primary cause of pathologic effects. The presence of double-stranded RNA replication complexes from viral origins causes the transcriptional activation of the interferon-α/β (IFN-α/β) or type-I IFN pathways (5). In this study on glioma cells, the activation of numerous interferon-induced proteins (such as IFIT1,

Table 1. Differentially regulated genes involved in pathogenesis of A172 cells infected with West Nile virus

Gene	Gene name	Fold change
Immune response related		
OAS3	2'-5'-oligoadenylate synthetase 3, 100 kDa	2.32
OASL	2'-5'-oligoadenylate synthetase-like	3.46
FIT1	Interferon-induced protein with tetratricopeptide repeats 1	10.74
IFIT2	Interferon-induced protein with tetratricopeptide repeats 2	3.76
IFI27	Interferon, α-inducible protein 27	4.03
IFITM1	Interferon-induced transmembrane protein 1 (9-27)	12.00
IFITM2	Interferon-induced transmembrane protein 2 (1-8D)	3.04
G1P2	Interferon, α-inducible protein (clone IFI-15K)	9.50
HLA-C	Major histocompatibility complex, class I, C	2.20
INDO	Indoleamine-pyrrole 2,3 dioxygenase	3.38
PTX3	Pentaxin-related gene, rapidly induced by interleukin-1β	3.44
Apoptosis related		
TNFSF14	Tumor necrosis factor (TNF) (ligand) superfamily, member 14	2.19
NFKBIA	Nuclear factor of kappa light polypeptide gene enhancer	4.13
TRAF1	TNF receptor-associated factor 1	2.01
SAT	Spermidine/spermine N1-acetyltransferase	2.18
Mitochondria related		
SDHC	Succinate dehydrogenase complex, subunit C	–2.31
COX5B	Cytochrome c oxidase subunit Vb	–2.13
COX6B	Cytochrome c oxidase subunit VIb	–2.41
ATP5G1	ATP synthase, mitochondrial F0 complex, subunit c, isoform 1	–2.64
ATP5C1	ATP synthase, mitochondrial F1 complex, γ polypeptide 1	–3.82
ATP5J	ATP synthase, mitochondrial F0 complex, subunit F6	–2.11
ATP5B	ATP synthase, mitochondrial F1 complex, β polypeptide	–2.17
ATP5A1	ATP synthase, mitochondrial F1 complex, α subunit, isoform 1	–2.21
ATP5O	ATP synthase, mitochondrial F1 complex, O subunit	–2.00
ATP5F1	ATP synthase, mitochondrial F0 complex, subunit b, isoform 1	–2.43
PRDX5	Peroxiredoxin 5	–2.74
PRDX3	Peroxiredoxin 3	–2.27
Protein biosynthesis related		
NACA	Nascent-polypeptide-associated complex polypeptide	–2.17

Table 2. Comparison of gene expression changes between microarray and qRT-PCR in A172 cells infected with West Nile virus*

Gene	Gene name	Microarray fold change	RT-PCR fold change
ARHI	DIRAS family, GTP-binding RAS-like 3	–2.72	–2.55
ATP5J	ATP synthase, mitochondrial F0 complex, subunit F6	–2.11	–2.60
CEB1	Hect domain and RLD 5	2.32	42.22
DNAJB1	DnaJ (Hsp40) homolog, subfamily B, member 1	–1.97	–2.14
DUSP1	Dual specificity phosphatase 1	1.92	5.66
EGR1	Early growth response 1	4.79	8.57
EIF4G2	Eukaryotic translation initiation factor 4 γ, 2	–2.11	–7.77
FLJ13855	Hypothetical protein FLJ13855	2.05	3.85
FOSL1	FOS-like antigen 1	2.08	6.50
IFITM1	Interferon-induced transmembrane protein 1 (9-27)	12.03	527.61
LTA4H	Leukotriene A4 hydrolase	–2.02	–8.10
RPL5	Ribosomal protein L5	–2.97	–9.03
RPL7A	Ribosomal protein L7a	–2.03	–3.42
RPLP0	Ribosomal protein, large, P0	–2.15	–1.52
TFPI2	Tissue factor pathway inhibitor 2	5.21	11.58

*qRT-PCR, quantitative reverse transcription–polymerase chain reaction.

IFIT2, IFI27, IFITM1, IFITM2, and G1P2) lends support to this mechanism of pathogenicity. Glial cells are useful in this study because they are immune cells of CNS origin. Activated glial cells have macrophagic activity and are primed to respond to the virus, therefore allowing the display of immune-mediated neuropathologic changes that reflect conditions in the natural CNS host cells. Glial cells can also activate the type-II (IFN-γ) pathway and modulate the immune response by regulating cell trafficking of various leukocytes, including macrophage activation and stimulation of specific T cells responsible for cytotoxic immunity (6).

An example of this activation was finding that the HLA-C gene coding for the major histocompatibility complex class I (MHC-I) antigens was upregulated in the A172 cells. Peptides derived from endogenous intracellular proteins are generally bound by the MHC-I molecules for presentation, thus paving the way for cell cytotoxicity in cellular immunity. In mice, the targeted killing of WNV-infected cells by CD8+ T cells may result in the severe neurologic disease often observed in WNV infections (7).

In addition, indoleamine 2,3 dioxygenase (INDO) was observed to be upregulated in WNV-infected A172 cells. Increased production of INDO by glial cells causes neuronal injury in neuroinflammatory diseases (8). The upregulation of the pentaxin-related gene (PTX3) is also implicated in local tissue damage through the amplification of inflammation in innate immunity (9).

A group of genes causing apoptosis was also found to be upregulated, thus elucidating pathways linking virus replication to apoptosis. These genes include the tumor necrosis factor superfamily (TNFSF14), nuclear factor of κ light-chain gene (NFKBIA), TNF receptor-associated factor (TRAF1), and spermidine/spermine N1-acetyltransferase (SAT). This highly conserved process of cellular self-destruction serves to limit the spread of WNV (10).

A major group of genes relating to mitochondria was found to be downregulated. Mitochondrial defects due to respiratory-chain dysfunction and free-radical formation have been associated with neurodegenerative diseases such as Huntington disease, Parkinson disease, and Friedreich ataxia (11). Neurologic symptoms of these diseases were also observed in WNV-infected patients (12), suggesting similar neurodegenerative pathways.

The activity of genes belonging to the energy synthesis pathways was decreased. These genes included succinate dehydrogenase (SDHC), cytochrome c oxidase (COX5B/COX6B), and various genes of the ATP synthase complex (ATP5G1, ATP5C1, ATP5J, ATP5B, ATP5A1, ATP5O, and ATP5F1). Decreased energy production from the downregulation of these genes is known to cause severe neurodegeneration (13). Two antioxidant enzymes of the peroxiredoxin family (PRDX5 and PRDX3) were also downregulated. The increase in oxidative stress induced by reactive oxygen species can create a proinflammatory condition that results in CNS pathology and leads to Alzheimer disease and Down syndrome (14). Downregulation of the nascent polypeptide-associated complex (NACA) can also lead to similar neurodegeneration (15).

In summary, this global transcriptional study showed a complex network of WNV-induced A172 cell interactions during infection. The examination of glial A172 cell response has provided insights into the molecular mechanisms behind the observed neuronal pathology in WNV encephalitis.

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Tickborne Meningoencephalitis, First Case after 19 Years in Northeastern Germany

To the Editor: Tickborne encephalitis virus (TBEV) is focally distributed in Europe and Asia (between 42° and 63° north latitude). Recently, 5 human tickborne encephalitis cases have been reported, and anti-tickborne encephalitis antibody prevalence in dogs has been observed in southern Norway (1). In the last 2 decades, mild winters may have favored a northbound spread and increased tickborne encephalitis incidence (2). Tickborne encephalitis is endemic in southern Germany, but no cases had been reported in northeast Germany since 1985 (3,4).

A 61-year-old man was bitten by a tick at Lake Woblitz, near the town of Neustrelitz in former East Germany, on May 31, 2004, between 8:00 a.m. and 4:00 p.m. The patient's history showed no other tick bites, no stays in tickborne encephalitis–endemic areas, and no tickborne encephalitis vaccination.

On June 9, transient fever and headache developed in the patient, followed 6 days later by difficulty in concentrating, apathy, and a strong urge to sleep. On June 23, the patient was hospitalized with fever (temperature 39.2°C) and mental confusion. Because he had cruised the Nile in December 2003, he was transferred to the Tropical Medicine Division of Rostock University to exclude a diagnosis of malaria. Somnolence, slurred speech, amnestic dysphasia, and impaired fine motor control, but no meningism, focal signs, pyramidal tract, or sensation impairment, were observed. Results of magnetic resonance imaging brain scan and electroencephalogram were normal.

Laboratory tests showed leukocytosis of 9,400 leukocytes/μL and lymphocytopenia of 12%. Alpha-amylase was 254 U/L, lipase 84 U/L, sodium 131 mmol/L, and fibrinogen 4.4 g/L. C-reactive protein and all other routine laboratory parameters were normal.

In the serum, immunoglobulin (Ig)G, but not IgM, was detected against *Borrelia burgdorferi.* Tests of cerebrospinal fluid (CSF) specimens, including polymerase chain reaction (PCR) for herpes simplex virus types 1 and 2, varicella zoster virus, Epstein-Barr virus (EBV), cytomegalovirus, and human herpesvirus 6 were negative. Antibody tests for *Borrelia burgdorferi*, *Mycoplasma pneumoniae*, *Bartonella*, *Brucella*, *Leptospira interrogans*, HIV, EBV, and arboviruses were negative. VDRL (Venereal Disease Research Laboratory) tests, Gram stains, and routine bacterial cultures for common pathogens were also negative (5).

Tests on CSF showed mild pleocytosis (9 leukocytes/μL) and high protein concentration (1,322 g/L). Most cells were lymphocytes (89%) and monocytes (10%). Protein analysis showed blood-brain barrier impairment and intrathecal IgM synthesis.

Anti-tickborne encephalitis virus IgG and IgM antibodies were detectable in the serum by enzyme-linked immunosorbent assay 29 days after the tick bite; corresponding CSF titers were borderline. One week later, IgG and IgM antibodies were positive in serum and CSF, while CSF leukocyte count and protein concentration were normal. The anti-tickborne encephalitis serum immunofluorescence titer rose from 1:80 (June 29) to 1:640 (July 14); titers against dengue, West Nile, yellow fever, and Japan B encephalitis were not elevated. Even though CSF specimens were negative for TBEV genome on 2 occasions, a confirmed tickborne encephalitis case had to be reported to the health authorities.

With symptomatic therapy, the patient's condition improved, and he was discharged on July 7 with slight mental slowing. By July 14, he had recovered completely.

Our patient had acquired tickborne encephalitis at a popular tourist site (Figure), where no human cases had

Figure. European tickborne encephalitis–endemic areas (shaded areas) and new infection site of case in northeastern Germany (arrow). Map provided by Baxter Germany GmbH.

occurred for 19 years. From 1959 to 1983, numerous TBEV foci existed in northeastern Germany (3). From 1960 to 1985, a total of 4 human cases were seen 10 km east of Neustrelitz. From 1983 to 1989, numerous attempts to cultivate TBEV from ticks or small mammals failed (3). In 1992, TBEV genome was detected by PCR in 3 tick pools from the island of Usedom, and in 2 pools from the Darss peninsula, 100 km northeast of Neustrelitz. From 1993 to July 2004, TBEV genome was not detected in 16,098 ticks collected from 275 regions of northeastern Germany, including the county where Lake Woblitz is situated, as part of a statewide surveillance program (State Health Services, unpub. data). However, during 2004, this county reported 24 cases of Lyme disease (2003: 10 cases; 2002: 8 cases; 2001: 1 case). Therefore, our tickborne encephalitis case might represent intensified amplification cycles of tickborne infectious agents in 2004.

The absence of tickborne encephalitis cases for 20 years does not likely represent a lack of data before or a lack of interest after the reunification of Germany. Tickborne encephalitis was a reportable disease under East German regulations, and tickborne encephalitis surveillance was intensified after reunification (3).

Eight weeks after our patient's tick bite, 160 *Ixodes ricinus* ticks were collected from 10 pools near Lake Woblitz. RNA was isolated in 5 mol/L guanidium isothiocyanate solution, extracted by phenolchloroform, and precipitated with ethanol. cDNA was amplified by nested reverse transcription–PCR and detected by electrophoresis (6). In 2 of these pools, PCR directed towards the 5′ terminal noncoding region of the TBEV genome yielded a 104-bp fragment, but the sequence was not specific for flaviviruses.

This case does not prove a northbound spread of tickborne encephalitis in northeastern Germany. Rather, it shows that after years of negative tickborne encephalitis test results in ticks, old tickborne encephalitis foci may retain activity. Thus, tickborne encephalitis should be included in the differential diagnosis of meningoencephalitis in northeastern Germany, even if the patient has not been in tickborne encephalitis–endemic areas.

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Social Impact of Leishmaniasis, Afghanistan

To the Editor: For almost a decade, Kabul, Afghanistan, has had the highest incidence of cutaneous leishmaniasis in the world, with an estimated 67,500 to 200,000 cases each year (1–3). Because of sandfly vector exposure, most leishmaniasis lesions occur on the face; anecdotal reports of severe stigma are associated with the disease (3). To prioritize aspects of operational activities and before developing a disease-specific health education strategy, we collected data on knowledge, attitudes, and perceptions regarding leishmaniasis.

In October 2002, we randomly chose 5 of Kabul's 14 administrative districts to carry out a house-to-house survey (HHS) as well as 13 focus group discussions (FGDs) with women. The 5 districts chosen were Karti-Seh (HHS) and Dasht-e-Barchi (4 FGDs), Karti-Now (3 FGDs), Arzam Qemat (3 FGDs), and Rahman Mena (3 FGDs). The survey was conducted by using a standardized, multiple-choice questionnaire. The most senior, available family member in 252 neighboring households was interviewed, after the first household was randomly selected (2). We focused on women in FGDs because they have greater risk for leishmaniasis than men (2,3) and are often the primary caregivers in Afghan culture

(4). The same HHS questions were used in the FGDs. Surveyors randomly chose a house in each district and explained the study's purpose to residents. When residents agreed to host an FGD, women from neighboring households were invited to join. FGDs had a maximum of 12 participants and lasted 2 hours; answers to questions were recorded on paper. FGD moderators were instructed to pose questions, encourage free discussion, and ask participants to emphasize personal experiences. FGD data were analyzed by thematic analysis of the transcripts. Surveys were carried out by experienced surveyors, who have been involved in previous leishmaniasis prevalence surveys or intervention trials (2–4). Written approval for the study was obtained from the Afghan Ministry of Health, and oral consent was given by all surveyed persons. Active case-patients surveyed were offered free antileishmanial treatment at the HealthNet International leishmaniasis clinics.

A total of 252 and 108 persons were surveyed in the HHS and FGDs, respectively, although not all respondents answered every question. Our study confirmed the prevalence of cutaneous leishmaniasis in Kabul; 128 (51%) of 252 HHS respondents reported a family member with leishmaniasis. Respondents were knowledgeable about leishmaniasis: of 360 total HHS and FGD respondents, 287 (80%) said that it was a disease, and 160 (44%) said that it was acne. Of 66 FGD respondents who knew that leishmaniasis was a disease, 29 (44%) knew that it was transmitted by mosquitoes. Of 104 FGD respondents, 41 (43%) could describe the clinical symptoms of leishmaniasis (each was asked to give 1 answer only), i.e., an open wound (n = 17) that is not painful (n = 7) and takes a long time to cure (n = 17).

The principal finding of our study is that we show, for the first time, the extent of the disease's social impact in Kabul. Because erroneous beliefs exist that the disease can be transmitted by person-to-person physical contact (of 360 respondents, the most common answers were "touching" [n = 86] and "sharing meals and household goods" [n = 26]), affected people are excluded from communal life. This exclusion can consist of minor domestic restrictions (40 [46%] of 89 FGD respondents said they would not share plates, cups, or towels with leishmaniasis patients) or more severe measures that lead to physical and emotional isolation. FGDs showed that leishmaniasis caused trauma; of 83 respondents who had children with leishmaniasis, 45 (54%) said their children felt disfigured because of lesions or scars (n = 20), because of painful treatment (intralesional or intramuscular injections with pentavalent antimony, n = 19), or because they were excluded from play with other children (n = 6). Of 96 FGD respondents, 21 (22%) said that a mother with leishmaniasis should not breast-feed her child; 48 (51%) of 94 FGD respondents would prevent someone with leishmaniasis from touching or hugging their children; 55 (57%) of 96 respondents said that a person with leishmaniasis should not be allowed to cook for the family; and 21 (22%) of 94 respondents said that a woman with a leishmaniasis lesion or scar will have difficulty finding a husband. Severity and visibility of the lesions as well as past experience of leishmaniasis within the family influenced respondents' answers.

The study yielded 2 other important findings. First, 245 (97%) of 252 HHS respondents knew that leishmaniasis does not resolve without treatment and that patients should seek professional assistance. Of 344 HHS and FGD respondents, 322 (94%) said that leishmaniasis patients should seek a doctor or clinic for treatment (as opposed to a traditional healer or self-medication). Second, 205 (57%) of 358 HHS and FGD respondents use methods to prevent exposure to sandfly vectors, i.e., screens for windows and doors (n = 108), nets around beds (n = 63), indoor insecticide spraying (n = 24), or other method of personal protection (n = 10); 152 (78%) of 252 HHS respondents said that they did not have a net over their bed because it was too expensive.

Kabul residents are knowledgeable about leishmaniasis; they are able to describe its symptoms and the necessity for professional treatment. However, we show that while many FGD respondents knew that leishmaniasis is transmitted by "mosquitoes," severe stigma and trauma are associated with the disease, particularly in children and women. Our operational experience corroborates this finding, which underlines the disease's social effect on the local population and refuting the belief that leishmaniasis is of little health importance (5). Half of the 15,983 leishmaniasis patients treated at HNI clinics in 2003 were women. Although women are at greater risk for leishmaniasis, they do not typically attend healthcare programs in Afghanistan because of sociocultural constraints (e.g., husbands not allowing their wife or daughters to attend) (6). In addition to diagnosing and treating active cases, HealthNet International will now focus on leishmaniasis education activities in Kabul, outlining aspects of disease transmission and prevention, as well as disseminating messages to reduce the disease's social impact.

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Methicillin-resistant *Staphylococcus aureus* Toxic Shock Syndrome

To the Editor: Toxic shock syndrome (TSS), which can be life threatening, is defined by clinical and laboratory evidence of fever, rash, desquamation, hypotension, and multiple organ failure caused by *Staphylococcus aureus* toxins. TSS caused by methicillin-resistant *S. aureus* (MRSA) strains has been found extensively in Japan (1), rarely in the United States (2), and, thus far, not in Europe.

We report a case of TSS due to an MRSA strain that produced a TSS toxin 1 (TSST-1). A 54-year-old woman was admitted to the emergency ward of Brugmann University Hospital, Brussels, with a 2-day history of myalgia, diarrhea, and vomiting. She had undergone surgery for a palate neoplasia 2 months earlier, and again 2 weeks earlier, in another hospital. After the second operation, she had been treated for a local scar infection with amoxicillin–clavulanic acid for 1 week.

On physical examination, the patient was conscious, tachypneic, pale, and sweating. Her temperature was 38.2°C and her blood pressure was 70/50 mm Hg. Abdominal examination findings were normal. The cutaneous operative wound was red and swollen. Laboratory results included the following: leukocyte count 19,830/mm^3 with 97% polynuclear neutrophils, platelets 90,000/mm^3, creatinine 2.1 mg/dL, bicarbonate 13 mEq/L, cyclic AMP receptor protein 43.7 ng/mL, creatine kinase 514 U/L. Cultures of blood, stool, and urine samples were negative for microbial agents. Puncture of the wound released 12 mL of pus; culture of the pus sample yielded an MRSA strain harboring a TSST-1 gene, detected by multiplex polymerase chain reaction as previously described (3).

By molecular typing, the strain belonged to the epidemic MRSA pulsed-field gel electrophoresis clone G10 and carried the staphylococcal chromosome cassette *mec* (SCC*mec*) type II. This clone belongs to the sequence type (ST) 5-SCC*mec* II clone, formerly named "New-York/Japan clone," which has been associated with neonatal TSS–like exanthematous disease in Japanese hospitals (4–6). This epidemic clone, which is widely disseminated in the United States, Japan, and Europe, has been found in 12% of Belgian hospitals during a national survey conducted in 2001 (6).

The treatment included aggressive intravenous fluid resuscitation, administration of dopamine, and antimicrobial agent therapy with teicoplamin and clindamycin. The treatment outcome was favorable. On the second day, a diffuse cutaneous macular rash appeared. The acute renal failure and the biological abnormalities resolved. On the fifth day, the patient was transferred back to the hospital where she had undergone surgery; extensive peeling then developed on both of the patient's hands.

Our patient met the criteria of TSS: she had fever, rash, desquamation, hypotension, vomiting, diarrhea, myalgias, elevated creatine kinase, acute renal failure, and thrombocytopenia. The diagnosis of staphylococcal TSS was confirmed by bacteriologic results.

Although TSST-1 production by MRSA strains has been described in Europe (7), this case is the first of TSS due to TSST-1–producing MRSA in Europe. Recently Nathalie van der Mee-Marquet et al. (8) described the first case of neonatal TSS–like exanthematous disease due to a MRSA strain containing the TSST-1 gene in Europe. They emphasized the risk of emergence of neonatal toxic shock syndrome–like exanthematous disease outside Japan.

We would also like to emphasize the rising risk of TSS due to virulent MRSA strains outside Japan and particularly in Europe. The usual recommendations for the treatment of staphylococcal TSS do not consider this possibility and consist of a β-lactamase–resistant anti-staphylococcal agent and clindamycin in some cases (to decrease the synthesis of TSST-1) (9–11).

We immediately treated our patient with teicoplanin and clindamycin because we suspected a nosocomial

infection with *S. aureus*, possibly MRSA. The possibility of MRSA must be considered when initiating antimicrobial agents to treat TSS.

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Are SARS Superspreaders Cloud Adults?

To the Editor: The primary mode of transmission of severe acute respiratory syndrome (SARS) appears to be through exposure to respiratory droplets and direct contact with patients and their contaminated environment. However, in summarizing their experiences during the SARS outbreaks in Toronto and Taiwan, McDonald et al. (1) note that certain persons were very efficient at transmitting SARS coronavirus (SARS-CoV), and that in certain settings these so-called "superspreaders" played a crucial role in the epidemic. Airborne transmission by aerosols may have occurred in many of these cases. The same observation has been made by others (2–4), but the causes of these superspreading events and the reasons for the variable communicability of SARS-CoV are still unclear. Possible explanations include specific host characteristics (e.g., altered immune status, underlying diseases), higher level of virus shedding, or environmental factors (1–3).

We hypothesize that superspreading events might be caused by coinfection with other respiratory viruses. Such a mechanism has been identified in the transmission of *Staphylococcus aureus*. Eichenwald et al. (5) showed that newborns whose noses are colonized with this bacterium disperse considerable amounts of airborne *S. aureus* and become highly contagious (i.e., superspreaders) after infection with a respiratory virus (e.g., adenovirus or echovirus). These babies caused explosive *S. aureus* outbreaks in nurseries. Because they are literally surrounded by clouds of bacteria, they were called "cloud babies" (5). We have shown that the same mechanism also occurs in certain adult nasal carriers of *S. aureus* ("cloud adults") (6,7). Reports indicate that viral infections of the upper respiratory tract facilitate the transmission of other bacteria, including *Streptococcus pneumoniae*, *S. pyogenes*, *Haemophilus influenzae*, and *Neisseria meningitidis* (8). Moreover, superspreading events have also been reported in outbreaks of viral diseases such as Ebola hemorrhagic fever and rubella (3).

Some observations suggest that coinfection with other respiratory viruses might cause superspreading events with airborne transmission of SARS-CoV. First, other viral pathogens, including human metapneumovirus, have been detected together with SARS-CoV in some patients with SARS (4). Second, few patients with SARS are superspreaders, and upper respiratory symptoms such as rhinorrhea and sore throat are a relatively uncommon manifestation of SARS (with prevalences of 14% and 16%, respectively) (4). Thus, some

patients with SARS and upper respiratory symptoms might be coinfected with other respiratory viruses and become superspreaders. Interestingly, the report on a SARS superspreading event in Hong Kong explicitly states that the superspreader had presented with a "runny nose" (in addition to fever, cough, and malaise) (3). Therefore, upper respiratory symptoms might be a marker for highly infectious SARS patients. Future investigations, based upon either existing specimens from the last outbreak or newly collected specimens from any future outbreak, should focus on whether an association exits between SARS superspreading events and coinfection with other respiratory viruses.

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Route of Infection in Melioidosis

To the Editor: Melioidosis is an emerging tropical infectious disease, the incidence of which is unknown in many developing countries because of the lack of diagnostic tests and medical practitioners' lack of awareness of the disease. It is a potentially fatal disease caused by the soil bacterium *Burkholderia pseudomallei*. Clinical manifestations, severity, and duration of *B. pseudomallei* infection vary greatly (1).

Melioidosis develops after subcutaneous infection, inhalation, or ingestion of contaminated particles or aerosols. Infection has occurred after near-drowning accidents (1–3) and transmission of *B. pseudomallei* in drinking water (4). The route of *B. pseudomallei* infection is at least 1 of the factors that influences disease outcome, thus contributing to the broad spectrum of clinical signs associated with melioidosis. Researchers use different routes of delivery of *B. pseudomallei* in experimental models to study the pathogenesis of the disease and the induction of host protection. Infection by different routes exposes a pathogen to different components of the host immune system and may subsequently influence disease outcome. Despite this difference, no comprehensive investigation has compared the pathogenesis of melioidosis established by different routes of infection.

Following intravenous (IV) injection, BALB/c mice are highly susceptible, and C57BL/6 mice are relatively resistant to *B. pseudomallei* infection (5). Using this murine model, we compared the pathogenesis of *B. pseudomallei* infection after introducing the bacterium by IV, intraperitoneal (IP), intranasal, oral, and subcutaneous (SC) routes of infection. The virulence of 2 *B. pseudomallei* strains (NCTC 13178 and NCTC 13179) was compared in BALB/c and C57BL/6 mice by using a modified version of the Reed & Meunch (1938) method. Compared to BALB/c mice, C57BL/6 mice are less susceptible to *B. pseudomallei* infection, regardless of the portal of entry, thus validating the model of differential susceptibility for various routes of infection (Table). However, as demonstrated by others (5–7), C57BL/6 mice are not completely resistant to infection by *B. pseudomallei*. Systemic melioidosis can be generated in C57BL/6 mice by using different routes of infection, if a high dose is used. When injected IV into BALB/c mice, NCTC 13178 is highly virulent since the 50% lethal dose (LD_{50}) is <10 CFU. However, if BALB/c mice are injected SC with NCTC 13178, the LD_{50} value increases 100-fold to 1 x 10^3 CFU. This value is equivalent to the LD_{50} of the less virulent NCTC 13179 delivered SC The results emphasize that virulence depends on the route of infection.

The pathogenesis of *B. pseudomallei* NCTC 13178 infection was compared after infection by the IV, IP, SC, intranasal, and oral routes. BALB/c and C57BL/6 mice were administered 570 CFU (equivalent to 60 x LD_{50} delivered IV) or 3 x 10^5 CFU (equivalent to 60 x LD_{50} delivered IV), respectively. At 1, 2, and 3 days postinfection, bacterial loads were measured in blood, spleen, liver, lungs, lymph nodes (right and left axillary and inguinal), and brain by using methods described previously (5).

A tropism for spleen and liver was demonstrated following infection by

Table. Ten-day LD_{50}* values (given in CFU) after intravenous, intraperitoneal, subcutaneous, intranasal, or oral introduction of NCTC 13178 or NCTC 13179 strains of *Burholderia pseudomallei* into BALB/c or C57BL/6 mice

	NCTC 13178		NCTC 13179	
Route of infection	BALB/c	C57BL/6	BALB/c	C57BL/6
Intravenous	<10	5×10^3	9×10^3	6×10^6
Intraperitoneal	1.2×10^1	9.7×10^3	4.7×10^5	2.1×10^7
Subcutaneous	1×10^3	9×10^5	9×10^2	$>10^8$
Intranasal†	1.4×10^2	1.8×10^3	1.9×10^6	$>10^8$
Oral‡	7.2×10^3	$1.8\ 10^6$	4.8×10^6	$>10^8$

*50% lethal dose.
†20 μL of challenge dose was introduced intranasally onto the nostrils of the mice by using a pipette tip.
‡20 μL of challenge dose was introduced orally to the back of the throat of the mice by using a pipette tip.

each of the 5 routes. *B. pseudomallei* could be detected in the tissues of IV- and IP-infected mice earlier and in higher numbers than in those of intranasally and orally-infected mice, despite the fact that all mice received equal numbers of bacteria. This finding reflects differences in the innate immune response, depending on the route of infection. Bacterial numbers in mice infected by the IV or IP route reached $>10^6$ CFU by day 2 postinfection, which indicates a failure of the innate immune response to control infection, leading to overwhelming sepsis and death.

Bacterial loads in tissues after challenge with a lethal dose of highly virulent NCTC 13178 did not indicate any tropism for the lung after intranasal infection. As early as day 1, bacterial loads were greatest in the liver and spleen, not lungs, of C57BL/6 and BALB/c mice following intranasal challenge. This finding suggests a very early systemic spread of *B. pseudomallei* from the lungs to other organs.

Bacteria were detected in the brains of all mice after infection by either the IV, IP, intranasal, or oral route. Colonies recovered from the brains of C57BL/6 mice infected by the intranasal or oral routes were mucoid in appearance. In comparison, bacteria recovered from brains of C57BL/6 mice that were challenged by the IV or IP route demonstrated the characteristic wrinkled shape on Ashdown agar and may have been a consequence of the overwhelming septicemia that spilled over to all organs. Variation in colonial morphology of *B. pseudomallei* has been documented previously (8), and biofilm formation may be an adaptation of *B. pseudomallei* that enables it to evade host immune responses or to survive within unfavorable environments (9,10). The variation in colonial morphology on Ashdown agar observed in bacteria isolated from brains of C57BL/6 mice infected by the intranasal or oral route may reflect a change to biofilm formation of *B. pseudomallei* in this tissue.

In summary, the results of this study reiterate the validity of the mouse model for differential susceptibility to *B. pseudomallei*, regardless of the route of infection. The data also emphasize that virulence depends on the portal of entry of *B. pseudomallei*. Researchers should, therefore, be particularly cautious when comparing and extrapolating data from studies that use different methods of infection.

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Bordetella pertussis in Adult Pneumonia Patients[1]

To the Editor: Although *B. pertussis* infection is well-characterized in children, the epidemiology and clinical spectrum of pertussis in ado-

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lescents and adults are less well defined. Instances of pneumonia complicating adult pertussis have been reported (1,2), yet the role of *B. pertussis* in adult pneumonia has not been rigorously evaluated.

This study searched specifically for evidence of *B. pertussis* infection in 304 adults (≥18 years) admitted to Christchurch Hospital (Christchurch, New Zealand) with community-acquired pneumonia from August 1999 to July 2000 (3). Nasopharyngeal samples and paired serum samples from these patients were stored and later tested for *B. pertussis* DNA and *B. pertussis* antibodies. Culture for *B. pertussis* was not performed because *B. pertussis* was not part of the original pneumonia study protocol.

Nasopharyngeal samples were centrifuged and tested for *B. pertussis* DNA by using the IS481 hemi-nested polymerase chain reaction (PCR) assay described previously (4). Serum samples taken from the patients during acute and convalescent phases of disease were tested for immunoglobulin (Ig)A and IgG antibodies against *B. pertussis* whole cell antigens by using enzyme-linked immunosorbent assay (ELISA) (Pan Bio, Queensland, Australia). All positive serum samples were tested for pertussis toxin (PT) IgG antibodies, the most specific serologic marker for recent *B. pertussis* infection (5). This assay, which uses highly purified PT as antigen, has been described in detail elsewhere (6).

Of the 304 adults, both acute and convalescent phase serum samples were available from 257 patients, only acute phase samples were available from 46 patients, and no samples were available from 1 patient; nasopharyngeal swabs samples were available for testing for 275 patients. Overall, 8 (3%) patients had definite recent *B. pertussis* infection based on *B. pertussis* DNA in nasopharyngeal samples (8 patients) or elevated levels of anti-PT IgG antibodies (single sample with an anti-PT IgG level ≥100 EU/mL, or demonstration of a ≥4-fold rise in anti-PT IgG level) (5 patients). Eighteen (6%) additional patients had evidence of possible recent *B. pertussis* infection based on elevated levels of IgA antibody or demonstrated IgG antibody seroconversion to whole cell lysate *B. pertussis* antigens, but had low levels of anti-PT IgG antibodies. A moderate degree of pertussis existed in the community during the study period, with 4–73 notifications per month in the Christchurch region (population 421,000).

Table. Characteristics of adults with pneumonia and evidence of recent *Bordetella pertussis* infection

Characteristic	Definite evidence of recent *B. pertussis* infection (n = 8)	Possible evidence of recent *B. pertussis* infection (n = 18)
Median age (range)	68 (37–86) y	71 (34–95) y
Male:female	4:4	13:5
Current or ex-smokers	6 (75%)	16 (89%)
Median (range) duration of symptoms at admission	8.5 (1–21) d	3.5 (1–30) d
Presence of cough	8 (100%)	15 (83%)
Sputum production	6 (75%)	7 (39%)
Other respiratory tract pathogens identified	6 (75%)	11 (61%)

Characteristics of the patients with evidence of recent *B. pertussis* infection are shown in the Table. Other respiratory pathogens identified from the patients with definite recent *B. pertussis* infection were *Streptococcus pneumoniae* (2 patients), *Haemophilus influenzae* (2 patients), respiratory syncytial virus (1 patient), and influenza A virus (1 patient). Respiratory pathogens identified in the group with possible recent *B. pertussis* infection were *S. pneumoniae* (6 patients), *H. influenzae* (2 patients), respiratory syncytial virus (2 patients), influenza A virus (2 patients), *Legionella pneumophila* (1 patient), adenovirus (1 patient), and *Pseudomonas aeruginosa* (1 patient). No patients died, but 2 were admitted to the intensive care unit. No clinical or laboratory variables distinguished patients with recent evidence of pertussis from other patients in the study, although the former group had higher proportion of current or ex-smokers (85% vs. 65%; 95% confidence interval for the difference 5%–35%).

This study is the first to systematically search for evidence of *B. pertussis* infection in adults with community-acquired pneumonia. We found evidence of recent *B. pertussis* infection in 3% of adults admitted to the hospital with well-defined pneumonia during a period of increased pertussis activity, and weaker evidence in an additional 6%. In comparison, a community-based study of 122 adults with respiratory tract infections found serologic evidence of *B. pertussis* infection in 7% of the patients (7). Other studies have reported that pneumonia complicates ≈4% of *B. pertussis* infections in adults (1,2), with the disease increasing with age (1).

B. pertussis infection can be difficult to diagnose, especially if symptoms have been present for many days, and we may have underestimated the number of patients with recent pertussis. However, the combination of PCR and serologic testing is one of the most sensitive approaches for diagnosing pertussis in adolescents and adults (5). The nasopharyngeal samples may not have been optimal for PCR testing because they were placed in viral transport media and had already been processed for viral studies. Although the viral transport media was not inhibitory to the PCR, the amount of cellular material may have decreased after this processing.

Our findings indicate that a small proportion of adults admitted to the

hospital with pneumonia had evidence of recent *B. pertussis* infection. In these persons, whether *B. pertussis* is a primary or secondary pathogen or an innocent bystander is not clear. Further work is needed to clarify the precise role of *B. pertussis* in developing adult pneumonia, the risk factors for *B. pertussis*–associated pneumonia, and the value of specific *B. pertussis* therapy in this setting. These data will also help inform about the role of pertussis vaccination in adults.

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SARS Risk Perception and Preventive Measures, Singapore and Japan

To the Editor: Healthcare workers accounted for 21% of all cases of severe acute respiratory syndrome (SARS) during the 2002–2003 outbreak (1). We studied perceptions of risk for SARS infection and preventive measures among healthcare workers in Singapore, who handled cases of SARS and where >41% of the cases occurred among healthcare workers, and in Japan, a SARS-free country.

A self-administered questionnaire was distributed to healthcare workers in various healthcare settings in Singapore (n = 15,025) and Japan (n = 9,978) from May to September 2003. Healthcare workers in Singapore were from 9 primary healthcare hospitals and 9 major institutional healthcare settings, including 3 tertiary hospitals where cases of SARS occurred among healthcare workers, 1 specialized women and children's hospital, 2 community hospitals, and 2 tertiary dental centers. In Japan, study participants were healthcare workers at 7 tertiary-level hospitals distributed throughout Japan. Four of these are university-attached, 2 are municipal hospitals, and 1 is a private hospital.

A total of 10,511 (70% response) and 7,282 (73% response) valid questionnaires were returned in Singapore and Japan, respectively. A total of 43% and 45% of the healthcare workers in Singapore and Japan were nurses; others were doctors, physiotherapists, pharmacists, attendants, cleaning staff, and administrative or clerical staff. In terms of sociodemographic characteristics, the mean ages of the healthcare workers were 36.6 years in Singapore and 35.6 years in Japan, while the gender distribution was 82% female in Singapore and 70% female in Japan, respectively. Approximately half (57% and 45%, respectively) of healthcare workers in Singapore and Japan were married.

A similar proportion (about two thirds) of healthcare workers in both countries felt at great risk of exposure to SARS. However, a higher proportion (76%) was afraid of contracting SARS in Singapore as compared to Japan (55%). Nearly all healthcare workers (96%) in Singapore felt that implementation of protective measures at work was generally effective, and 95% were satisfied with the explanation of their necessity and importance. Slightly fewer (93%) agreed that clear policies and protocols for everyone to follow were in place. In contrast, among Japanese healthcare workers, only 65% agreed that clear policies and protocols were in place, and many fewer (31%) felt that protective measures at work were generally effective (Table).

As to the national experiences with the SARS outbreak, healthcare work-

ers in Singapore managed 238 cases of SARS, while those in Japan did not encounter any cases. Furthermore, preventive measures were strictly enforced and effective in Singaporean, and the outbreak was contained successfully. In contrast, preventive measures were in place in Japan, but workers lacked confidence in an untested system (2). These differences are probable explanations for the varying responses in the Singapore and Japanese healthcare workers. The perceived need for adherence to prescribed measures and willingness to follow protocols were quite different, given the difference in perceived risks. In SARS-free Japan, most healthcare workers were aware that institutional policies and protocols existed, but less than a third were confident of their effectiveness. The degree of implementation and adherence endorsed by healthcare workers was also lower in Japan.

Infections of healthcare workers at the onset of an outbreak may be due to perceptions that recommended policies and measures are unnecessary or excessive. Thus, efforts to educate and communicate the rationale and importance of protective measures may be especially important when outbreaks seem distant and perceived danger is low.

Table. Singaporean and Japanese healthcare workers' risk perceptions about severe acute respiratory syndrome (SARS)

	Workers	
Perceptions	Singapore (n = 10,511),%	Japan (n = 7,282),%
Felt at great risk of exposure to SARS	66	64
Were afraid of contracting SARS	76	55
Felt that protective measures were effective	96	31
Thought that protective measures were necessary and important	95	88
Felt that policies and protocols were clear	93	65
Thought that policies and protocols were implemented	90	50
Felt that recommended measures were adhered to	92	43

Healthcare workers in both Singapore and Japan perceived a risk for exposure to SARS at work, which reflected the global reach of the illness. However, in Singapore, where cases existed and where the disease was eventually contained, the perceived danger of contracting the illness was higher, and most healthcare workers were reassured by the preventive measures taken, which they viewed as effective. This situation was in contrast to the healthcare workers' perceptions of infection risk and confidence in preventive measures against SARS in Japan, where the measures for infection control were untested.

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Pathogenic Fungi—Structural Biology and Taxonomy

Giocondo San-Blas and Richard A. Calderone

Caister Academic Press
ISBN: 0954246470
Pages: 371, Price: US$199.00

Structural Biology and Taxonomy is the first volume in a series authored by leading medical mycologists. The series' scope is to review progress in basic research on zoopathogenic fungi, a timely effort as medical mycology moves into the genomics era. General knowledge of fungal pathogens is assumed. This volume begins by discussing the cell wall which, besides its roles in pathogenesis, is now a practical drug target. The molecular architecture of fungal walls remains elusive because of difficulty in correlating chemical composition with the ultrastructural layers and uncertainty concerning the linkages connecting major cell wall polymers: glucans, mannan, and chitin.

The basis for morphogenesis is the holy grail of medical mycology because temperature-sensitive dimorphism is a stratagem used by several deep-seated fungal pathogens. The fungal cell cyle is considered with respect to the mechanism of sequential gene expression in *Candida albicans*, since little is known about the cell cycle in pathogenic molds. Important interpretation is provided about the hyphal form of *C. albicans*, which clarifies the germ tube's role in morphogenesis and, potentially, in disease.

The molecular genetics of morphogenesis in *C. albicans* follows. Hyphal growth during infection is arguably a pathogenic factor since it thwarts phagocytosis. Genes controlling hyphal development include ones that are upregulated during cell elongation and adherence to epithelia. Fine tuning of morphogenesis is illustrated by the "enhanced filamentous growth" gene which, when knocked out, blocks the transition to the mycelial form. Early steps in this transition are complex, with at least 2 signaling pathways identified: 1 stops yeast growth and another, with a heat shock protein 70-type profile, initiates the assembly of proteins necessary for mycelial growth.

As the focus on morphogenesis continues, dimorphism in several endemic mycoses is concisely reviewed. Heat shock proteins are emphasized because of the temperature-sensitive morphogenesis to the tissue form. Yeast-form–specific genes identified in *Histoplasma capsulatum* function in calcium/calmodulin signaling pathways and sulphur metabolism. Calcium-dependent signaling pathways and heat shock protein expression regulate dimorphism in *Paracoccidioides brasiliensis* and have broad implications for other pathogens. *Coccidioides immitis* dimorphism involves the construction and rupture of the spherule, or tissue form, for which chitin and glucan synthetases and hydrolases are key enzymes. A further chapter discusses how surface membrane G-protein receptors in *C. albicans* and other fungi transmit external stimuli through 2 major protein kinase cascades. These govern multiple functions, including hyphal development and the secretion of pathogenic factors.

Departing from the morphogenesis theme, chapters on phylogenetic analysis help clarify the taxonomy of noncultivatable medical fungi (excluding *Pneumocystis*). The evolution and population genetics of 3 agents of major deep-seated mycoses, which follow, are of more general interest. Finally, in a return to the original cell wall theme, the fine structure of mannans and galactomannans is explained as a useful adjunct in classifying fungi.

Pathogenic Fungi—Structural Biology and Taxonomy is an important interpretation of recent literature, a valuable addition to collections, and recommended reading for investigators seeking a broad appreciation for the current state of the art.

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Albrecht Dürer (1471–1528). Stag Beetle (1505) (detail)
Watercolor and gouache;
upper left corner added with tip of left antenna painted in by a later hand
(14.1 cm × 11.4 cm)
The J. Paul Getty Museum,
Los Angeles, California, USA

Fearsome Creatures and Nature's Gothic

Polyxeni Potter

"In Venice, I am treated as a nobleman…. I really am somebody, whereas at home I am just a hack," (1) said Albrecht Dürer of his life and studies abroad. During his travels, he came under the influence of Italian Renaissance, which had a transforming effect on the way he viewed art. Challenging his Gothic roots as well as his use of color, travel opened the door to the work of Leonardo da Vinci, engraver Andrea Mantegna, and founder of the Venetian school of painting, Giovanni Bellini, "…the best painter of them all" (2).

Dürer was born in Nürnberg, Germany, a bustling humanist center of the Reformation. The third of 18 children in a Hungarian family of goldsmiths, he apprenticed as metalworker from a very young age, his exacting skills evident later in the unparalleled detail and precision of his famed woodcuts, engravings, and etchings. Intellectually gifted and versatile, he was as comfortable with mathematics and writing as with art. His confidence and charisma were immortalized in a series of striking self-portraits and captured in his epitaph: "Whatever was mortal in Albrecht Dürer lies beneath this mound" (3).

Synthesizing Gothic traditions of the North with theories and practices of Italy, Dürer flourished as exponent of Northern Renaissance (4). He became an exceptional painter, but his greatest impact was on printmaking, which he elevated to art form. Introducing new tonal and dramatic features to graphic images, he increased their conceptual scope and intensity as well as technical perfection.

Even before his travels to Italy, which during this period enjoyed a revival of mathematics, Dürer came to believe that "…art must be based upon science—in particular, upon mathematics, as the most exact, logical, and graphically constructive of the sciences" (5). He studied geometric principles, from Pythagoras, Plato, and Euclid to Piero della Francesca, Luca Pacioli, and da Vinci. Specifically, Dürer was interested in Platonic and Archimedean solids and the golden mean and how these mathematical concepts influenced proportion and geometric ratios in art, affecting beauty and meaning.

Dürer had access to Europe's best-known theologians and scholars, including Erasmus, and his diverse portfolio contained portraits of Holy Roman Emperors Maximilian I and Charles V. Many of his works had religious themes, but he was also partial to nature. In his Treatise on Proportion, he commented, "Life in nature makes us recognize the truth of these things, so look at it diligently, follow it, and do not turn away from nature to your own thoughts…. For, verily, art is embedded in nature; whoever can draw her out, has her" (6).

Within larger themes or alone in spectacular nature scenes, exotic animals were a large part of Dürer's work. In Rhinoceros, one of his most popular animal engravings, the artist meticulously detailed a creature he had never seen in an image that served as scientific model for the species for more than two centuries (7). In other works, insects and beasts symbolized doubt, temptation, or other failings and tormented people, as if in contest for the human soul. In Christ in Limbo, the tormentor was a half-human pig (8).

Among the best-known of Dürer's nature works, The Stag Beetle on this month's cover is startling, and not only for its artistic presentation. Insects, though much in line with the artist's Renaissance interest in nature, were

thought the lowest of creatures by his contemporaries, hardly suitable focal points for period art.

The Stag Beetle is not the scientific study of a curious creature. It is a finished painting, and one likely executed from observation. The beetle, structured, modular, and richly colored after the rotting matter it consumes, arches backward lifting the curve of its spiky mandibles. In this icon of natural design, the artist mimics nature not only with respectful attention to detail but also with talent at illusion: the shadow cast beneath the stationary armored trunk makes the beetle seem to strut across the canvas—just as the crablike claws make its harmless frame seem ferocious and menacing.

Dürer's realistic rendering of this humble bug is a tribute to the minutest in nature—that which is often overlooked or summarily destroyed, its importance lost to ignorance or neglect. Such is the case with the endangered stag beetle, thoroughly benign but seemingly ominous, all too readily squashed in its disappearing woodsy habitat.

Other critters, not so benign or visible, are also easy to ignore, their pestiferous history relegated to the past and quickly forgotten. Blood-thirsty ticks, bed bugs, and other insects, as if caught in some Gothic time machine, continue to torment humans, still claiming their lives, if not their souls. Renewed infestations of ticks causing meningoencephalitis in Germany (9) and of bed bugs compromising health in Canada and elsewhere (10) warn against ignorance and neglect regarding visible or invisible tiny creatures of nature.

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EMERGING INFECTIOUS DISEASES

A Peer-Reviewed Journal Tracking and Analyzing Disease Trends
Vol. 11, No. 5, May 2005

Upcoming Issue

Look in the May issue for the following topics:

Dengue Fever, Hawaii, 2001–2002

Dengue Risk among Visitors to Hawaii

Dengue Type 3 Virus, Saint Martin, 2003–2004

Leptospirosis during Dengue Outbreak, Bangladesh

Retinal Hemorrhage in Patients with Dengue Fever

Venezuelan Equine Encephalitis Virus Infection of Spiny Rats

Kala-Azar Risk Factors, Bangladesh

Avian Influenza Risk Perception, Hong Kong

Assessing Parents' Perception of Children's Risk for Recreational Water Illness

Adenovirus Peptide Diversity during Outbreak, Korea, 1995–2000

Salmonella Derby Clonal Spread from Pork

Probable Tiger-to-Tiger Transmission of Avian Influenza H5N1

Complete list of articles in the May issue at http://www.cdc.gov/ncidod/eid/upcoming.htm

Upcoming Infectious Disease Activities

April 9–12, 2005
Society for Healthcare Epidemiology of America (SHEA) Annual Meeting
Los Angeles, CA, USA
Contact: 703-684-1006
Web site: http://www.shea-online.org

May 1, 2005
International Society of Travel Medicine (ISTM) offers certificate of knowledge in travel medicine exam (Given prior to the opening of 9th Conference of the ISTM)
Contact: exam@istm.org
http://www.ISTM.org/

May 1–5, 2005
9th Conference of the International Society of Travel Medicine
Lisbon, Portugal
Contact: 49-89-2180-3830
http://www.ISTM.org/

May 3–5, 2005
Controlling Infectious Agents and Other Contaminants in Healthcare Facilities through Planning and Design
Madison, WI, USA
Contact: 800-462-0876
http://epd.engr.wisc.edu/emaG041

May 9–11, 2005
The Eighth Annual Conference on Vaccine Research
Baltimore Marriot Inner Harbor Hotel
Baltimore, MD, USA
Contact: 301-656-0003 ext. 12 or vaccine@nfid.org
http://www.nfid.org

May 11–14, 2005
7th International Meeting on Microbial Epidemiologic Markers (IMMEM7)
Victoria, British Columbia, Canada
http://www.asm.org/Meetings/index.asp?bid=27725

EMERGING INFECTIOUS DISEASES www.cdc.gov/eid

JOURNAL BACKGROUND AND GOALS

What are "emerging" infectious diseases?

Infectious diseases whose incidence in humans has increased in the past 2 decades or threatens to increase in the near future have been defined as "emerging." These diseases, which respect no national boundaries, include

- New infections resulting from changes or evolution of existing organisms.
- Known infections spreading to new geographic areas or populations.
- Previously unrecognized infections appearing in areas undergoing ecologic transformation.
- Old infections reemerging as a result of antimicrobial resistance in known agents or breakdowns in public health measures.

Why an "Emerging" Infectious Diseases journal?

The Centers for Disease Control and Prevention (CDC), the agency of the U.S. Public Health Service charged with disease prevention and health promotion, leads efforts against emerging infections, from AIDS, hantavirus pulmonary syndrome, and avian flu, to tuberculosis and West Nile virus infection. CDC's efforts encompass improvements in disease surveillance, the public health infrastructure, and epidemiologic and laboratory training.

Emerging Infectious Diseases represents the scientific communications component of CDC's efforts against the threat of emerging infections. However, even as it addresses CDC's interest in the elusive, continuous, evolving, and global nature of these infections, the journal relies on a broad international authorship base and is rigorously peer-reviewed by independent reviewers from all over the world.

What are the goals of Emerging Infectious Diseases?

1) Recognition of new and reemerging infections and understanding of factors involved in disease emergence, prevention, and elimination. Toward this end, the journal

   - Investigates factors known to influence emergence: microbial adaptation and change, human demographics and behavior, technology and industry, economic development and land use, international travel and commerce, and the breakdown of public health measures.
   - Reports laboratory and epidemiologic findings within a broader public health perspective.
   - Provides swift updates of infectious disease trends and research: new methods of detecting, characterizing, or subtyping pathogens; developments in antimicrobial drugs, vaccines, and prevention or elimination programs; case reports.

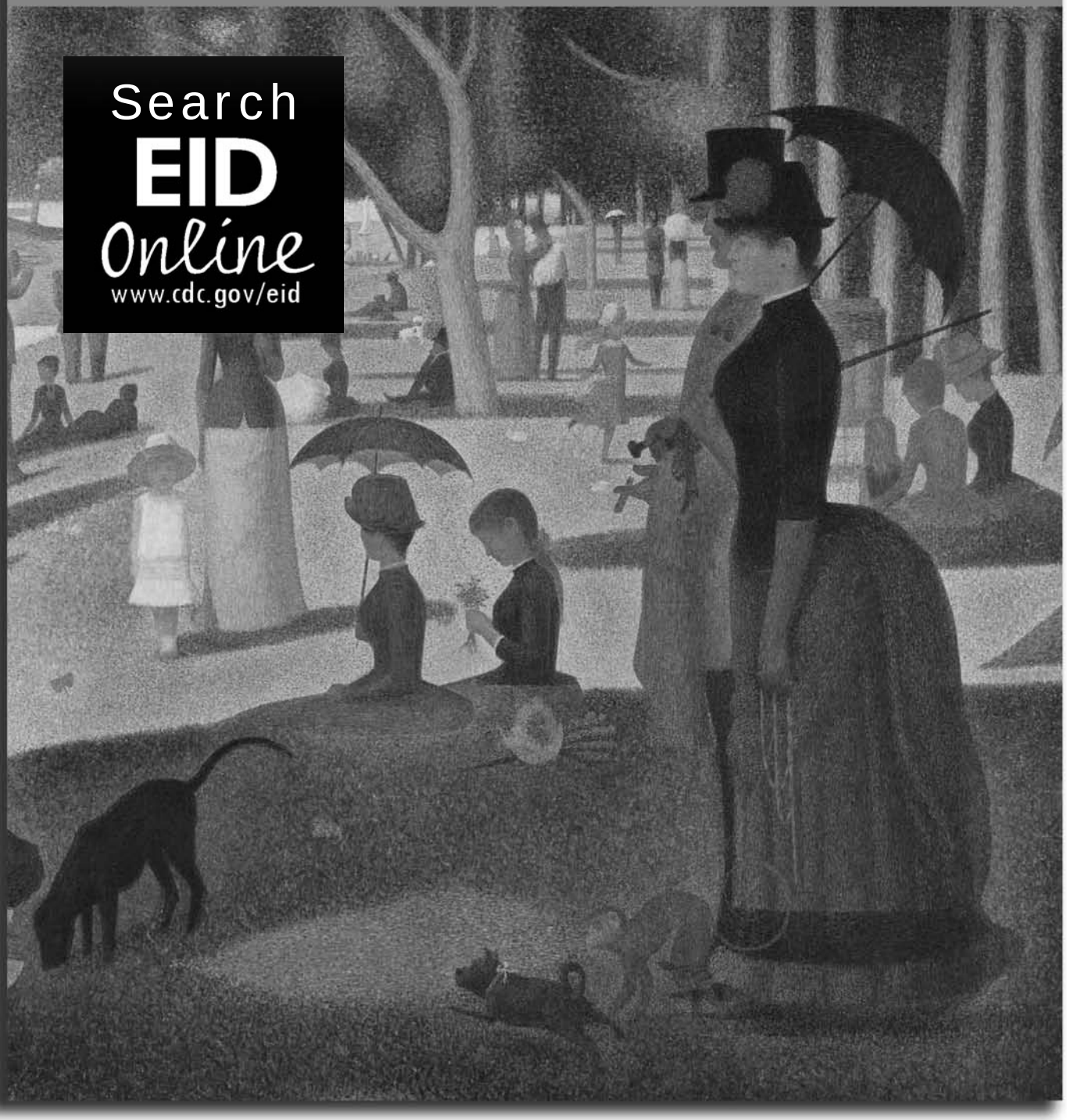
2) Fast and broad dissemination of reliable information on emerging infectious diseases. Toward this end, the journal

   - Publishes reports of interest to researchers in infectious diseases and related sciences, as well as to public health generalists learning the scientific basis for prevention programs.
   - Encourages insightful analysis and commentary, stimulating global interest in and discussion of emerging infectious disease issues.
   - Harnesses electronic technology to expedite and enhance global dissemination of emerging infectious disease information.

EMERGING INFECTIOUS DISEASES

EID Online www.cdc.gov/eid

A Peer-Reviewed Journal Tracking and Analyzing Disease Trends Vol.11, No.3, March 2005



EMERGING
INFECTIOUS DISEASES
A Peer-Reviewed Journal Tracking and Analyzing Disease Trends

Editorial Policy and Call for Articles

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 http://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 (http://www.cdc.gov/eid/ncidod/EID/trans.htm).

Instructions to Authors

Manuscript Preparation. For word processing, use MS Word. Begin each of the following sections on a new page and in this order: title page, 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 and Figures. Create tables within MS Word's table tool. Do not format tables as columns or tabs. Send graphics in native, high-resolution (200 dpi minimum) .TIF (Tagged Image File), or .EPS (Encapsulated Postscript) format. Graphics should be in a separate electronic file from the text file. For graphic files, use Arial font. Convert Macintosh files into the suggested PC format. Figures, symbols, letters, and numbers should be large enough to remain legible when reduced. Place figure keys within the figure. For more information see EID Style Guide (http://www.cdc.gov/ncidod/ EID/style_guide.htm).

Manuscript Submission. Include a cover letter indicating the proposed category of the article (e.g., Research, Dispatch) and verifying that the final manuscript has been seen and approved by all authors. Complete provided Authors Checklist. To submit a manuscript, access Manuscript Central from the Emerging Infectious Diseases web page (www.cdc.gov/eid).

Types of Articles

Perspectives. Articles should be under 3,500 words and should include references, not to exceed 40. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), a one-sentence summary of the conclusions, and a brief biographical sketch of first author. 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 of first author—both authors if only two. 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 of first author—both authors if only two. 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 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 1,000–1,500 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 two); and a brief biographical sketch of first author—both authors if only two. 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 one Figure or Table and should not be divided into sections. All letters should contain material not previously published and include a word count.

Book Reviews. Short reviews (250–500 words) of recently published books on emerging disease issues are welcome. The name of the book, publisher, and number of pages should be included.

Announcements. We welcome brief announcements (50–150 words) of timely events of interest to our readers. (Announcements may be posted on the journal Web page only, depending on the event date.)

Conference Summaries. Summaries of emerging infectious disease conference activities are published online only (effective January 2005). 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.