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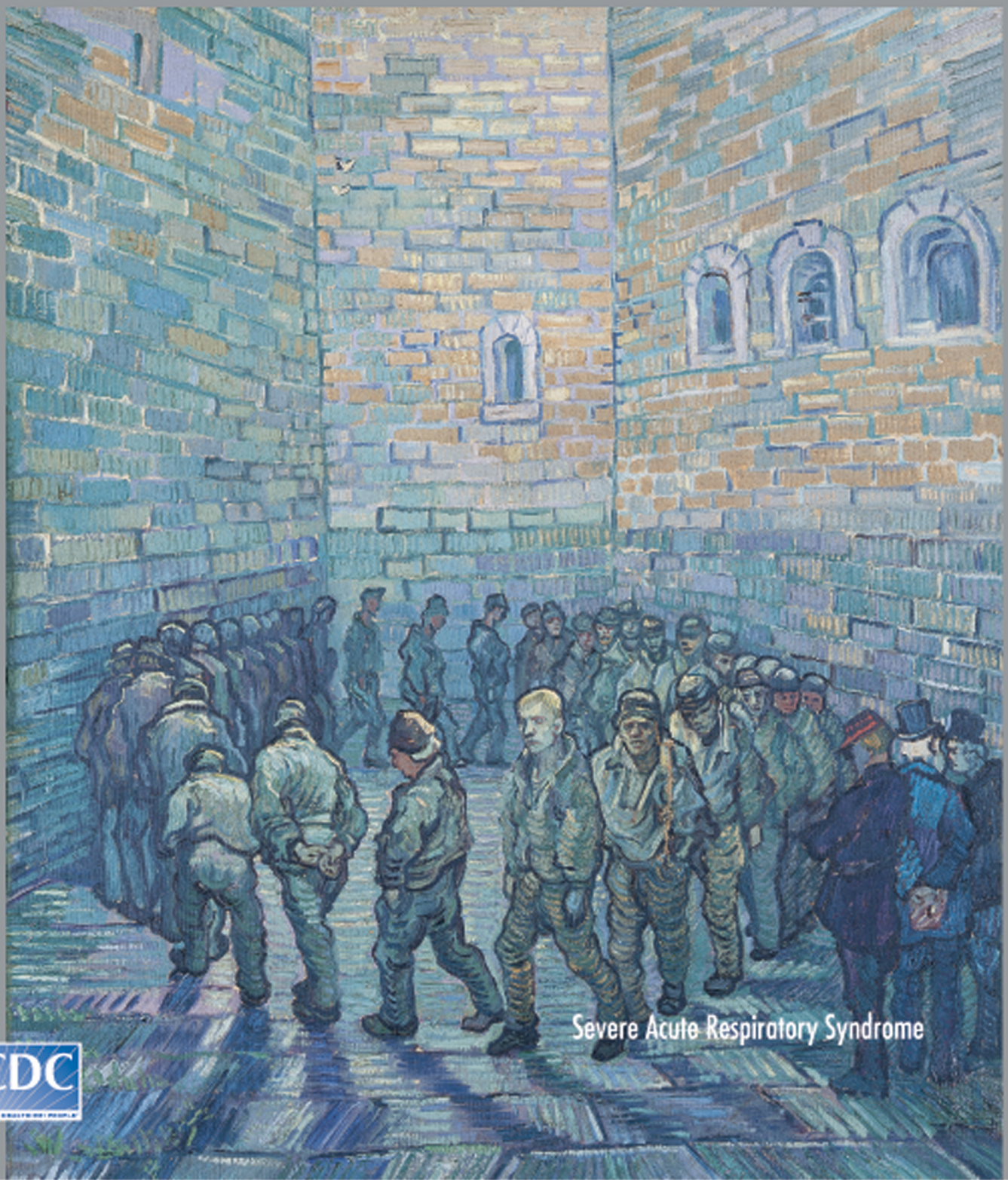

Severe Acute Respiratory Syndrome

CDC

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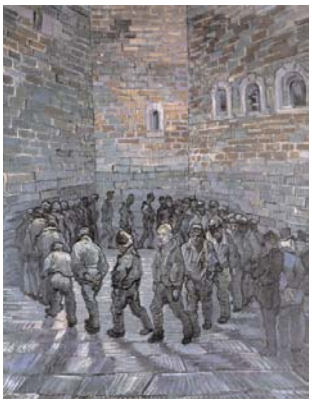

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The Prison Courtyard (1890)
Oil on canvas, 80 cm x 64 cm.
The State Pushkin Museum
of Fine Arts, Moscow, Russia

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Role of China in the Quest To Define and Control Severe Acute Respiratory Syndrome

Robert F. Breiman,* Meirion R. Evans,† Wolfgang Preiser,‡ James Maguire,§ Alan Schnur,¶ Ailan Li,¶ Henk Bekedam,¶ and John S. MacKenzie#[1]

China holds the key to solving many questions crucial to global control of severe acute respiratory syndrome (SARS). The disease appears to have originated in Guangdong Province, and the causative agent, SARS coronavirus, is likely to have originated from an animal host, perhaps sold in public markets. Epidemiologic findings, integral to defining an animal-human linkage, may be confirmed by laboratory studies; once animal host(s) are confirmed, interventions may be needed to prevent further animal-to-human transmission. Community seroprevalence studies may help determine the basis for the decline in disease incidence in Guangdong Province after February 2002. China will also be able to contribute key data about how the causative agent is transmitted and how it is evolving, as well as identifying pivotal factors influencing disease outcome.

Severe acute respiratory syndrome (SARS) is a newly emerged disease, caused by a previously unknown coronavirus. The first known cases occurred in Guangdong Province in southern China in November and December 2002. During late February 2003, a physician who was incubating SARS traveled from Guangzhou, the provincial capital, to Hong Kong, Special Administrative Region of China, and stayed at a hotel. There, the virus was transmitted from him to local residents and to travelers, who became ill and transmitted disease to others when they returned to Vietnam, Singapore, Canada, and Taiwan, Province of China (1). SARS has now occurred in >8,450 people with >800 deaths worldwide.

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The tally of SARS climbed rapidly in China through May 2003, then decelerated markedly during June. The disease has now been reported in 24 of China's 31 provinces. By June 26, 2003, a total of 5,327 SARS cases and 348 deaths had been reported from mainland China, including 2,521 cases in Beijing and 1,512 in Guangdong Province.

Since February 2003, teams of technical consultants for the World Health Organization have been working in China to provide assistance to the Ministry of Health and provincial governments on public health responses to the SARS outbreak. A team that began working in China in March reviewed considerable clinical, epidemiologic, and laboratory data with scientists and officials from a variety of settings in Guangdong Province and Beijing. The team worked closely with colleagues from the National and Guangdong Provincial Centers for Disease Control, and together were able to establish that cases occurring in Guangdong beginning in November were clinically and epidemiologically similar to subsequent cases of SARS documented elsewhere.

The team observed detailed, comprehensive data collection forms, which are completed for activities and behaviors and clinical manifestations of patients with SARS. The team was informed that serum and respiratory secretion specimens collected from many patients from Guangdong were being held under appropriate storage conditions, awaiting further laboratory testing.

While a dedicated, collaborative international effort has resulted in substantial understanding of this disease with remarkable speed, critical information is still lacking. We detail a variety of knowledge gaps that should be addressed through a set of activities to optimize prevention and control of SARS.

[1]Drs. Breiman, Evans, Preiser, Maguire, and MacKenzie were consultants for the World Health Organization, assisting its Beijing office.

Emergence of SARS-associated Coronavirus in Humans

Available evidence suggests that SARS emerged in Guangdong Province, in southern China. How and when did it emerge? Did the causative agent evolve in an animal species and jump to humans (or perhaps first to other animal species), or did the virus evolve within humans? The genetic sequence of the virus has been obtained in several laboratories, and phylogenetic analyses have shown that it is unlike other coronaviruses of animal and human origin. Indeed, the virus has been tentatively placed in a new fourth genetic group (2,3).

Why is it so important to answer the question of how SARS emerged? Most recently recognized novel emergent viruses have been zoonotic, usually with a reservoir in wildlife (4,5). Thus, SARS coronavirus, if zoonotic, may provide the basis for modeling and predicting the appearance of other potential zoonotic human pathogens. More importantly, the information may be crucial for control of SARS. If this disease is to be curtailed or eliminated by strict public health measures, blocking further animal-to-human transmission is indicated. Only about half of the cases in Guangdong are attributed to contact with a SARS patient. Transmission from an unknown, but persisting animal reservoir might explain this finding; however, a nonspecific case definition (i.e., many "cases" might not actually be SARS) and limitations in contact-tracing capacity are other potential explanations.

Finding a potential animal source is, however, a daunting task. The province is famous for its "wet markets," where a bewildering variety of live fauna are offered for sale (sometimes illegally) for their medicinal properties or culinary potential. The opportunity for contact, not only with farmed animals but also with a variety of otherwise rare or uncommon wild animals, is enormous. More than one third of early cases, with dates of onset before February 1, 2003, were in food handlers (persons who handle, kill, and sell food animals, or those who prepare and serve food) (Guangdong Province Center for Disease Control and Prevention, unpub. data,).

Hypothesis-generating epidemiologic studies are indicated to focus on early cases of SARS and cases in persons without known contact with infected persons. These studies should also collect information from appropriately selected controls (i.e., matched by categories such as community and age), regarding exposures to animals of any kind in any setting (including food preparation, dietary habits, pets, and a variety of other activities and behaviors in the community).

Plausible hypotheses generated by epidemiologic studies should be briskly followed by intensive, focused, laboratory studies where relevant, including surveys of specific animal populations to identify SARS-associated coronaviruses (by culture and polymerase chain reaction [PCR]) or to measure specific antibodies. Some virologic surveys have already been conducted among prevalent animal populations, including those known to harbor other coronaviruses or other viruses transmissible to humans or wild animals, handled and sold in the markets; a variety of animals, most notably masked palm civets, have been reported to harbor SARS-associated coronavirus. However, whether these animals are transmitting virus or are recipients of virus transmission is not yet clear. Solutions will lie with identifying epidemiologic links, which should guide targeted animal studies. Molecular epidemiologic and genetic studies can then be helpful in evaluating viruses isolated from animals and from humans.

Natural History of the Epidemic

Since the earliest known cases were in Guangdong Province, China has had more time than any other location to observe disease incidence over time. Evidence from Guangdong Provincial Centers for Disease Control suggests that the disease incidence peaked in mid-February, and declined weekly through May. What were the reasons for the decline? Introduction of stringent infection-control measures in hospital settings undoubtedly resulted in reduced incidence in healthcare settings but would not likely have accounted for reductions in community transmission. Efforts have been made to reduce the interval between onset of illness and hospitalization (minimizing the potential for community transmission). This effort likely had substantial impact in reducing disease incidence, as shown elsewhere (6).

The initial hypothesis was that the virus attenuated after multiple generations of transmission; this hypothesis now seems unlikely. We note several other considerations. Were there a limited number of susceptible people within the population to begin with? Such a concept is possible if there had been earlier spread of a less virulent coronavirus, providing some immunity to a proportion of the population. If so, whether this occurrence was unique to Guangdong will be important to determine.

Alternatively, did the population develop widespread immunity to the causative agent itself? This scenario would require a good deal of asymptomatic or mildly symptomatic disease. At this stage, no reason exists to exclude the possibility of a much wider spectrum of disease than is currently appreciated, since the spectrum of illness has not been fully evaluated.

Another possibility is that a second agent might be required, in addition to coronavirus, to produce severe illness; if this is the case, the epidemiology (like seasonality) of the second agent (perhaps a less recently emerged pathogen for which there is already fairly widespread immunity), rather than coronavirus, may actually be

responsible for the decline of the incidence of SARS in Guangdong.

Extensive seroprevalence studies will be helpful for sorting through these possibilities. Analyzing stored serum samples, collected before the onset of this outbreak, could be of immense value in evaluating the possibility of preexisting immunity. Some researchers have found human metapneumoviruses (7) and species of *Chlamydia* in patients with SARS, but the importance of these findings is unclear. Systematic evaluation of specimens available from all cases, severe cases, and healthy controls in China regarding the presence of antibodies to coronavirus, as well as hypothesized co-infecting agents, should be done.

Important clues may come from seroprevalence and other epidemiologic studies in children. As in other affected countries, children were disproportionately less affected by SARS than adults. Carefully working through the bases for reduced incidence and severity may uncover cross-protecting infectious or immunizing agents or crucial host factors for protection.

Super-Spreading Events

When documenting the source of person-to-person transmission of SARS has been possible, a substantial proportion of cases have emanated from single persons, so-called super-spreaders (l). While contact tracing is undoubtedly incomplete, most infected patients have transmitted illness to few other people. Understanding the differentiating characteristics of persons who transmit, especially patients who are able to transmit to several other people, often after minimal contact, may provide important clues for public health strategies focused on preventing transmission. In addition, better defining environmental settings or circumstances that facilitate high transmission rates would be helpful. China is not unique in documenting super-spreaders. The country could participate in multinational studies to define the characteristics of super-spreaders and their role in the epidemiology of SARS. Of particular interest is the virus load of super-spreaders, compared with those of other infected persons.

Little is known about the importance of fecal-oral transmission or about the length of time that infectious virus shedding occurs in the gastrointestinal tract. Virus shedding in feces has major implications for control strategies and for the possibility of continued carriage and shedding by clinically recovered patients. China has the opportunity to explore the role of fecal spread in the transmission of SARS.

Evolution of the Virus

The causative agent is a coronavirus (8–10), and the entire genome of several strains has been fully sequenced by many laboratories globally (2,3,11). Tests have been developed to detect coronavirus genetic sequences by PCR. In addition, tests to detect SARS-associated coronavirus antibodies have been developed, but the sensitivity and specificity of these tests are low, especially early in the illness when public health and clinical needs are greatest. A good test for SARS would be important not only for diagnosis and management but also for investigating the origin of the disease and for defining its epidemiology.

If the causative agent can be isolated from stored specimens from the earliest group of patients (from November 2002 to January 2003), how their genetic sequences compare with those from viruses isolated later from various parts of China and elsewhere, and from animals from Guangdong and Guanxi Provinces, would be useful to know. Mutations may be important for a number of reasons. They may affect transmissibility and virulence; they may provide (or frustrate) therapeutic targets for new drugs; and they may pose challenges for development of diagnostic tests and vaccines. Specimens from Chinese patients provide the longest observation window with which mutational tendencies can be evaluated.

An analysis of 14 full-length sequences suggests that two genetic lineages might have arisen from Guangdong. One lineage is represented by the chain of transmission associated with the physician from Guangzhou who traveled to Hong Kong, Special Administrative Region, in February. The other lineage is associated with isolates from Hong Kong, Guangzhou, and Beijing (11). If two genetic lineages arose in Guangdong, were there two separate transmission events from an animal host to humans, or did the lineage diverge within humans? Specimens from early cases in Guangdong may be helpful in addressing this question.

Outcomes of Infection

Epidemiologic, immunologic, and microbiologic factors associated with severe outcome are not fully defined. Clearly, though, a principal determinant for poor outcome is advancing age. As with other respiratory diseases, age-related coexisting conditions reduce the capacity to compensate to conditions associated with severe disease. Understanding other specific factors that result in poor outcome will have value for optimizing therapeutic approaches.

Clinicians disagree about the value of early treatment with ribavirin and high-dose corticosteroids, and some are reticent to ventilate patients because of high risk for transmission to healthcare workers associated with intubation. More data are needed to help define the most effective treatment strategy, particularly for areas with limited resources.

Extraordinary clinical expertise exists among health professionals in Guangdong Province. They have substantial experience with a variety of antivirals, antibiotics,

alternative (herbal) medicines, and corticosteroids, and with using assisted ventilation in the treatment of patients with SARS (12). While randomized clinical trials have not been conducted, careful compilations of existing case series data would be helpful in evaluating the potential effectiveness of various management regimens.

The store of clinical data, accumulated from treating hundreds of SARS cases, needs to be put to good use. One priority is to investigate clinical, epidemiologic, and laboratory predictors of poor outcome. Such experience will supplement other recently published data from Hong Kong, Special Administrative Region (1,13–15), and Singapore (16).

Several questions remain unanswered. Do patients exposed to high viral doses (for which a short incubation period may be a surrogate) or to a co-infecting pathogen have poorer outcomes? What is the impact of multiple exposures to SARS-associated coronavirus, like that which occurred among healthcare workers early in the epidemic? Do patients infected early in the transmission cycle perform more poorly than those infected during subsequent cycles of transmission?

Learning from the SARS Epidemic

Seldom have intersections between politics, economic development, and public health been more graphically demonstrated. While awaiting the development of effective prophylactic and therapeutic options, many countries have had to muster substantial political will for quick and transparent steps to declare the presence of a lethal pathogen within their borders; conduct surveillance and report the results; use contact tracing, quarantine, and border control measures when needed; and apply stringent infection control measures in healthcare settings. Providing the general public with timely and candid information about the magnitude of the problem, the known risks, and how persons can protect themselves has also been necessary. These actions were necessary even when they appeared contrary to economic interests in the short run. Delaying implementation can result in major public health consequences, in addition to damage to the economy and national image.

The work outlined here involves descriptive and epidemiologic inquiry, fundamental to establishing an understanding of this new pathogen and disease. While refined and esoteric research will likely also be conducted, support must first be established for systematically addressing these basic questions and rapidly disseminating results through publication in international journals, presentations at international meetings, and in public communications. In China, in contrast with many other settings globally, scientific inquiry and dissemination of results to the international community are subject to institutional interference. The SARS pandemic has shown that virulent pathogens are beholden to no political philosophy or edict. Only careful and rapid application of knowledge and reason through a variety of public health measures has been effective in minimizing the spread and severity of the SARS epidemic. More information and data generated from studies of the epidemic in China are needed immediately to save lives and to prevent fear and disease, both in China itself and elsewhere in the world.

SARS became a public health emergency for China, where investment in health services has been given low priority for many years. Maintaining control in a country so large and diverse will be a major challenge for the months, and perhaps years, to come. Each of China's mainland provinces (including municipalities with equivalent status, autonomous regions, and special administrative regions) is like a country within a country. Many are larger than most countries in Europe. Some, such as Shanghai, are wealthy and highly developed, while others such as Guangxi (bordering Guangdong and Vietnam) are poor and typical of developing countries. Given the potential for reemergence of SARS in the future, if sustained control measures are not in place in China, the possibility of controlling the global threat posed by the disease until new technology (i.e., an effective vaccine) is available may be slight. Key strategies include effective disease surveillance and reporting with early detection and isolation; hospital infection control during triage and treatment of cases; and transparent, open public communication about risk and disease magnitude.

China has recently begun to vigorously address the need for better surveillance, accurate reporting, and forthright public communication. Substantial epidemiologic, clinical, virologic, and immunologic expertise and interest are available within China to address the fundamental questions. International expertise is also available to provide guidance, feedback, and assistance when requested. Identifying the modest resources needed to implement the work should not be a barrier. Support from the government will be needed to carry out valid, transparent studies, and for permission to report the findings, regardless of the conclusions. SARS provides a jarring reminder of the preparedness that is needed to respond to emerging and existing disease threats; it highlights the need to reinvest in health in China, and strengthen public health programs, including surveillance systems and response capacity.

While disease incidence has abated in China and in other locations globally, the disease may still represent an important threat in the future. Many of the solutions to solve the multifaceted puzzle of SARS and to prevent future epidemics must come from China. Without solutions from that country, the degree of difficulty for sustained control of the problem globally is raised still higher.

Dr. Breiman is head of the Programme on Infectious Diseases and Vaccine Sciences at the International Centre for Diarrheal Disease Research, Bangladesh–Centre for Health and Population Research in Bangladesh. His research focuses on evaluating new vaccines for use in developing countries and on the epidemiology of emerging infectious diseases. Previously he directed the United States National Vaccine Program Office and was chief of the Epidemiology Section of the Childhood and Respiratory Diseases Branch, Division of Bacterial and Mycotic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention.

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Lessons from the Severe Acute Respiratory Syndrome Outbreak in Hong Kong

Abu S.M. Abdullah,* Brian Tomlinson,† Clive S. Cockram,† and G. Neil Thomas*[1]

Severe acute respiratory syndrome (SARS) is now a global public health threat with many medical, ethical, social, economic, political, and legal implications. The nonspecific signs and symptoms of this disease, coupled with a relatively long incubation period and the initial absence of a reliable diagnostic test, limited the understanding of the magnitude of the outbreak. This paper outlines our experience with public health issues that have arisen during this outbreak of SARS in Hong Kong. We confirmed that case detection, reporting, clear and timely dissemination of information, and strict infection control measures are essential in handling such an infectious disease outbreak. The need for an outbreak response unit is crucial to combat any future outbreak.

Severe acute respiratory syndrome (SARS) originated in November 2002 in the Guangdong Province of China and, by February 2003, had spread to Hong Kong and subsequently to 32 other countries or regions, infecting approximately 8,459 patients and resulting in >800 deaths (1). The overall mortality rate is approximately 14% to 15%, ranging from <1% in persons <24 years of age to >50% in persons >65 years of age (2). The cause of SARS is not yet confirmed, but a novel coronavirus has been identified and resembles the virus found in civet cats (3,4). SARS is the latest in a series of new infectious diseases (e.g., HIV/AIDS, Ebola, Nipah, and Avian H5N1 influenza) that are adding additional stress to a healthcare system already dealing with the resurgence of established conditions (e.g., dengue, malaria, and tuberculosis). As global air travel is now commonplace and has facilitated the international spread of SARS, identifying and globally publicizing the lessons learned from the latest outbreak are important.

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Risk Factors for Spread of SARS

Mode of Transmission

The mechanism of transmission of the agent or agents causing SARS is not yet fully understood but is probably mainly by droplet secretions, fomites, or person-to-person contact. To date, no evidence of airborne transmission exists. In the Amoy Gardens outbreak in Hong Kong, aerosolization of fecal waste contaminated with the SARS agent has also been proposed to have contributed to transmission. The virus has been reported to be stable in feces and urine at room temperature for at least 1–2 days, and up to 4 days in stool from patients experiencing diarrhea (5). After drying on plastic surfaces, the virus can survive for up to 48 hours, although commonly used disinfectants and fixatives are effective against it (5).

Super-spreading patients may play a role in the spread of the disease. For instance, the Hong Kong index patient is thought to have infected persons who transmitted the virus worldwide, subsequently resulting in outbreaks of >300 patients in Amoy Gardens in Hong Kong and >60 cases in Singapore (6–10). These last two clusters may have been started by two persons undergoing hemodialysis. Another hemodialysis patient has been involved in the transmission of SARS in Toronto; therefore, such patients, who may have a relatively depressed immune system with associated high viral loads, may be unduly facilitating transmission of the virus. A more direct role of hemodialysis patients in the spread of viral infections has been previously observed in Edinburgh, Scotland, in the late 1960s, where transmission of hepatitis B was associated with mortality rates of 24% and 31% in renal patients and staff members, respectively (11).

[1]Abu S.M. Abdullah and G. Neil Thomas, the guarantor, collated the data and drafted the manuscript. Brian Tomlinson and Clive S. Cockram have been treating SARS patients since the beginning of the outbreak. All authors were involved with the final production of the manuscript.

Existing Medical Practices

Procedures, such as the use of ventilators and nebulized bronchodilators, have been reported to have led to spread by droplet transmission and aerosolization of virus-containing particles (12,13). Similarly other procedures, such as cardiopulmonary resuscitation, use of positive airway pressure devices, bronchoscopy, endotracheal intubation, airway suction, and sputum suction are thought to increase risk for infection (7). Although the use of such equipment in the treatment of most pneumonias, except influenza, presents no risk to staff, the emergence of SARS has thrown into sharp focus the general safety of such routine practices, particularly when dealing with novel infectious agents. The SARS outbreak is unique in its propensity to infect healthcare workers; for instance, in China approximately 20% of cases are in healthcare workers, and early in the outbreak the rate was closer to 90% (14,15).

Simple measures such as hand washing after touching a patient, the use of an appropriate and well-fitted facemask, and the introduction of infection control measures at an early stage, as well as quarantine of patients, may have reduced transmission (12). Restricting visitors to the hospital would further reduce the risk for transmission into the community. However, despite stringent use of full infection control procedures, breakthrough cases of SARS still occurred in healthcare workers.

Coronavirus infections have been reported to infect lymphocytes, reducing their numbers in the Hong Kong patients by 30% (13). Immune-mediated cellular damage to the lungs has been reported (7) and has prompted the use of steroids in these patients. Given the role of super-spreading patients (10), who have relatively depressed immune systems, steroid use may further increase the viral load and prolong shedding of viable viral particles past the 1–2 weeks after symptoms disappear, potentially increasing the transmission of the disease and the duration of infectivity of the patient.

Complexity of the SARS Outbreak

Two overlapping sets of disease signs and symptoms have been reported, with some patients having varying degrees of enteric disease. Patients from China and at a number of Hong Kong hospitals have had relatively low rates of diarrhea (10% to 20%) (3,13), whereas patients from Amoy Gardens and Canada have had higher rates, 50% to 70% (9,16,17). Some of these differences may result from the timing of data collection, with collection of data later in the course of the patient's illness including symptoms of diarrhea that may be associated with antibiotic therapy. However, these data suggest that possible differences in the mode of transmission, such as respiratory droplet compared to fecal-oral, or differences in the specificity of the organism to the respiratory or gastrointestinal tracts may also be present. Mutations in isolates from respiratory or gastrointestinal tracts from the same cattle infected with coronavirus have been previously reported (18); such mutations may contribute to the observed differences in symptoms.

The nonspecific disease signs and symptoms, long mean incubation period (6.4 days), long time between onset of symptoms and hospital admission (from 3 to 5 days) (6), and lack of a reliable diagnostic test in the early phase of the illness (19) can lead to potential transmission to frontline healthcare workers and the community. Similarly, the signs and symptoms in elderly patients, in whom the primary disease phase may be muted without evident fever, may further contribute to the spread of SARS. Additionally, as with other diseases, misdiagnosis can have fatal consequences. For example, in 2001, an airline cabin crew member infected with malaria was misdiagnosed by two physicians, who did not identify the fact that she had recently traveled to a malaria-endemic area (20). She was treated for a common cold and died within 1 week of a malaria diagnosis by a tropical medicine specialist. Misdiagnosis of a case of SARS, particularly in a super-spreader in whom the disease symptoms may differ, could lead to rapid dissemination through the population. The nonspecific features and lack of an early diagnostic test have also led to difficulty of diagnosis with a potential threat to the community if such patients are discharged.

Disseminating Information

The accuracy and timeliness of the reporting and dissemination of data relating to SARS are important issues affecting public perception, and hence, fear, as well as the implementation of programs to limit spread of the disease. Inadequate reporting of cases (21) may have hindered implementation of preventative measures. Similarly, media attention, which plays a major role in the widespread dissemination of information, has a tendency to sensationalize information, leading to misconceptions over community preventative strategies, government and institutional procedures, and the magnitude of the outbreak. On the other hand, lack of information led to the development of public myths, with people in Guangdong believing that boiling white vinegar would protect them from infection and leading to carbon monoxide poisoning from charcoal burning to heat the vinegar (22).

Challenges to the Medical Community and Future Directions

SARS presents formidable challenges to the healthcare community with medical, social, political, legal, and economic implications. All countries have to be prepared at a number of levels to deal with the threat posed by the SARS epidemic and any other novel infectious disease. The

healthcare sector should consider a few issues: 1) SARS has emphasized the need for stringent infection control measures in hospitals on a regular basis, in anticipation of the next epidemic. While the measures may be in place, are we sure that they are being properly implemented at all times? 2) Healthcare workers should always follow simple, but stringent hygienic practices (e.g., washing hands before and after seeing a patient, even when no epidemic is apparent). 3) Appropriate history taking, to obtain important information, such as recent travel history or contacts with possibly infected persons, when a patient with a fever is seen, could help to quickly identify persons at risk and reduce spread. 4) Given the association with a number of super-spreaders and renal dialysis patients, strict quarantine procedures should be implemented if such persons are suspected of having SARS. 5) The concepts of specificity and sensitivity need to be widely understood and applied. Although the need for rapid diagnostic tests is important, introducing tests with inadequate sensitivity and unknown specificity should be prevented, as the data cannot be interpreted. A negative test does not always exclude a disease, and discharging patients later diagnosed and readmitted could have serious consequences. 6) The use of high-risk medical procedures that may inadvertently spread the disease through aerosolization of the agent should be evaluated with potential new diseases in mind. Other high-risk procedures should also be reconsidered with regard to infection control to limit risk from the use of intubation, cardiopulmonary resuscitation, and positive airway pressure devices. 7) Quarantine and isolation procedures and contact tracing need to be instituted early in the outbreak, and access to hospitals treating such patients needs to be restricted to limit spread into the community. 8) Environmental hygiene needs to be maintained. In the wake of the SARS outbreak, the Hong Kong government has introduced a number of measures to improve public hygiene, including closely monitoring the integrity of sewage disposal systems (deficiencies that were a possible source of the Amoy Gardens outbreak). The government has increased penalties for spitting, which still remains a commonplace habit.

As with the outbreak of avian influenza, in which humans became infected through the purchase of live poultry, a process that still continues, the SARS virus appears to have been contracted from an animal source (possibly civet cats [23]) used for human consumption. Close contact between humans and animal vectors in the southern China region has been responsible for a number of epidemics, including influenza A. A reduction in exposure to animal viral reservoirs should reduce the occurrence of such events. To that end, the Chinese government has increased implementation of laws that prevent the consumption of wild animals.

Timely communication and exchange of complete, accurate information are important during any epidemic. Difficulties in obtaining information from all relevant sources could delay appropriate analyses, reporting of the situation, and implementation of necessary actions. Plans for integration of appropriate agencies should be made in advance of any epidemic. The data collected should be two-tiered to include essential information required to control the outbreak, such as clinical details and contact information, as well as more detailed data that will enable ongoing or retrospective evaluation to determine, for instance, mode of transmission, which remains unconfirmed.

An epidemic like SARS has an impact on many sectors of the society. Leadership is essential to coordinate activities and information dissemination in order to minimize confused messages and public panic. Coordination should be maintained with all relevant sectors including the health professionals, policymakers, community leaders, media, and the public.

Early detection and handling systems need to be consolidated to prepare for future epidemics. To this end, the Hong Kong government has announced the allocation of HK$1 billion (US$1=HK$7.8) to fund a center for disease control. The role for the center remains to be clarified but should include monitoring for novel infections and research into existing agents. The center should also include an outbreak response unit that can be called on to spearhead coordinated action in a timely manner. The team should include infectious disease and public health specialists, epidemiologists, media spokespersons, administrators with suitable connections to frontline healthcare units, and other statutory bodies to enable collation and dissemination of important information and risk communication to relevant stakeholders. The unit will require legislative power to enable the rapid initiation of control measures both in the hospitals and the community.

As no prophylaxis vaccination or specific proven treatment is yet available against SARS, prevention is the only measure that one can take to prevent epidemics. Communicating the risks and preventive measures in an effective and acceptable manner is important.

SARS has had a significant impact on the local healthcare system; a high proportion of patients require intensive care, coupled with prolonged hospitalization, overloading the system. Similarly, the ready transmission to hospital care workers reduced the availability of knowledgeable healthcare workers to treat other patients and colleagues, and this further limited the ability of the hospitals to cope with the current outbreak. In summary, the current SARS outbreak provides a timely reminder of the importance of maintaining basic healthcare practices at all times so that, when the next new disease strikes, we are well prepared to

deal with it. Establishing an outbreak response unit within the healthcare sector should be a priority with appropriate resources.

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Automated, Laboratory-based System Using the Internet for Disease Outbreak Detection, the Netherlands

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Rapid detection of outbreaks is recognized as crucial for effective control measures and has particular relevance with the recently increased concern about bioterrorism. Automated analysis of electronically collected laboratory data can result in rapid detection of widespread outbreaks or outbreaks of pathogens with common signs and symptoms. In the Netherlands, an automated outbreak detection system for all types of pathogens has been developed within an existing electronic laboratory-based surveillance system called ISIS. Features include the use of a flexible algorithm for daily analysis of data and presentation of signals on the Internet for interpretation by health professionals. By 2006, the outbreak detection system will analyze laboratory-reported data on all pathogens and will cover 35% of the Dutch population.

Rapid detection of outbreaks on a time scale compatible with disease incubation periods is recognized as crucial to maximize the effect of control measures. Most outbreaks are rapidly detected and controlled locally. However, outbreaks involving cases over a wider area or in several local health jurisdictions may have only few local cases and thus be easily missed, especially if the outbreak has a slowly rising number of cases. Outbreaks of certain pathogens with common signs and symptoms (e.g., gastroenteric disease) can also be missed. The role of national laboratory data in detecting such outbreaks has been increasingly recognized in the last few years as modern typing techniques give more precision on the pathogen type and subtype, routinely unearthing outbreaks by linking cases either locally, nationally, or internationally (1–4) that otherwise would probably not be detected. In addition, surveillance of a wide range of pathogens is essential in identifying emerging disease threats (5,6). The increasingly perceived threat of bioterrorism recently has made more urgent the need for rapid detection of increases in laboratory diagnoses of common and uncommon pathogens to complement clinician-based reporting systems. Increasing computational power in the last 10 years has resulted in the development of mathematical algorithms to routinely and rapidly detect significant clusters within large amounts of surveillance data (7–12). Automated electronic laboratory reporting is frequently promoted to improve data quality and timeliness of collection (13). More recently, the general availability of the Internet permits feedback to many users, who can have continuous, simultaneous, and even interactive access to information. The Internet allows for immediate communication of signals of possible outbreaks to relevant professionals for interpretation and action.

In the Netherlands, these developments have led to the implementation of automated laboratory-based surveillance system integrated with the Internet in a project named the Infectious Disease Surveillance Information System (ISIS). We describe the development of an automated outbreak detection system within ISIS for all laboratory-reported pathogens in the Netherlands. The system is updated daily with Web-based feedback.

Overview of National Laboratory Surveillance

In the Netherlands, >90% of the 76 microbiologic laboratories are associated with public hospitals; <10% are private laboratories not associated with hospitals. Other than 10 notifiable infectious diseases, microbiologic laboratories have no legal requirement to provide data for surveillance. Since 1994, ISIS has collected anonymous positive and negative test results on more than 350 pathogens directly from voluntarily participating laboratories on a daily basis in a fully automated system that uses electronic data interchange. The raw information is then processed by applying a set of criteria based on the diagnosis of a

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particular infection. Laboratory results are thus combined into surveillance diagnoses by the removal of results of duplicate testing of the same case by the same or a different microbiologic technique and then classified by the type of infection.[1] Surveillance diagnoses are then presented as feedback on a password-protected Internet site within 24 hours. At present, information on 40 of the 350 pathogens is presented on this site (Table) (available with password from: URL: http://www.isis.rivm.nl). Currently, 11 laboratories located throughout the country are connected to ISIS, covering 16% of the total Dutch population of 16 million. The coverage of each laboratory is calculated from the coverage of each hospital exclusively served by that laboratory, which in turn is calculated by a national organization that calculates the government subsidy to each hospital. One laboratory (the National Institute for Public Health and the Environment [RIVM]) is also the national reference laboratory for *Salmonella*, *Escherichia coli*, and *Mycobacterium tuberculosis*, for which the coverage is much higher. The coverage of the *Salmonella* reference laboratory, for example, is estimated to be 64% of the national population. Since 1996, an algorithm has been used to detect outbreaks in the surveillance data resulting from *Salmonella* (sub)typing (14).

Apart from ISIS, two other systems collect laboratory data. Fifteen regional public health laboratories provide a weekly report of aggregated data of positive diagnoses for nine bacterial pathogens. These same laboratories and two other laboratories form a network of 17 virologic laboratories that report weekly aggregated numbers of positive diagnoses of 37 virologic pathogens. Four of the 15 public health laboratories contribute data electronically to ISIS.

Design of the Outbreak Detection System

The overall objective of the system was the automated detection of an unexpected national increase of any one pathogen reported by laboratories in a determined period, for feedback to all interested parties by means of the Internet, followed by interpretation and communication to relevant authorities for decisions on control to be taken. The system thus comprises three components: detection of clusters in time or unusual disease events (e.g., one case of rabies) and signal generation; feedback of the signals on the Internet to relevant professionals; and interpretation of signals on a weekly basis with communication to relevant authorities.

Table. List of 40 current surveillance diagnoses generated on ISIS with type of threshold[a]

Surveillance diagnosis	Threshold type
Adenovirus infection	H
Entamoeba histolytica, intestinal infection	H
E. histolytica, extraintestinal infection	H
Campylobacter spp. infection	H
Campylobacter jejuni infection	H
Chlamydia trachomatis infection	H
Enterovirus infection	H
Escherichia coli O157 infection	F (4)
Giardia lamblia infection	H
Neisseria gonorroeae infection	H
Haemophilus influenzae, invasive infection	H
Hepatitis A virus infection	H
Hepatitis B virus infection	H
Hepatitis C virus infection	H
Bordetella parapertussis infection	H
B. pertussis infection	H
Hantavirus infection	F (0)
Listeria monocytogenes infection	H
Malaria, *Plasmodium* spp. infection	H
Malaria, *P. ovale* infection	H
Malaria, *P. malaria* infection	H
Malaria, *P. falciparum* infection	H
Malaria, *P. vivax* infection	H
N. meningitis, invasive infection	H
Parainfluenza virus infection	H
Salmonella enterica Paratyphii group A infection	H
S. Paratyphii group B infection	H
S. Paratyphii group C infection	H
S. Typhi infection	F (3)
Respiratory syncytial virus infection	F (10)
Rhinovirus infection	F (10)
Salmonella spp. (nontyphoid) infection	H
S. Typhi infection	H
Shigella spp. infection	F
Staphylococcus aureus, invasive infection	H
Streptococcus group A, invasive infection	H
Streptococcus group B, invasive infection	H
Streptococcus pneumoniae infection	H
Yersinia spp., non-*pestis*	H
Yersinia enterocolitica	H

[a]ISIS, Infectious Disease Surveillance Information System; H, historical algorithm-defined threshold; F, fixed user-defined threshold (cases/week); F(0), zero threshold where one case is signaled.

Cluster Detection and Generation of Signals

Approach

Our approach was to design a system to detect outbreaks that otherwise would probably be missed altogether and detect more rapidly the outbreaks that would also probably be eventually detected by other means. We

[1]Example 1: A surveillance diagnosis for a case of respiratory syncytial virus infection is a positive culture or positive polymerase chain reaction (PCR) or positive direct immunofluorescence or positive enzyme immunoassay, with all positive tests on the same case-patient within a 6-week period reported as one surveillance diagnosis. Example 2: A surveillance diagnosis for a case of invasive *Haemophilus influenza* infection is a positive culture from a normally sterile site, with all positive results from the same case in 3 months considered one surveillance diagnosis.

designed the system with sensitivity and timeliness as the priority features, especially since small increases in laboratory data often indicate larger communitywide outbreaks. Sensitivity in this context would be defined as the number of relevant outbreaks found from all relevant outbreaks. Clearly, this distinction depends on how "relevant" is defined. All relevant outbreaks, however, should include those outbreaks of public health importance that are missed by conventional means; therefore, the denominator will always be unknown. Thus, absolute sensitivity of the automated system will be impossible to calculate. The system, however, can be designed to maximize sensitivity and detect more outbreaks than other mechanisms such as clinical observation, without resulting in an unmanageable number of signals. The system was also intended to be more timely, by detecting the same outbreaks as other mechanisms but more quickly. The specificity of the system was considered less important in the initial phase, since false-positive results could be filtered out when signals were interpreted.

We also decided that the system should be sensitive enough to detect even one case of certain critical infectious diseases (e.g., hantavirus infection) or unusual infections of current interest (e.g., hepatitis E virus infection), which might indicate an outbreak, and to detect expected seasonal increases of diseases caused by selected pathogens (e.g., influenza) as they occur. This design would allow for rapid action to verify the signal and institute case-finding or put in place certain public health measures (e.g., prompting nursing homes to vaccinate residents against influenza).

Generation of Signals

Signals generated by the system are produced by comparing observed values with a predefined threshold value. Threshold values are calculated from values expected from historical data (for most pathogens) or are fixed, user-defined thresholds, set by epidemiologists for detecting seasonal increases or monitoring critical pathogens.

Algorithms Using Historical Data

Several algorithm types applied to outbreak detection have been described in the literature, based either on Cumulative Sums (12,15) linear regression (7), or Fourier regression and autocorrelative models such as Box-Jenkins (8,9). Fourier analysis and autocorrelative methods require model building or the setting of many parameters, processes considered too labor-intensive for a generic algorithm for all type of pathogens. We decided to base the ISIS system on the algorithm currently run each week on *Salmonella* data, which has been successfully detecting outbreaks since 1998 in the Netherlands (14,16–20) but is not automatic and requires an operator to periodically update data. The algorithm is a simple linear regression model, adjusted for seasonality, secular trends, and past outbreaks in a similar manner as described by Farrington et al. (7) and requires little parameter resetting or model checking. Briefly, to calculate an expected total value for the current epidemiologic week, a regression line is plotted through the totals in the nine epidemiologic weeks centered on the same epidemiologic week in the previous 5 years. For example, to calculate an expected value for week 20, a regression line is plotted through the values at weeks 16–24 of the previous 5 years.

To maximize sensitivity we decided, after preliminary testing with *Salmonella* data, on two variations of the same algorithm, using two different window periods. The first is a 7-day total calculated daily. This variation is based on an algorithm that calculates expected week totals of a certain pathogen and a threshold value of 2.56 standard deviations from the mean (equivalent to a 99% confidence interval). A 7-day window advances day-by-day as new data enter the system and a new 7-day observed total is calculated daily and compared with the expected value for that epidemiologic week (Monday to Sunday). If the observed total is over the threshold, a signal is generated. The second algorithm variation is a 4-week total calculated daily. Each week, this algorithm calculates an expected total for the previous 4 weeks and a 99% threshold value. A 4-week window advances day-by-day and a new 4-week observed total is compared with the expected total for the four epidemiologic weeks ending with the current week.

Most outbreaks would be detected in a timely manner by the 7-day total system. However, comparison of the two algorithms using *Salmonella* data has shown that small sustained increases $\geq$1 month would be missed by a 7-day total system, since the threshold value would not be exceeded in any one 7-day period. Including a 4-week total algorithm in the system produces 10% extra signals of outbreaks with slowly increasing numbers of cases, which otherwise would not be detected.

If the 4-week total is <5, or the 7-day total is <3, no signals are generated, even if above threshold. Though reducing sensitivity, this cutoff greatly reduces the number of signals of sporadic cases of infrequent infections that are of little public health significance. The system uses the date the sample was taken for calculation of observed and expected totals since for any one pathogen a variable delay between date of disease onset and date of reporting of result to ISIS is likely. In the case of an outbreak, the use of date of reporting for surveillance would result in a lower peak number of cases spread over a longer period (a "smeared" epidemiologic curve), reducing the sensitivity of the system. Using date of sampling entails retrospective examination of data to ensure that data reported in 1 week and plotted by date of sampling do not produce a signal in weeks previous to reporting. The "look-back" period (i.e.,

the period of retrospective examination) has been set at 10 epidemiologic weeks. This window allows enough time for most pathogens to be sampled, tested, and reported. New signals of an excess of cases at time of sampling >10 weeks previous to reporting are unlikely to signify unrecognised outbreaks that can still be investigated and controlled.

User-Defined or Fixed Threshold

Algorithms depending on automated evaluation of historical data are often unreliable in detecting seasonal increases in pathogens whose seasonality shifts. A flexible, user-defined, fixed threshold was chosen to detect such increases in selected pathogens. For instance, with present historical data on respiratory syncytial virus, 10 positive laboratory results in any epidemiologic week have always indicated the beginning of the epidemic season. Thus, the threshold for that virus is set at 10 positives in a 7-day period. Some pathogens (e.g., hantavirus) have been defined as zero-tolerance, where one positive result is considered worth a signal. Although such cases are often communicated faster by other means, in some of these situations the system can be considered as a backup.

Data Used

At present, signals are generated from both the *Salmonella* database (data from the national reference laboratory stored in ISIS but not processed into surveillance diagnoses and presented only internally) and the database of 38 surveillance diagnoses (data on pathogens stored in ISIS and processed into surveillance diagnoses for Internet feedback). Signals are generated from the *Salmonella* database with algorithms that use historical data and from the surveillance diagnoses database with both user-defined and algorithm-defined thresholds (Figure 1).

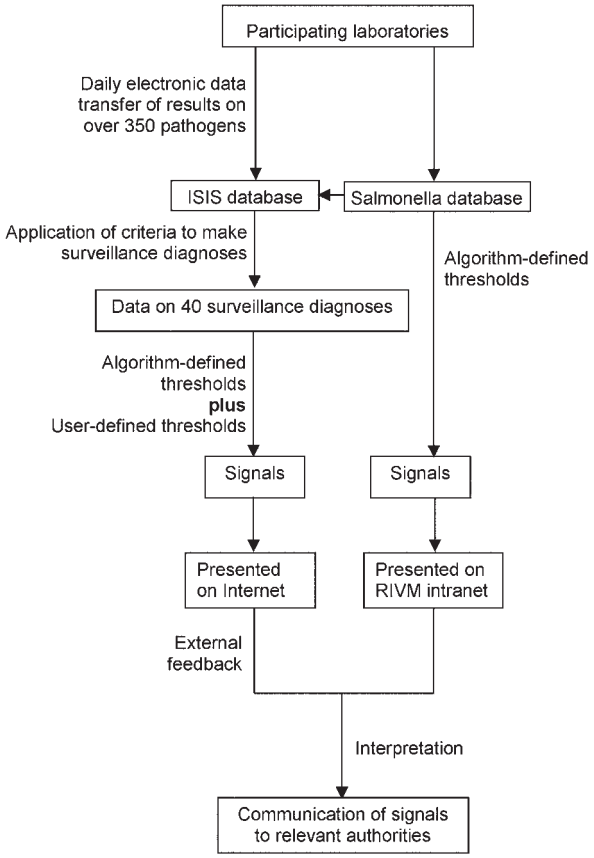


Figure 1. Flow diagram showing flow and processing of laboratory data in the Infectious Disease Surveillance Information System (ISIS) and means by which signals generated by the ISIS database and the Salmonella database are created and handled. RIVM, National Institute for Public Health and the Environment, Bilthoven, the Netherlands.

Internet Feedback of Signals

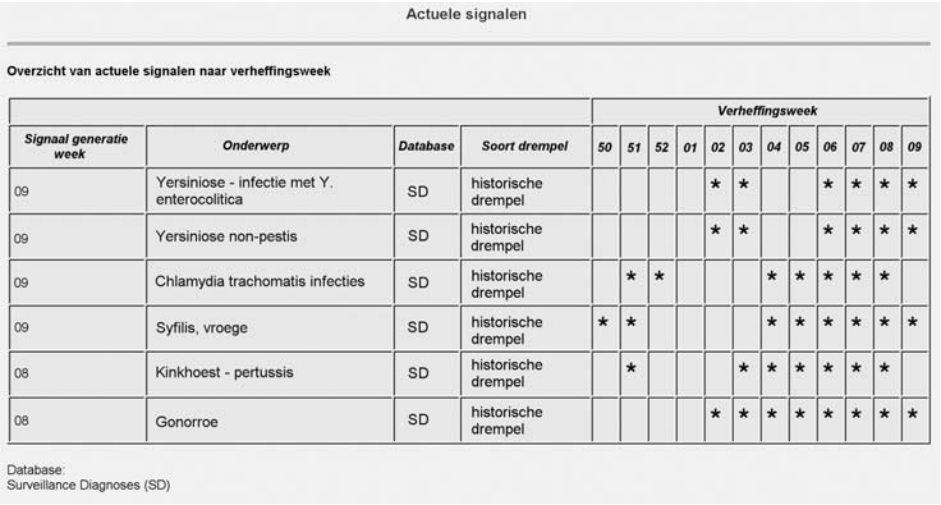
Currently, signals from the *Salmonella* database are presented only on an internal RIVM site. Signals are listed and incidence by municipality mapped. The signals generated from surveillance diagnoses, however, are available on the Internet for all local health authorities, Ministry of Public Health staff, and all registered microbiologists to access. The signals are presented first in a table (Figure 2) that displays, for each signaled pathogen, the week in which the increase occurred (by week of sampling), the type of algorithm used, and the epidemiologic week in which the signal was generated.

Signals remain in this table for one epidemiologic week after they are signaled. For each signaled pathogen, a link can be made to a graph showing the observed and threshold for the previous 2 years. Historical signals by week of signaling are also listed on the site. Age and sex breakdown of all cases of a pathogen in the previous 4 weeks can be compared with that of all data, allowing an idea of which age or sex may be affected in an outbreak. Those with access to the site can also subscribe to automatically receive an email of a new signal.

Signal Interpretation and Action

Signals that were produced during the previous 7 days are interpreted formally on a weekly basis in a meeting of members of RIVM and the National Co-ordination Centre for Communicable Disease Outbreak Management. Since 1999, this group has interpreted all signals of potential national importance, from informal and formal sources. In addition, the algorithm-generated signals are monitored on a daily basis. The accessibility of the site allows input from many other health professionals who can contact ISIS should they have some information to help interpret any signal.

Every week a meeting report is written and disseminated to all 46 regional health authorities as well as to the



Actuele signalen

Overzicht van actuele signalen naar verheffingsweek

				Verheffingsweek											
Signaal generatie week	Onderwerp	Database	Soort drempel	50	51	52	01	02	03	04	05	06	07	08	09
09	Yersiniose - infectie met Y. enterocolitica	SD	historische drempel					*	*			*	*	*	*
09	Yersiniose non-pestis	SD	historische drempel					*	*			*	*	*	*
09	Chlamydia trachomatis infecties	SD	historische drempel		*	*				*	*	*	*	*	
09	Syfilis, vroege	SD	historische drempel	*	*					*	*	*	*	*	*
08	Kinkhoest - pertussis	SD	historische drempel		*				*	*	*	*	*	*	
08	Gonorroe	SD	historische drempel					*	*	*	*	*	*	*	*

Database:
Surveillance Diagnoses (SD)

Figure 2. View of Web page listing surveillance diagnoses ("onderwerp") flagged on week 9 of 2002. The asterisks in the columns labeled "verheffingsweek" indicate the week of sampling when the number of a particular surveillance diagnosis exceeded the threshold defined by a historical algorithm ("historische drempel"). The surveillance diagnosis for syphilis ("syphilis, vroege") is flagged at the end of 2001 (weeks 51 and 52) and 2002 (weeks 4–9).

Ministry of Health and other interested parties. The investigation and control of outbreaks within one area is the legal responsibility of that area's health authority. For outbreaks that span one or more health authorities, the RIVM coordinates and supports investigation, while RIVM and the National Co-ordination Centre for Communicable Disease Outbreak Management coordinate implementation of control measures.

The early-warning system was implemented in January 2002. In early March 2002, the system signaled an increase in diagnoses of syphilis. This increase was subsequently found to represent a sustained outbreak of syphilis that had begun the previous year in a large Dutch city. The outbreak was subsequently investigated, and prevention strategies were implemented (21) (Figures 2 and 3).

Limitations

This system is designed to complement, not replace, any conventional methods of outbreak detection (e.g., clinician-based surveillance of notifiable diseases). Laboratory-based surveillance will be less timely and sensitive than conventional methods in detecting many local outbreaks of disease, particularly those clearly associated with a certain setting, and in detecting many widespread outbreaks of disease with unusual signs and symptoms (e.g., acute flaccid paralysis in a polio outbreak). Local outbreaks may also be more rapidly detected from local, not national, laboratory data. In addition, though expansion is planned, many laboratories are likely never to participate in ISIS, limiting the coverage of the electronic system.

Analysis of large amounts of laboratory data will likely signal many clusters of no significance, and the work generated in interpreting signals meaningfully may be overwhelming and so mask true signals. Thoroughly evaluating and adjusting parameters such as the minimum number required to trigger a signal may be required to prevent this but at a cost of losing sensitivity. Conversely, the ability to detect clusters of commonly reported pathogens that are not routinely subtyped (e.g., *Campylobacter*) will always be limited because the signal will be likely smaller than the variability of the large amount of data routinely submitted. One solution to this problem is to apply the algorithm to subsets of reduced amounts of data on common pathogens such as data collected by a group of regional laboratories.

Future Work

Evaluation of the System

The ISIS outbreak detection system needs to be evaluated to demonstrate a clear advantage over conventional means for detecting outbreaks of infection of all types of pathogens, not just salmonellae (for which the algorithm has already proved its usefulness). The sensitivity and timeliness of algorithms in other outbreak detection systems relative to a variety of standards such as formal records of investigated outbreaks or informal epidemiologic judgment, have been assessed retrospectively (11,12). However, no records of investigated outbreaks in the Netherlands exist, and the minutes from the signals meeting have only recently been put in a format that allows easy interpretation of signal outcome. In addition, retrospective analysis does not allow evaluation of the extra sensitivity nor of the specificity of an algorithm. This limitation exists because any signals from historical data produced by the algorithm, and not detected by other means, are classified as false positives, when many may have been genuine. Nonetheless, some idea of the value of the algorithm is given by the fact that since 1998, no national outbreak of *Salmonella* has been detected by means other than by the *Salmonella* outbreak detection system. Additionally, the feedback on the Internet and comments from the public health community are impor-

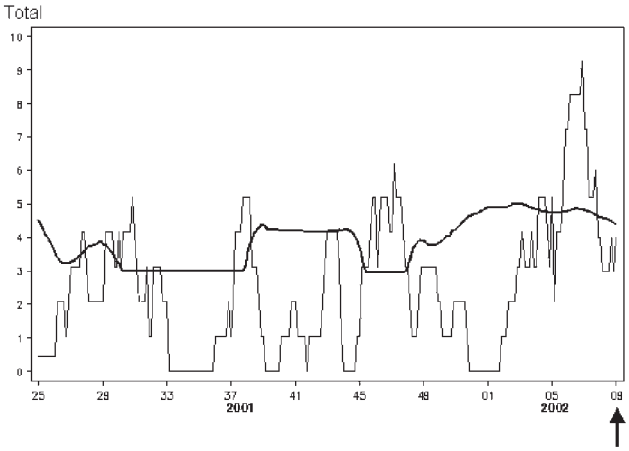


Figure 3. Graph showing sharp increase of weekly totals (week of sampling) of syphilis diagnoses (black line) exceeding the 99% threshold (red line). Arrow (week 9) marks when submitted laboratory reports resulted in signal generation and subsequent investigation.

tant factors that affect the sensitivity, specificity, and timeliness of the whole system since they will impact the eventual interpretation of a signal.

ISIS will, therefore, be evaluated prospectively at the weekly signal meeting, comparing signals detected by the algorithm to signals detected by other means. This comparison will allow assessment of the following: 1) how many signals detected by the algorithm are not of public health interest, as decided in the weekly meeting (a measure of specificity), and 2) the number of relevant signals detected by other means that should have been detected by the algorithm (a measure of relative sensitivity and timeliness). Assessing the number of outbreaks that the algorithm detects that would not have been detected otherwise will not be possible, since once a signal is detected by algorithm it can never be known with certainty that it would not have been detected later by other means. However, if the first detection of a signal is by algorithm, this will give some measure of timeliness of the system.

Expansion

At present, 40 surveillance diagnoses in ISIS are available for use in the automated outbreak detection system. Much incoming data are as yet not formatted for daily signal generation and feedback as described. A priority, therefore, is to adapt the system to directly analyze raw data (those not processed as surveillance diagnoses) on the other 300 pathogens currently collected, and, in particular, to make the current *Salmonella* outbreak detection system part of the automated ISIS. By 2004, a total of 25 laboratories are scheduled to be connected, increasing the coverage of the system for all pathogens to at least 35% of the Dutch population. We also hope that regional health authorities will eventually have access to their own Web page, presenting the results of applying the algorithms to their data. This improvement would allow smaller regional outbreaks of common pathogens to be detected.

Conclusion

We describe an automated outbreak detection system that uses laboratory data electronically collected in the Netherlands by ISIS. The system assesses data as soon as they are made available and disseminates the information by means of the Internet to all involved health professionals to help in the rapid interpretation and subsequent action to control any suspected outbreak. Much still needs to be done, and efforts are now concentrated on increasing the data available to ISIS, system evaluation, and subsequent modifications, with the aim of having a flexible, automated outbreak detection system for all laboratory-reported pathogens in the Netherlands by 2006.

Acknowledgments

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Dr. Widdowson is a veterinary public health epidemiologist now based at the Centers for Disease Control and Prevention. He is responsible for the foodborne virus epidemiology program, with a particular focus on Norwalk-like viruses. His other research interests include all aspects of zoonotic infections.

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Automated Laboratory Reporting of Infectious Diseases in a Climate of Bioterrorism

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While newly available electronic transmission methods can increase timeliness and completeness of infectious disease reports, limitations of this technology may unintentionally compromise detection of, and response to, bioterrorism and other outbreaks. We reviewed implementation experiences for five electronic laboratory systems and identified problems with data transmission, sensitivity, specificity, and user interpretation. The results suggest a need for backup transmission methods, validation, standards, preserving human judgment in the process, and provider and end-user involvement. As illustrated, challenges encountered in deployment of existing electronic laboratory reporting systems could guide further refinement and advances in infectious disease surveillance.

The primary purpose of reporting diseases is to trigger an appropriate public health response so that further illness can be prevented and public fears allayed. The threat of emerging infections and bioterrorist attacks has heightened the need to make disease surveillance more sensitive, specific, and timely (1,2). Recent advances in provider and laboratory information management have facilitated one step towards the modernization of surveillance: the development of automated reporting systems (3,4). With recent funding for activities to defend the public's health against terrorism and naturally occurring diseases, development of automated reporting systems has been accelerated (5).

However, technologically innovative reporting systems need to be consistent with the purpose of disease reporting. Wholesale adoption of automated electronic reporting systems in their current form might instead represent a quick response to the pressures of the moment rather than a fully considered decision that acknowledges some of the documented problems with the new technology. We review here current limitations of systems that provide automated notification of reportable conditions identified in clinical laboratories. A more thorough understanding of the pitfalls of such existing systems can provide insights to improve the development and implementation of new media in infectious disease surveillance.

With the computerization of patient and clinical laboratory data, automated notification of reportable events to health departments is often assumed to be more effective than conventional paper-based reporting (6). In recent years, the Centers for Disease Control and Prevention (CDC) has been funding several states to develop electronic laboratory reporting (7). With electronic reporting, laboratory findings (e.g., *Escherichia coli* O157:H7 test results) are captured from clinical laboratory data and transmitted directly to the state. In turn, the state routes messages to local health units, as illustrated in the Figure. The National Electronic Disease Surveillance System (NEDSS) and bioterrorism preparedness initiatives are expected to further enhance disease surveillance by supporting integration of electronic data from various sources (4,8). Evidence from deployed systems shows promise in the ability of electronic laboratory reporting to deliver more timely and complete notifications than paper-based methods (9–12).

At the same time, experiences in Pennsylvania, New York, Hawaii, California, and other states indicate that implementation of automated reporting also poses unanticipated challenges. Five problem areas have been identified: sensitivity, specificity, completeness, coding standards, and end-user acceptance.

Sensitivity

To achieve the objective of triggering local public health response, automated electronic systems should consistently report cases that would have been reported by conventional methods. Contrary to expectations, automated reports seldom replicate the traditional paper-based system. Errors in data transmission reduce sensitivity in automated electronic reporting systems. An evaluation of electronic laboratory reporting in Hawaii documented that automated reports were not received for almost 30% of the days on which the paper-based method generated a report,

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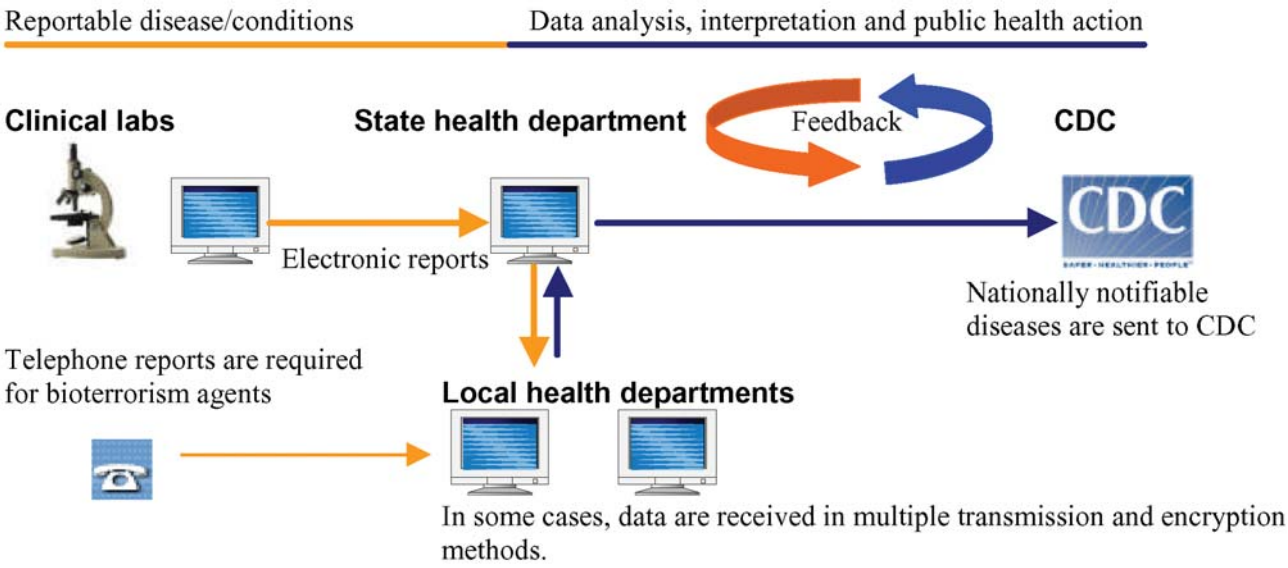

Figure. Steps in automated reporting of infectious disease data. The process begins with abstraction of reportable conditions using a software program. Data are stored in a file for future transmission or sent directly to the health department in the case of automated reporting systems. Typically, there are multiple clinical laboratories, and reports are transmitted in a variety of methods including file transfer protocol and dial-up modem at arranged intervals. State health departments review data and forward them to local health departments, where investigations are done and reportable conditions are determined. Local health departments forward data back to the state, where further analysis and interpretation are accomplished. The state uploads nationally notifiable diseases data to a secure data network at the Centers for Disease Control and Prevention. That agency sends data quality feedback to the state immediately. The level of feedback among states, laboratories, and local health departments is unknown but suspected to vary widely.

suggesting that automated reporting alone was potentially suboptimal. Lapses in electronic reporting were due to various causes including ongoing adjustments to the data extraction program (11). In California, lapses in a semiautomated electronic laboratory reporting were traced to a failure in forwarding reports from the county of diagnosis to the county of residence (12). In Pennsylvania, lapses in automated notification have resulted from the occasional failure of data extraction at the clinical laboratory computer, difficulties deciphering reportable diseases from test results which used local terminology rather than Logical Observation Identifier Names and Codes (LOINC) codes (available from: URL: http://www.regenstrief.org/loinc), and problems in the transmission of data files to and access by local health jurisdictions. To prevent interruption of reports while the automated system was being refined, Pennsylvania opted to continue conventional paper-based reports for 8 months after initiating electronic reporting.

Specificity

Typically, automated reporting increases not only reportable events data but also the number of extraneous reports (e.g., nonreportable conditions, unnecessary negative reports, or duplicate reports). In addition, false-positive results are increased by automated abstraction of culture results entered in free-text. For example, in an evaluation of an electronic laboratory reporting system in Allegheny County, Pennsylvania, negative results of *Salmonella* isolates were automatically transmitted as positive *Salmonella* results because the software recognized the organism name (9). Often, automated reporting transmits preliminary test results followed by results of confirmatory tests for the same condition. This method is desirable because some duplicates may actually provide useful preliminary test results that might trigger timely responses (9,10). However, multiple test results increase time for data processing. In addition, low specificity attributable to extraneous records of nonreportable culture results is also problematic. While over time automated programs can be expected to improve, initially erroneous or missing data will continue to arise and require manual checking and recoding.

Programming solutions might offer relief in eliminating extraneous records. But in a climate of bioterrorism, a complete replacement of human judgment is probably unacceptable for many. Therefore, in planning new systems, accounting for the time and effort of an experienced epidemiologist to review electronic laboratory data before routing them to investigators will be essential.

Completeness of Case Records

To be useful, case-reports received through conventional or automated methods must contain data in key fields identifying patient and physician (e.g., name, address, and

telephone number) and specimen (e.g., collection date, type, test, and result). Lack of sufficient identifying information for follow-up investigations is a serious limitation in many currently operating automated systems.

In addition, experiences in New York and Pennsylvania indicate that the lack of a patient's address is a barrier to routing electronic laboratory data to local health departments. Locating a patient's residence is also useful for recognizing clusters of diseases attributable to natural causes or intentional acts of terrorism. Automated means were intended to improve completeness of case record data by duplicating required fields, but this has not always been the case (13). Whether the laboratories fail to report missing data or whether data elements are not provided in the initial forms submitted with specimens is unclear. Widespread dissemination of standardized disease reporting forms specifying information required by health departments to both clinical laboratories and providers could reduce this problem. Such information could also be made readily available through the Internet. An example of what laboratories and providers are required to include in Minnesota is available (URL: http://www.health.state.mn.us/divs/dpc/ades/surveillance/card.pdf).

Data Standards

To facilitate use of state-of-the-art electronic surveillance tools as envisioned in the NEDSS initiative, adoption of Systemized Nomenclature of Human and Veterinary Medicine (SNOMED) (available from: URL: http:// www.snomed.org/), LOINC, and Health Level 7 standards (a national standard for sharing clinical data, available from: URL: http://www.hl7.org/) by clinical laboratories is essential. However, in practice clinical laboratories often use locally developed coding schemes or a combination of codes and free text. Data often arrive in multiple file formats or even with multiple formats within one mapping standard (Figure). In practice, file messages from multiple laboratories are mapped into a standardized database with desired variables including patient, physician contact information, specimen identifiers, test name, and results.

To increase use of uniform data coding and Health Level 7 as the standard for automated electronic reporting, further studies are needed to understand barriers encountered by clinical laboratories and ways to overcome them. Cost or lack of information technology resources might be factors contributing to slow adoption of standard coding in small-size clinical laboratories. In addition, variations in reporting requirements across states may be an extra cost to laboratories that serve multiple health jurisdictions. In addition to understanding and assisting in reducing barriers to use of standards, public health officials could help promote use of coding standards by demonstrating their benefits to laboratories and providers. For example, use of standards such as LOINC facilitates integration of microbiologic culture data, minimizes chances for data errors in translating free text or handwritten test results, and makes it easier for laboratories to monitor antimicrobial resistance patterns. This could be reinforced by introducing regular data quality feedback to all the stakeholders, as illustrated in the Figure.

User Acceptance

The entire process for detecting diseases relies on acceptance and appropriate intervention by those working on the front-line of the public health system. As shown on the Figure, public health surveillance largely depends on investigation at the local level, where a determination is made that reported events meet case definitions for reportable and notifiable conditions. Local health departments report data to the state level, where nationally notifiable diseases (available from: URL: http://www.cdc.gov/epo/dphsi/phs/infdis.htm) are transmitted to CDC. That agency in turn reports internationally quarantinable diseases to the World Health Organization (available from: URL: http://www.who.int/emc/IHR/ int_regs.html). The process begins with receiving, managing, and using surveillance data. Automated reports in the form of electronic-mail attachments could be cumbersome for some local health departments with limited information technology support. Also, encryption of data for confidentiality reasons increases complexity of the data retrieval process. Acceptance of automated electronic reporting systems is likely when assistance on data analysis and management is given to disease investigators.

During the 2001 bioterrorism outbreak investigation, labor-intensive methods (i.e., faxes and emails) were used for surveillance of cases with clinical syndromes compatible with anthrax among patients in selected counties in New Jersey, Pennsylvania, and Delaware (14). Because of personnel time demands, automated electronic systems are attractive in surveillance of syndromes suggestive of bioterrorism agents. While automated electronic surveillance systems using patient encounter records for syndromic surveillance might offer relatively low costs of adoption for physicians (15), other persons in the system may become unduly burdened. For example, when automated reports of syndromes are forwarded to local public health officials, who should interpret and act upon the results remains unclear. The key to the success of such innovative systems outside investigational settings will be their ability to offer meaningful results at an acceptable marginal cost to both reporters and local health departments. Integration of syndromic surveillance into local public health surveillance is less understood and needs attention.

Discussion

Responding to and anticipating the difficulties encountered by existing automated reporting systems could be used to improve current systems and guide development of future infectious disease surveillance. Addressing limitations of automated reporting systems by continuing conventional notification methods during the adjustment period, promoting use of coding standards, validating data, and involving end-users is essential.

As illustrated in this study, lapses in data transmission occur during initial deployment of automated reporting systems. The potential risks attributable to lapses or errors in automated electronic reports are great, as are costs associated with misdiagnoses and treatment of healthy persons (16). Experiences in Hawaii and Pennsylvania indicate the need for continuing with existing reporting mechanisms during the first year while new systems are being refined.

Our study calls for evaluations to validate new automated systems before they are integrated into public health surveillance. While health departments and CDC have typically collaborated in such efforts, involvement of providers and laboratorians is likely to yield additional insights. Participation of public health officials is indicated in evaluations of automated methods that are being developed in research settings to capture nonreportable syndromes for bioterrorism detection.

Partnerships among state health departments, clinical laboratories, providers, CDC, and other diagnostics systems are needed to promote widespread use of uniform coding standards (LOINC and SNOMED) and Health Level 7 for messaging. As demonstrated in New York State, involving all users early in the planning stages enhances the success of automated electronic reporting system (13). CDC could facilitate laboratory participation in use of standards by assisting health departments in identifying benefits such as use of LOINC-coded data for antimicrobial resistance monitoring.

Current federal funding for emergency preparedness surveillance and epidemiology capacity (17) is expected to stimulate widespread use of automated systems in infectious disease reporting. However, automated systems are a complement rather than a substitute for human involvement in interpreting laboratory findings and screening for errors. Furthermore, the requirement that providers and laboratories report immediately by telephone when they detect organisms indicating an outbreak or an unusual occurrence of potential public health importance (18) is expected to continue even when automated reporting systems are implemented. Complete replacement of human judgment in reporting conditions suggestive of CDC category A bioterrorism agents (available from: URL: http://www.bt.cdc.gov/Agent/Agentlist.asp) or other conditions that require immediate investigation is unrealistic.

Despite the limitations we have described, automated electronic systems hold promise for modernizing infectious disease surveillance by making reporting more timely and complete. Modern technology can translate into better public health preparedness by enhancing and complementing existing reporting systems.

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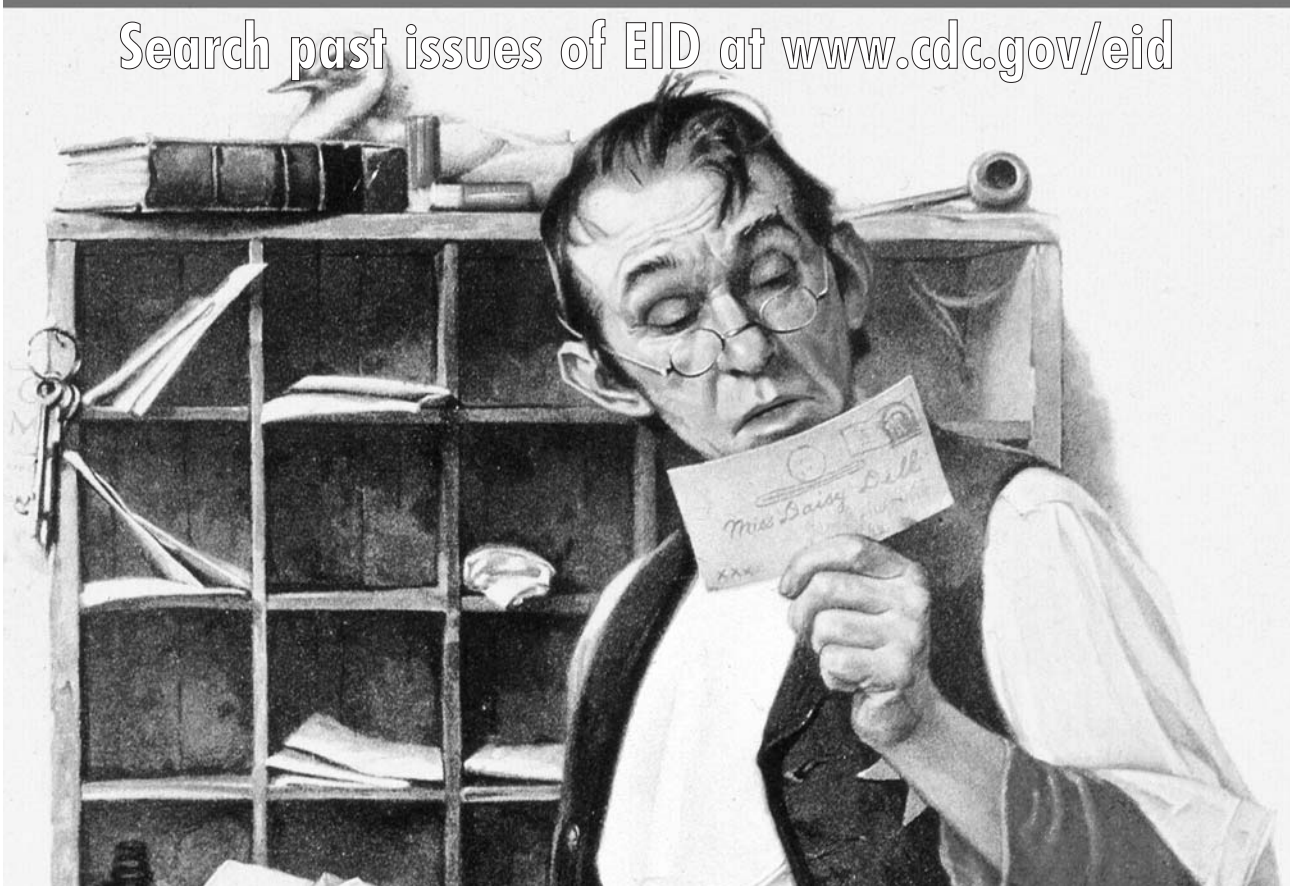

Human Metapneumovirus Detection in Patients with Severe Acute Respiratory Syndrome

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We used a combination approach of conventional virus isolation and molecular techniques to detect human metapneumovirus (HMPV) in patients with severe acute respiratory syndrome (SARS). Of the 48 study patients, 25 (52.1%) were infected with HMPV; 6 of these 25 patients were also infected with coronavirus, and another 5 patients (10.4%) were infected with coronavirus alone. Using this combination approach, we found that human laryngeal carcinoma (HEp-2) cells were superior to rhesus monkey kidney (LLC-MK2) cells commonly used in previous studies for isolation of HMPV. These widely available HEp-2 cells should be included in conjunction with a molecular method for cell culture followup to detect HMPV, particularly in patients with SARS.

Human metapneumovirus (HMPV) was first identified in 2001 in samples from children with respiratory tract diseases (1). Subsequent studies showed that the virus is responsible worldwide for a proportion of community-acquired acute respiratory tract infections in children (2–4), as well as other age groups (5–9). Co-infection of HMPV with respiratory syncytial virus (RSV) in infants has been suggested to be a factor that influences the severity of bronchiolitis (10).

HMPV is a new member of the family *Paramyxoviridae*, subfamily *Pneumovirus*. The overall percentage of amino acid sequence homology between HMPV and avian metapneumovirus (APV) ranges from 56% to 88% for open reading frames N, P, M, F, M2-1, M2-2, and L (11). Phylogenetically, RSV is the closest human virus related to HMPV, and the clinical symptoms of HMPV may share an overlapping spectrum with RSV (2,4,7,9,10). The epidemiology and symptoms of HMPV infection have not been fully elucidated; one obstacle in establishing these data is the difficulty in establishing a laboratory diagnosis of the infection. We describe our experience of detecting HMPV during an outbreak of severe acute respiratory syndrome (SARS).

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Methods

Study Population

In early March 2003, an outbreak of SARS occurred in the Prince of Wales Hospital (the teaching hospital of The Chinese University of Hong Kong). Our study participants were patients admitted to our hospital for suspected SARS during the first week of the outbreak (12). These patients fulfilled the World Health Organization definition for probable SARS cases (13). Briefly, patients had an acute onset of fever (>38°C, most with chills or rigor), dyspnea, myalgia, headache, and hypoxemia. Peripheral air-space consolidation subsequently developed in all study patients as observed on chest radiographs or thoracic computed tomographic scan; patients showed no response to antimicrobial drugs prescribed for typical and atypical pneumonia (β-lactams, macrolides, and fluoroquinolones).

During our study, we examined 48 patients who comprised our first group of SARS patients and had a clear history of exposure. Forty-five participants were adults (26 men, 19 women) 21–69 years of age (mean 35.4 years of age; standard deviation 11.5 years). The group included 26 healthcare workers and 7 medical students who worked in a ward (index ward) in the hospital where a few patients with SARS had stayed. The remaining 12 patients had been hospitalized or were visitors to the same ward. Three study participants were children (two boys, one girl) 2–7 years of age. All these children were living with persons who had been hospitalized or were visitors to the index ward and who had contracted SARS.

Virus Isolation

Nasopharyngeal aspirate (NPA) samples were taken from all patients by inserting a suction catheter into the nasopharyngeal area via the nostril. A low suction force was applied to collect approximately 0.5 mL fluid, which was then transferred into 2 mL of viral transport medium. All NPAs were added onto rhesus monkey kidney (LLC-MK2), human laryngeal carcinoma (HEp-2), Mardin Darby Canine Kidney (MDCK), human embryonic lung fibroblast, Buffalo green monkey kidney (BGM), and African green monkey kidney (Vero) monolayers. All cell cultures were incubated at 37°C, except for MDCK, which was incubated at 33°C. All NPAs were added to an additional LLC-MK2 cell culture tube and incubated at 33°C. Cell monolayers were examined daily for cytopathic effect. After 14 days of incubation, a hemadsorption test for LLC-MK2 and MDCK monolayers was performed. All cell cultures materials were kept frozen for subsequent analyses.

HMPV Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

To detect HMPV, we used a nested RT-PCR focused on the F-gene. This RT-PCR was applied on all cell cultures, regardless of cytopathic effect. After one cycle of freeze-and-thaw, RNA was extracted from cell cultures by using the QIAamp Viral RNA Mini Kit (QIAGEN GmbH, Hilden, Germany), according to the manufacturer's protocol. The outer primers were 5′-AGC TGT TCC ATT GGC AGC A-3′ for RT and amplification and 5′-ATG CTG TTC RCC YTC AAC TTT-3′ (R = A or G, Y = C or T) for amplification. These primers were designed on the basis of HMPV sequences available from GenBank. The reaction was carried out in a single-tube (Superscript One-Step RT-PCR and Platinum Taq; Invitrogen Corp., Carlsbad, CA) by using 0.2 μM of each primer and thermal cycling conditions of 50°C for 30 min and 94°C for 3 min; followed by 40 cycles of 94°C for 30 s, 52°C for 30 s, 72°C for 45 s, and a final extension at 72°C for 7 min. For the second round of amplification, we used 0.2 μM of inner primers 5′-GAG TAG GGA TCA TCA AGC A-3′ and 5′-GCT TAG CTG RTA TAC AGT GTT-3′. The PCR was conducted at 95°C for 15 min for denaturation of DNA templates and activation of the hot-start DNA polymerase (HotStarTaq, QIAGEN GmbH), followed by 40 cycles at 94°C for 30 s, 54°C for 30 s, and 72°C for 45 s, and a final extension at 72°C for 7 min. PCR products detected by agarose gel electrophoresis were analyzed for sequence homology with known HMPV strains. In addition to virus isolation, RNA was extracted directly from NPAs for HMPV RT-PCR by using the same protocol as for cell cultures.

Coronavirus RT-PCR

RNA was extracted from the supernatant of Vero cell cultures showing cytopathic effect by using the same method as for HMPV. Coronavirus was detected by RT-PCR with primers COR-1 (sense) 5′ CAC CGT TTC TAC AGG TTA GCT AAC GA 3′ and COR-2 (antisense) 5′ AAA TGT TTA CGC AGG TAA GCG TAA AA 3′, which had been shown to be specific for the novel coronavirus detected from patients with SARS (14). The RT-PCR for coronavirus was conducted similarly to HMPV (by using 0.6 μM of each primer and thermal cycling conditions of 54°C for 30 min, 94°C for 3 min; 45 cycles of 94°C for 45 s, 60°C for 45 s, 72°C for 45 s; and 72°C for 7 min).

Sequence Analysis

The nucleotide sequence of purified PCR products was determined by PCR-based cycle sequencing performed with the inner primers. Sequencing reactions were performed according to the manufacturer's protocol (BigDye Terminator Cycle Sequencing Kit version 3.1, Applied Biosystems, Foster City, CA) and run on the ABI Prism 3100 Genetic Analyzer. All sequences were confirmed by repeated PCRs and sequencing from both directions.

Electron Microscopy

Selected cell cultures that showed cytopathic effect were examined by electron microscopy. Cell culture supernatants were coated on formvar-carbon grids and stained with 2% phosphotungstic acid.

Antibody Detection

To ascertain the HMPV culture results, we obtained paired serum samples (first sample collected within 5 days and second sample collected >14 days after onset of illness) and tested for HMPV antibody. HMPV–infected LLC-MK2 cells were coated on 12-well glass slide and fixed in acetone. The presence of antibody in serum samples was tested for by using the direct immunofluorescence technique.

Exclusion of Cross-Contamination and Test for Reproducibility

Specimen processing, viral culture inoculation, RNA extraction, RT-PCR amplification, and PCR product analyses were conducted in different rooms. Special care was taken to avoid contamination with RNase, and to avoid cross-contamination between reactions. During the inoculation of cell monolayers, we placed a negative control using the same cell line injected with maintenance medium after every fifth cell culture tube. These negative control cell culture tubes were also incubated, examined for cytopathic effect, and processed for RT-PCR as for cell culture tubes injected with specimens. For RNA extraction and

RT-PCR procedures, we placed negative controls using cell culture medium to replace cell supernatant injected with NPAs or double distilled water to replace NPA sample after every fifth reaction. These negative controls did not show positive results, which indicated the absence of cross-contamination. To test the reproducibility of RT-PCR results, we repeated the testing of all positive samples and 30 randomly selected negative samples; all results were reproducible. We also spiked negative NPA samples with HMPV RNA and repeated the extraction and RT-PCR procedures. The results showed no inhibitors were present in the extracted RNA preparations.

Results

Of the 48 NPAs studied, we observed no cytopathic effect on HEp-2, MDCK, human embryonic lung fibroblast, and BGM monolayers. Eleven (22.9%) specimens showed cytopathic effect of diffuse refractile rounding of cells on Vero cell monolayers 2–4 days after incubation, progressed rapidly, and involved the whole monolayer within 12–24 hours. The same cytopathic effect was reproducible on passage to Vero cells, and appeared 1 to 2 days after incubation. These Vero cell cultures were all positive by the coronavirus RT-PCR. The Vero cell culture supernatants showing cytopathic effect were randomly selected for electron microscopy examination, and coronavirus particles were seen.

Five specimens showed cytopathic effect of focal refractile rounding of cells in LLC-MK2 monolayers. All these LLC-MK2 cell cultures had been incubated at 37°C. Unlike the cytopathic effect observed in Vero cells attributable to coronavirus, this cytopathic effect developed after 10 to 12 days of incubation and progressed slowly to detachment from the cell monolayer.

The HMPV RT-PCR examination of cell cultures was negative for human embryonic lung fibroblast, BGM cells, and Vero cells (including those positive for coronavirus). In contrast, HMPV RT-PCR showed a PCR product of the expected size (89 bp) from 25 (52.1%) isolation materials injected with specimens. The nucleotide sequences of the PCR products were identical to the F-gene fragment of HMPV (GenBank accession no. NC 004148) (1). We retrospectively examined the first round of PCR products of all positive samples. Those positive samples derived from direct NPAs did not show positive band, indicating a nested RT-PCR was necessary. However, most (27 [90%] of 30) of those derived from cell cultures showed a positive band of the expected size from the first round of PCR. The distribution of HMPV RT-PCR results on direct detection of NPAs and from different cell culture types is shown in the Table. Overall, the sensitivity of direct detection of NPAs using HMPV RT-PCR was 2 (8.0%) of 25 samples. In one of these two samples, we isolated the virus from three cell lines. In the other sample, we isolated virus from HEp-2 and LLC-MK2. Overall, HEp-2 was the most sensitive cell lines (22 [88.0%] of 25 HMPV positive samples); LLC-MK2 cells detected 6 (24.0%) of 25 samples, and MDCK cells detected 2 (8.0%) of 25 samples. Most (with the exception of three LLC-MK2–positive samples) showed positive results in HEp-2 cells. All six LLC-MK2 cell cultures positive for HMPV were incubated at 37°C; three of these positive cultures that had had the corresponding LLC-MK2 cell cultures incubated at 33°C showed positive results.

To ascertain that cell cultures with HMPV RT-PCR–positive results represented the isolation of HMPV, all LLC-MK2 (incubated at 37°C), HEp-2, and MDCK cell cultures, regardless of the HMPV RT-PCR findings, were passaged to LLC-MK2 cells for a prolonged incubation of 28 days. HEp-2 cells were not used for this purpose because HEp-2 cell monolayers are often difficult to maintain for >2 weeks. The results showed that all passages from HMPV RT-PCR–positive cell cultures showed cytopathic effect of focal refractile rounding of cells that occurred after 10 to 22 days of incubation (Figure 1). The cytopathic effect progressed slowly to detachment from the cell monolayer (Figure 2). The supernatants of these passages were also positive by the HMPV RT-PCR and had visible HMPV viral particles on electron microscopy examination (Figure 3). The passages from HMPV RT-PCR–negative supernatants did not show positive results by the above tests. We also passaged five Vero cell cultures

Table. Distribution of human metapneumovirus reverse transcription–polymerase chain reaction results among 25 positive nasopharyngeal aspirates[a,b]

No. of patients (%)	Human metapneumovirus F-gene sequence detected by RT-PCR			
	Nasopharyngeal aspirate	HEp-2 cells	LLC-MK2 cells	MDCK cells
1 (4.0)	Positive	Positive	Positive	Positive
1 (4.0)	Positive	Positive	Positive	Negative
1 (4.0)	Negative	Positive	Positive	Negative
1 (4.0)	Negative	Positive	Negative	Positive
18 (72.0)	Negative	Positive	Negative	Negative
3 (12.0)	Negative	Negative	Positive	Negative

[a]RT-PCR, reverse transcription-polymerase chain reaction; HEp-2, human laryngeal carcinoma monolayer; LLC-MK2, rhesus monkey kidney monolayer; MDCK, Mardin Darby canine kidney monolayer; BGM, Buffalo green monkey kidney monolayer.
[b]The human metapneumovirus RT-PCR results for all human embryonic lung fibroblast, BGM, and Vero cell cultures were negative.

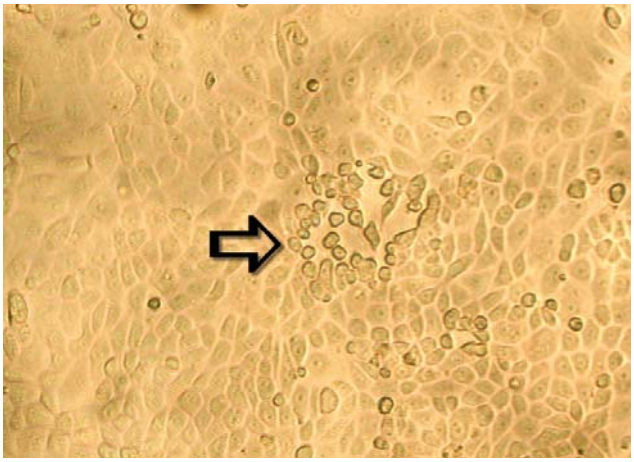

Figure 1. Early cytopathic effect of human metapneumovirus in rhesus monkey kidney (LLC-MK2) cell monolayers. A focus of infected cells that exhibit refractile rounding is indicated by an arrow (100X).

that were positive for coronavirus to LLC-MK2 cells in a similar way. All of these passages did not show cytopathic effect and were negative by the HMPV RT-PCR.

To reconfirm the fact that HMPV infections detected by this combination approach represented genuine infections, we coated HMPV-infected LLC-MK2 cells onto slides for antibody detection using the immunofluorescence technique. All HMPV culture–positive patients who had serologic evidence of infection had a more than fourfold rise in antibody titers, and 15 patients seroconverted.

Overall, our results indicated that the combination approach of using conventional virus isolation and molecular detection could be successfully applied to the isolation of HMPV (Figure 4). With this approach, we found that among the 48 study participants, 6 (12.5%) had both HMPV and coronavirus isolated from NPAs, 19 (39.6%) had HMPV, and 5 (10.4%) had coronavirus. Eighteen (37.5%) had no virus isolated from the cell lines that we used.

Discussion

On the basis of a combination of conventional virus isolation system and molecular techniques, we found that 52.1% (25/48) of patients with SARS admitted to our hospital had HMPV infection. Isolation of HMPV is known to be difficult, which is why the virus could not be detected until recently. The first report on HMPV by van den Hoogen et al. (1) showed that the virus produced syncytia formation in tertiary monkey kidney cells, followed by rapid internal disruption of the cells and subsequent detachment from cell monolayer. The virus replicated poorly in Vero cells and human lung adenocarcinoma (A-549) cells and could not be propagated in MDCK cells or chicken embryo fibroblasts (1). In the study from Boivin et al. (7), multiple cell lines including LLC-MK2, HEp-2, MDCK, human foreskin fibroblast, Vero, Mink lung, A-549, human rhabdomyosarcoma (RD), transformed human kidney (293), and human colon adenocarcinoma (HT-29), were used for isolation of HMPV. The results showed that HMPV only grew on LLC-MK2 cells with cytopathic effect of round and refringent cells but without syncytia formation in most cases, an observation in agreement with our results. In that study, HEp-2 cell monolayers did not show cytopathic effect. Since the HEp-2 cells were not tested for HMPV RNA, we do not know whether our findings on HEp-2 cells were also observed by Boivin et al. In another study reported by Peret et al. (6), LLC-MK2, MDCK, and NCI-H292 cells were used; those researchers found that only LLC-MK2 cells produced cytopathic effect of focal rounding and without syncytia formation, which is also similar to our observation. The major difference in our approach for HMPV isolation compared to previous studies is the use of RT-PCR to enhance the detection of HMPV isolated from cell cultures. With this approach, we found that HEp-2 cells, a widely available and commonly used cell line, support the growth of HMPV. When RT-PCR was used to follow up all cell cultures, the sensitivity of HEp-2 cells was higher than LLC-MK2 cells, the cell line most commonly used in previous studies for HMPV. However, even using our approach, LLC-MK2 cells cannot be discarded, as in 12% of cases HMPV was only isolated from LLC-MK2 cells. In contrast, in the presence of HEp-2 cells, MDCK cells gave little additional value, as both specimens positive by MDCK cells had the viruses isolated from HEp-2 cells. In addition, our initial incubation of 14 days might not be optimal for isolating HMPV because Boivin et al. reported that the cytopathic effect took a mean incubation time of 17.3 days to develop (7).

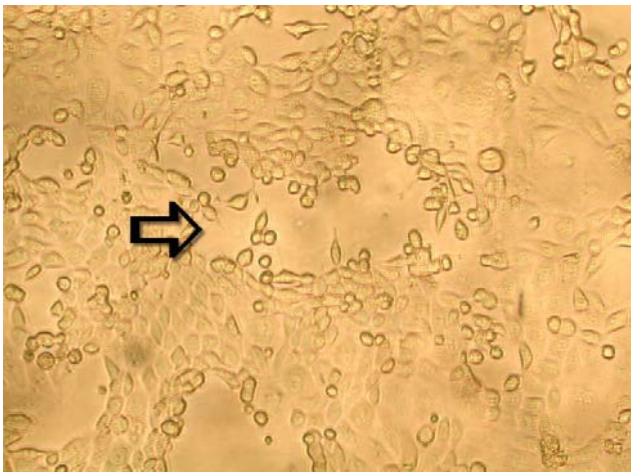

Figure 2. Late cytopathic effect of human metapneumovirus in rhesus monkey kidney (LLC-MK2) cell monolayers. Infected cells progressed slowly from focal rounding to detachment from cell monolayer indicated by an arrow (100X).

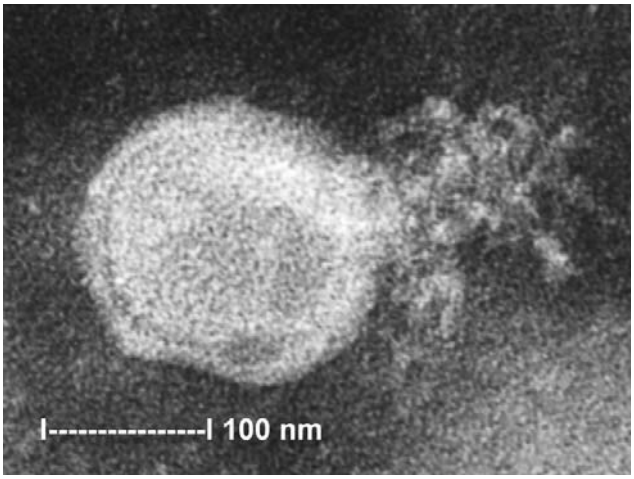

Figure 3. Electron micrograph of human metapneumovirus collected from the supernatant of rhesus monkey kidney (LLC-MK2) cell culture. A virion-releasing nucleocapsid is shown.

By prolonging the initial incubation of LLC-MK2 cells to 21 or 28 days, more HMPV infections might have been detected from our "negative" group.

Because all our study samples were collected from patients related to the outbreak of SARS that occurred in our hospital, one cannot simply infer that this in vitro growth property can be applied to all HMPV strains in general. Nevertheless, our approach of including HEp-2 cells, a widely available cell line, to search for HMPV, in particular for those cases related to SARS, needs to be considered. In our study, six patients were co-infected with HMPV and coronavirus. Although the number was limited, our findings suggest that HMPV and coronavirus have different in vitro tropisms, and the isolation of one virus does not affect the recovery of the other from different cell lines.

Overall, we confirmed that 25 (52.1%) of 48 patients admitted to our hospital with SARS had HMPV infections, with 6 also co-infected with coronavirus. However, the data on such high prevalence of HMPV should be interpreted cautiously. Our study population was based on persons and their family members who had been exposed in the index ward in our hospital. Thus, a co-circulation of two pathogens within our study group was possible. While the clinical presentations of all our study participants fulfilled the World Health Organization definition for a probable case of SARS (13), one should not infer, at this stage, that the prevalence of HMPV is similarly high in SARS outbreaks occurring elsewhere. On the other hand, the possibility of an important role of HMPV in the current worldwide outbreak of SARS should not be neglected. HMPV has also been detected in five of six SARS patients living in Canada (15). In that study series, coronavirus was also detected in five of six patients and four patients were co-infected with HMPV and coronavirus. A few recent studies implicate a strong association of a novel coronavirus with the worldwide outbreak of SARS (16–18). While both HMPV and coronavirus infections may result in severe respiratory tract diseases, their transmission efficiency may not be the same. This urgent question must be answered because the answer affects the priority for immediate development of control strategies.

During this outbreak of SARS, we have applied this combination approach of conventional virus isolation and molecular detection to establish the viral infection status of other patients hospitalized for SARS. We are in the process of analyzing a larger cohort to elucidate their clinical conditions, treatment responses, and epidemiologic links with

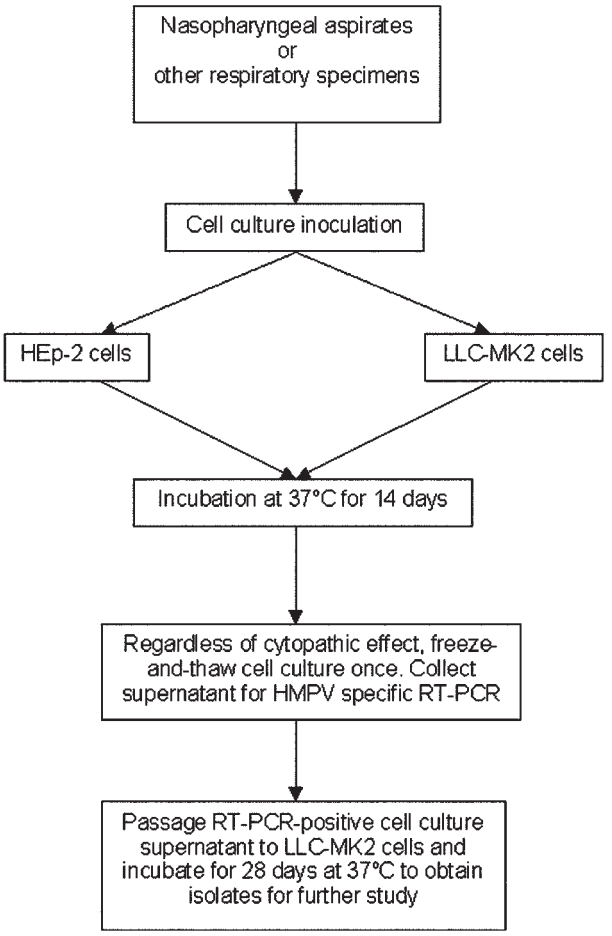

Figure 4. Combination approach of conventional virus isolation and molecular techniques to detect human metapneumovirus (HMPV) infection. Nasopharyngeal aspirates were examined in this study. This approach can be applied to other respiratory specimens. Prolonged incubation of rhesus monkey kidney (LLC-MK2) cells to 28 days for culture of original specimens may improve sensitivity of detection. Detection based on cytopathic effect is not sensitive for first-round culture from original specimens. All cell cultures should be examined by HMPV–specific reverse transcription-polymerase chain reaction. RT-PCR, reverse transcription-polymerase chain reaction.

respect to the infection status for both HMPV and coronavirus. Similar work in other parts of the world is needed.

Acknowledgments

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Severe Acute Respiratory Syndrome: Clinical Outcome and Prognostic Correlates

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Severe acute respiratory syndrome (SARS) poses a major threat to the health of people worldwide. We performed a retrospective case series analysis to assess clinical outcome and identify pretreatment prognostic correlates of SARS, managed under a standardized treatment protocol. We studied 127 male and 196 female patients with a mean age of 41±14 (range 18–83). All patients, except two, received ribavirin and steroid combination therapy. In 115 (36%) patients, the course of disease was limited. Pneumonitis progressed rapidly in the remaining patients. Sixty-seven (21%) patients required intensive care, and 42 (13%) required ventilator support. Advanced age, high admission neutrophil count, and high initial lactate dehydrogenase level were independent correlates of an adverse clinical outcome. SARS-associated coronavirus caused severe illnesses in most patients, despite early treatment with ribavirin and steroid. This study has identified three independent pretreatment prognostic correlates.

The outbreak of severe acute respiratory syndrome (SARS) in Hong Kong was caused by a novel virus belonging to the family *Coronaviridae* (1,2). The virus is transmitted through respiratory droplets, direct contact with fomites, and aerosolized respiratory secretions (3,4). The first outbreak was linked to an index patient treated in the Prince of Wales Hospital (4). The second wave of spread in the community was started by an infected patient with renal disease and amplified by the sewage system of Amoy Gardens, a densely populated condominium in Hong Kong (5). The floor drain traps in many apartments of Amoy Gardens were not filled with water and thus lost the sealing function. Therefore, the bathrooms of many apartments were openly connected with the soil stack. Virus-loaded droplets of an affected apartment could have been spread through the floor drain system. Hundreds of patients were then treated in public hospitals. The virus was highly contagious and caused substantial illness and death among the general population as well as among healthcare workers.

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The Hong Kong Hospital Authority, which provides more than 90% of inpatient care in Hong Kong, has been responsible for the management of all SARS patients (6). The Princess Margaret Hospital is a designated treatment center for SARS patients. Convalescent-phase SARS patients are treated in Wong Tai Sin Hospital. More than 500 SARS patients have been treated in these two hospitals since March 2003. The Hospital Authority has established a structured approach in the diagnosis, investigation, and treatment of SARS. The clinical diagnostic criteria of the Hospital Authority's SARS registry (defined in Table 1) were similar to the case definition of probable SARS by the World Health Organization (3).

Persons infected with the SARS-associated coronavirus may exhibit a wide spectrum of signs and symptoms and a varied clinical course. We have found asymptomatic cases and patients with spontaneous recovery without antiviral or steroid therapy; SARS is at the other end of the disease spectrum. The Hospital Authority's hypothetical disease model has three phases: viral replication, immune hyperactivity, and pulmonary destruction (7). Autopsy findings have supported the theory of cytokine deregulation in SARS (8). Using steroids in the treatment of SARS was based on this hypothesis and on initial clinical experience in the management of SARS in Hong Kong (4).

The recommended treatment regime at the time of the Amoy Gardens outbreak consisted of antibiotics, ribavirin, and steroid combination therapy. Patients without known epidemiologic contact with SARS patients were treated with antibiotics that would prevent both community-acquired pneumonia and hospital infections. If patients did not respond to antibiotics in 48 h, they would be given a combination of ribavirin and steroid. For patients with an epidemiologic history of contact with a SARS patient, this combination would be started together with the above

[1]P.T. Tsui and M.L. Kwok were coprincipal investigators and responsible for data collection. Dr. Tsui wrote the manuscript. H. Yuen and S.T. Lai led and fine tuned the research from hypothesis generation to writing the paper.

Table 1. Case definition of SARS, Hong Kong Hospital Authority SARS Registry, April 22, 2003

Inclusion criteria	Exclusion criterion
Radiographic evidence of infiltrates consistent with pneumonia	A case should be excluded if an alternative diagnosis can fully explain the illness
Temperature >38°C or history of such temperature at any time in the past 2 days	
At least two of the following:	
History of chills in the past 2 days	
Cough (new or increased cough) or breathing difficulty	
General malaise or myalgia	
Known history of exposure	

[a]SARS, severe acute respiratory syndrome.

antibiotic. Ribavirin would be given at a dose of 8 mg/kg intravenously every 8 h. For patients who appeared for treatment with extensive pneumonitis, a loading dose of 33 mg/kg of ribavirin, followed by 20 mg/kg every 8 h, was given intravenously. Hydrocortisone, 2 mg/kg every 6 h or 4 mg/kg every 8 h, would be administered, together with ribavirin. Oral equivalent doses of ribavirin and prednisolone could be prescribed at any stage of the disease. The total duration of therapy could range from 14 to 21 days. Besides administering steroids, we have tried in selected cases immunomodulation through the use of intravenous pentaglobin. Pulsed doses of methylprednisolone were restricted to those with disease progression and marked lung involvement. Lee et al. have made a comprehensive report of 138 cases of suspected SARS during a hospital outbreak in Hong Kong (4). Our study investigated the SARS patients after the Amoy Gardens outbreak to identify associated pretreatment prognostic factors for risk stratification and assess the clinical outcome of SARS under a standardized treatment protocol.

Methods

We performed a retrospective case series study. All reported SARS patients who stayed in the medical wards or intensive care unit of Princess Margaret Hospital and Wong Tai Sin Hospital on April 16, 2003, were screened. Patients were excluded if subsequent follow-up serologic tests showed no rise in antibody titer against SARS-associated coronavirus. All eligible SARS patients, except three, were recruited into the study. One healthcare worker refused to be studied, and two patients who were suspected of contracting the infection during their hospital stay were also excluded. This cohort was followed up until May 20, 2003. Data were collected through the Hong Kong Hospital Authority's computerized clinical management system, case record review, and a questionnaire survey assisted by the nursing staff of each SARS ward. Age, sex, occupation, residential address, smoking habit, time between onset of fever and start of antiviral therapy, coexisting conditions, and laboratory data were the variables under study. Outcome variables were the following: dependency on high amounts of oxygen (requiring at least 3 L/min of oxygen through a nasal cannula) and admission to an intensive care unit or death.

Statistical Analysis

Categorical variables were analyzed with the chi-square test and the means of continuous variables were compared with the Student t test. Association among continuous variables was assessed with Pearson correlation coefficient. Multivariate logistic regression by backward stepwise analysis was performed to identify independent variables that correlated with the clinical outcome as of May 20, 2003. Cox's regression model was used to study survival data. Plus-minus values are mean ± standard deviation; a p value of <0.05 was considered significant, and all probabilities were two-tailed. SYSTAT software (version 10.0, SPSS, Chicago, IL) was used for statistical analysis.

Results

The study population consisted of 127 male and 196 female patients, ranging in age from 18 to 83 (41±14). Forty-seven (15%) patients were healthcare workers. One hundred thirty-three (41%) were Amoy Gardens residents. Two hundred seventy-three (85%) patients were in good health. The coexisting conditions are listed in Table 2. Psychiatric illness, hepatitis B carrier status, and thalassemia trait status were not classified as coexisting conditions. Fifteen (14%) males and 7 (4%) females were current smokers. The overall prevalence of smoking among SARS patients was 7.6% (9.1% if healthcare workers are excluded). None of the affected healthcare workers smoked. The symptoms exhibited fulfilled the diagnostic criteria of the Hospital Authority's SARS registry.

All patients had lung involvement, documented either by chest x-ray or high-resolution computed tomographic scan of the thorax. Lymphopenia, found in 221 (68%) patients, was a prominent feature in those who sought treatment. Other initial laboratory findings included thrombocytopenia (41%), elevated creatine kinase level (14%), and elevated lactate dehydrogenase level (42%). Initial bacterial cultures were negative. Virus screening was negative for adenovirus, respiratory syncytial virus,

Table 2. Coexisting conditions in patients with severe acute respiratory syndrome

Coexisting condition	No. of patients
Hypertension	16
Diabetes mellitus	8
Chronic lung disease	6
Pregnancy	5
Neurologic disease	5
Renal disease	4
Cardiovascular disease	3
Immunologic disease	3
Malignancy	1

influenza A and B, and parainfluenza virus. Two hundred and seven (64%) patients had reverse transcriptase–polymerase chain reaction (RT-PCR) assays performed for SARS-associated coronavirus, and 128 (62%) of the results were positive. Two hundred and forty-two (75%) patients had completed serologic testing. The diagnosis of recent SARS-associated coronavirus infection was confirmed by either RT-PCR assays or serologic test in 286 (89%) patients. The sensitivity of RT-PCR assays was 58% (95% confidence interval [CI], 50% to 66%).

Our patients sought treatment 3.9±2.7 days after onset of fever. The interval between onset of fever and admission was positively correlated with admission neutrophil count (Pearson r=0.1, p=0.07), admission platelet count (Pearson r=0.1, p=0.06), and initial lactate dehydrogenase level (Pearson r=0.36, p<0.001). An antibiotic was started immediately after admission in all cases. Either levofloxacin, 500 mg once a day, or amoxicillin/clavulinate acid, 375 mg three times a day plus clarithromycin, 500 mg twice a day, was used to protect against community-acquired pneumonia. All patients were also treated with oral or intravenous ribavirin, according to protocol. Most (94%) were given either intravenous hydrocortisone or oral prednisolone, according to protocol. Five patients received intravenous methylprednisolone as a form of steroid therapy. The dose was administered at 3 mg/kg once a day and would be tapered down to 1 mg/kg if the patient showed a clinical response. Pulsed doses of methylprednisolone (500 mg per dose) were given as initial treatment in 12 patients, who then received maintenance steroid therapy. Two patients were treated with ribavirin only. Ribavirin plus steroid therapy was administered 1.2±1.7 days after admission. The interval between admission and initiation of antiviral therapy was negatively correlated with the interval between onset of fever and admission (Pearson r = –0.17, p=0.003).

Clinical Outcome

In 115 (36%) patients, the disease was limited with resolution of fever and pneumonitis. Two hundred and eight (64%) patients had either clinical or radiologic evidence of progression of pneumonitis, and they received 2.9±2 gm pulsed dose methylprednisolone therapy. Maintenance steroid was resumed after pulsed dose therapy. Patients who were given pulsed doses of steroids were treated with potent broad-spectrum intravenous antibiotics (piperacillin and tazobactam) to protect against hospital-acquired infection. Hyperglycemia, hypokalemia, flare up of hepatitis B infection, hospital-acquired infection, and steroid psychosis were the acute side effects encountered. Hepatitis B carriers were treated with lamivudine, 100 mg once a day; no liver failure occurred in members of this cohort. Disease progression was apparently arrested by pulsed dose steroid therapy in 98 (30%) patients. In the remaining 110 (34%) patients, the illness ran a severe and protracted course, and the patient needed high doses of oxygen. Sixty-seven (21%) had been admitted to intensive care unit, and 42 (13%) required ventilator support. Twenty-six patients died (12 males and 14 females). The crude mortality rate of our cohort after 47±8 days of follow-up was 7.9% (95% CI, 5% to 10.8%) and was an underestimation because of sampling bias. Those who died before April 16, 2003, were excluded from our sample, while long-term survivors were retained for study. Among them, 10 had concurrent medical illness. No healthcare worker in this cohort died. Diabetes was found in three patients who died, and hypertension in four who died. Eleven of those who died lived in Amoy Gardens. A young pregnant woman died after delivery, despite aggressive treatment.

Age, sex, healthcare worker status, Amoy Gardens resident status, presence of coexisting conditions, interval between onset of fever and therapy (ribavirin plus steroid), neutrophil and platelet count on admission, and initial creatine kinase and lactate dehydrogenase levels were the correlates of clinical outcome under study. Variables with a p value of <0.1 by univariate analysis were entered into the multivariate regression model. By multivariate logistic regression, advanced age, high neutrophil count on admission, and high initial lactate dehydrogenase level were independent correlates of high oxygen dependency as well as intensive care unit admission or death (Table 3). By Cox's backward stepwise regression, young age, low neutrophil count on admission, and healthcare worker status (p=0.05) were favorable independent correlates of survival time (Table 3). A dose-response relationship also existed between the independent correlates and clinical outcome (Figures 1–3). We used the term "correlates" instead of "predictors" of outcome because of the method we used, a case series.

The second serology titer obtained after the end of second week was negatively correlated with age (Pearson r=–0.13, p=0.05) and admission lymphocyte count (Pearson r=–0.17, p=0.01). Conversely, the neutrophil count on admission was positively correlated with the sec-

Table 3. Independent prognostic correlates and clinical outcome

	High oxygen dependency	ICU care or death	Survival time
Correlates	OR (95% CI) p value	OR (95% CI) p value	Hazard ratio (95% CI) p value
Age (per 10-y increase)	1.48 (1.21 to 1.8) p<0.001	1.57 (1.26 to 1.95) p<0.001	1.75 (1.38 to 2.2) p<0.001
Admission neutrophil (per $1x10^9$/L increase)	1.31 (1.14 to 1.5) p<0.001	1.28 (1.13 to 1.46) p<0.001	1.17 (1.09 to 1.26) p<0.001
Initial LDH level (per 100 IU/L increase)	1.49 (1.23 to 1.82) p<0.001	1.35 (1.11 to 1.64) p=0.003	p value not significant

[a]ICU, intensive care unit; LDH, lactate dehydrogenase; OR, odds ratio; CI, confidence interval.

ond serology titer (Pearson r=0.2, p=0.003). The pulsed dose of steroid was not shown to affect the second serology titer (Pearson r=0.1, p=0.18). Patients who depended on high oxygen therapy had a higher second antibody titer against SARS-associated coronavirus (p = 0.05).

Discussion

The virus attacked persons of both sexes and all ages. Many were previously in good health and the wage earners in their families. Not infrequently, several members of a family were admitted to the hospital. The need for isolation discouraged close social contact. Unfortunately, some of the patients were also stigmatized. The psychosocial effect of SARS is by no means a lesser problem.

RT-PCR assay for SARS-associated coronavirus is a new test, and its sensitivity and specificity have yet to be established. In our cohort, the sensitivity was 58%, and results depended on sampling technique and stage of disease (9). Contamination of specimen could lead to a false-positive result. A false-negative result could arise from performing the test in the very early or late stage of the disease. Diarrhea was common among Amoy Gardens SARS patients. The virus could be found in stool by RT-PCR assays. A negative test does not rule out the diagnosis, however. The serologic test remains as the standard criterion of definitive diagnosis. Pulsed doses of steroid did not seem to affect the humoral response of SARS patients. In retrospect, the intensity of antibody response was related to clinical outcome and associated pretreatment prognostic factors. The viral load could be a determinant of these prognostic association factors.

Our hematologic and biochemical data, as well as associated prognostic factors, agreed with the work of Lee et al. (4). Both advanced age and high neutrophil count on admission were associated with poor outcome. We found that initial lactate dehydrogenase level was also an associated prognostic factor. The early phase of SARS is characterized by lymphopenia and thrombocytopenia. As the disease progresses, both neutrophil and platelet counts rise, accompanied by an elevation in lactate dehydrogenase level. The neutrophilic response is important in the pathogenesis of hypersensitivity pneumonitis, and thus the initial neutrophil count could also indicate disease progression. Lactate dehydrogenase level reflects tissue necrosis related to immune hyperactivity in SARS and thus relates to poor outcome. Patients with high neutrophil counts and lactate dehydrogenase levels on admission could have been late in seeking treatment or have experienced heavy exposure to the virus.

Effect on Healthcare Workers

The spread of the disease to healthcare workers is a major problem in any country dealing with SARS. Intubation, nasopharyngeal aspiration, chest physiotherapy, handling of excreta, and even feeding become high-risk procedures. All healthcare workers working in Hospital Authority hospitals are required to follow the recommended personal protection equipment standards (10). The level of precaution depends on the risk in the work area and the type of procedure performed. All healthcare workers work-

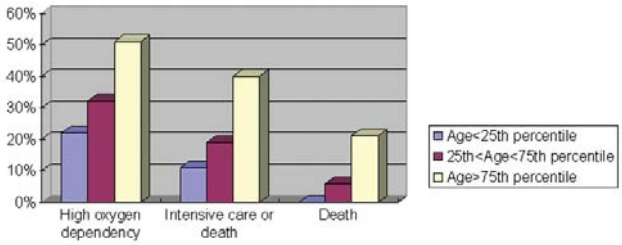

Figure 1. Relationship between age and fatal severe acute respiratory syndrome illness, Hong Kong, 2003.

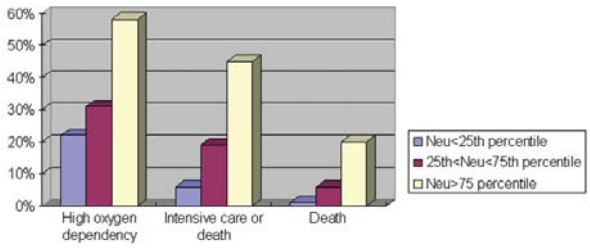

Figure 2. Relationship between neutrophil count and fatal severe acute respiratory syndrome illness, Hong Kong, 2003.

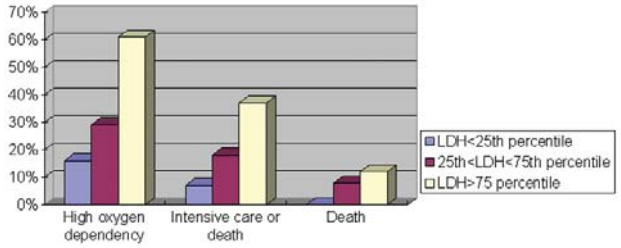

Figure 3. Relationship between lactate dehydrogenase level (LDH) and fatal severe acute respiratory syndrome illness, Hong Kong, 2003

Table 4. Number of infected healthcare workers treated in Princess Margaret Hospital, Hong Kong, 2003[a]

	ICU	SARS area	Non-SARS area	Total no. (%)
Doctor	2	1	1	4 (9)
Nurse	9	15	3	27 (57)
Other	6	3	7	16 (34)

[a]ICU, intensive care unit; SARS, severe acute respiratory syndrome.

ing in a SARS area wore N-95 masks, face shields, caps, gowns, and surgical gloves. The intensive care unit was high-risk area in this cohort (Table 4). However, healthcare workers working in a non-SARS area were not exempted. They contracted the disease from SARS patients who sought treatment early or exhibited atypical signs and symptoms. By univariate analysis, healthcare worker status was negatively correlated with death. Healthcare workers were younger. They sought treatment earlier and had a lower neutrophil count and lower initial lactate dehydrogenase level on admission (Table 5). Nevertheless, healthcare worker status was still an independent survival correlate after controlling these confounding variables. The current safety precaution could not prevent all frontline healthcare workers from contracting SARS, but minimizing individual exposure to the virus might reduce the viral load, subsequent immune hyperactivity, and the risk for a fatal outcome.

Benefit of Treatment

Most of the patients in this cohort were treated according to protocol. The clinical outcome did not represent the natural history of SARS. The only variable that was related to the benefit of treatment was the time from onset to treatment. Donnelly et al. found that the time between the onset of symptoms and admission to hospital did not affect the death rate (11). In this study, patients who sought treatment early and received antiviral and steroid combination therapy were not shown to do better by multivariate analysis.

The Hospital Authority adopted an aggressive treatment protocol during the peak of the SARS epidemic in Hong Kong. Broad-spectrum antibiotics and a combination of ribavirin and steroid were the mainstays of treatment. The dose of ribavirin used was small to prevent major side effects. The administration of steroids in SARS treatment is controversial, however. Theoretically, the early use of steroids promotes viral replication, enhances infectivity, and possibly causes a rebound of infection. Peiris et al. found that the viral load peaked at day 10 in SARS patients treated with both ribavirin and steroids (12). However, immunosuppression or, more precisely, immunomodulation, is believed to be an effective therapy at the second stage of SARS. The current consensus among the Hospital Authority's expert panel is to begin administering a steroid or pentaglobin at the second stage of SARS when a hypersensitivity immune reaction occurs (7).

Table 5. Relationship between healthcare worker status and other prognostic variables

Variable	Non-HCW[a]	HCW	p value
Age	42±14	37±11	0.007
Onset-to-treatment (d)	5.3±3.0	3.8±2.2	0.001
Neutrophil ($x10^9$/L)	4.5±2.8	3.9±1.5	0.04
Lactate dehydrogenase (IU/L)	276±161	188±63	<0.001

HCW, healthcare worker.

Patients who sought treatment early tended to receive antiviral therapy at a later time. This is understandable since the symptoms of SARS are nonspecific, and clinicians also rely on laboratory data for diagnosis. The sensitivity of current RT-PCR assays is not satisfactory. A more sensitive and rapid diagnostic test must be developed, particularly if we have an effective treatment regime in the future.

Conclusion

One third of the SARS patients in our study had a limited disease course. In the remaining two thirds, pneumonitis progressed rapidly after the early use of ribavirin and steroid combination therapy. Apparently, approximately one third responded to pulsed doses of steroids, while the other third depended on treatment with high amounts of oxygen. Intensive care was required for 21% of patients. Advanced age, high neutrophil count on admission, and elevated initial lactate dehydrogenase level were independent correlates of an adverse clinical outcome. Strong evidence to support early and routine use of ribavirin and steroid combination therapy in all SARS patients does not exist.

We need to investigate new antiviral agents and test the efficacy of steroids in randomized controlled trials. SARS is an entirely new emerging disease and its clinical course varies widely. By stratifying our patients according to risk, we could individualize our treatment protocol. In addition, we need a more sensitive and rapid diagnostic test for SARS-associated coronavirus infection, both for treatment and for forming cohorts of patients infected with this deadly disease.

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Dr. Tsui is an internal physician at Princess Margaret Hospital of Hong Kong. His research interests include epidemiology and disease prevention.

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Hantavirus Infection in Humans and Rodents, Northwestern Argentina

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We initiated a study to elucidate the ecology and epidemiology of hantavirus infections in northern Argentina. The northwestern hantavirus pulmonary syndrome (HPS)–endemic area of Argentina comprises Salta and Jujuy Provinces. Between 1997 and 2000, 30 HPS cases were diagnosed in Jujuy Province (population 512,329). Most patients had a mild clinical course, and the death rate (13.3%) was low. We performed a serologic and epidemiologic survey in residents of the area, in conjunction with a serologic study in rodents. The prevalence of hantavirus antibodies in the general human population was 6.5%, one of the highest reported in the literature. No evidence of interhuman transmission was found, and the high prevalence of hantavirus antibody seemed to be associated with the high infestation of rodents detected in domestic and peridomestic habitats.

Hantaviruses (family *Bunyaviridae,* genus *Hantavirus*) are zoonotic viruses of rodents that produce two major clinical syndromes in humans: hemorrhagic fever with renal syndrome (HFRS) in Asia and Europe and hantavirus pulmonary syndrome (HPS) in the Americas. Since HPS was initially characterized in the United States in 1993 and the associated hantavirus (Sin Nombre virus, or SNV) was identified, an increasing number of human cases and SNV-related viruses have been identified in different countries of North and South America (1). Three HPS-endemic areas have been recognized in Argentina: northern (Salta and Jujuy Provinces), central (Buenos Aires, Santa Fe, and Entre Rios Provinces), and southern (Rio Negro, Neuquén, and Chubut Provinces). In the North, cases of acute respiratory distress syndrome of unknown etiology have been reported since 1984 at Orán, Salta Province. The illness, known in the area as "Distress of Orán," had an unexplained etiology until the early 1990s, when these cases were first associated with *Leptospira interrogans* infections and later with hantaviruses. Of 21 patients tested between 1991 and 1993, eight showed serologic evidence of recent leptospira infection by microscopic agglutination test, and 4 had a positive immunoglobulin (Ig) M enzyme-linked immunosorbent assay (ELISA) using Hantaan virus antigen (2,3). Ultimately, these patients were recognized as having HPS, and a new SNV-related hantavirus, now designated Oran virus, was recognized in the region (4). The first HPS cases in Jujuy Province were confirmed in 1997, and since then, their number has been progressively increasing. Isolated cases were detected in several different localities (San Pedro, La Mendieta, Caimancito, Libertador General San Martín, Fraile Pintado, and San Salvador, the provincial capital), but most originated in the town of Yuto and surroundings. A high percentage of confirmed cases had the usual nonspecific prodrome but were not followed by a distress syndrome. The case death rate (4 [13.3%] of 30) was noticeably lower than that reported in other areas of the country and in the literature. Some strains of hantavirus were then hypothesized to produce subclinical disease. Only one hantavirus antibody-prevalence study had been performed among inhabitants of the Gran Chaco of Paraguay and Argentina (Salta Province), and hantavirus antibodies were found in 20% to 40% of participants (5).

Some differences in the clinical signs and symptoms of HPS have been recognized in other areas of the Americas compared with those described after infections with SNV; these differences have included possible person-to-person transmission, a different spectrum of clinical illnesses, an elevated incidence of infections in children, and higher antibody prevalence (6). For instance, patients from the area under study had unusually mild clinical symptoms and low death rates, supporting the idea that a less pathogenic hantavirus could be circulating in that area or that host or environmental factors might be responsible for the observed pattern. The objectives of this study were to determine the prevalence of hantavirus antibodies in the

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general population, identify risk factors, and investigate the rodent species implicated in hantavirus transmission in Yuto.

Material and Methods

Study Area

Yuto is located in the Ledesma Department, in the northeastern portion of Jujuy Province (23° 38′ S, 64° 28′ W). General topography is determined by the outlying spurs of the Andes range, and the area is covered by dense subtropical vegetation. The easternmost part of the study area is flat or slightly undulated, very fertile, with numerous rivers and streams and an average elevation of 349 m. Mean annual temperature is 20.7°C, ranging from 14.5°C in July (winter) to 25.8°C in January (summer). The rainy season starts in November as an annual monsoon, which lasts through the summer and into early fall; mean annual rainfall is 862 mm with a maximum monthly mean of 191 mm in January and a minimum monthly mean of 4 mm in July. Similar habitats and topography continue to the South (to Tucumán Province) and the North (to Oran, in Salta Province). The original biome of the area is a subtropical forest called "the yungas forests," with numerous tree species of high economic value (*Anadenanthera colubrina, Calycophyllum multiflorum, Phyllostylon rhamnoides, Astronium urundeuva, Maclura tinctoria, Cordia trichotoma,* among others). This forest area is now considerably fragmented and modified by human agricultural activities. The main cultivated crop is sugar cane, which is grown from May to November. Other products include citrus fruits, avocados, pears, bananas, mangos, papayas, cherimoyas, and vegetables. Agriculture is the main source of employment, mostly involving manual labor. Housing for agricultural laborers is typically of very poor construction, in many cases consisting of shacks of salvaged wood and sheet metal. This type of domestic and peridomestic habitat offers prime conditions for rodent infestations, providing easy rodent access and poor sanitation, and is found even in the urban area of Yuto.

Population Survey

A cross-sectional study was performed on a sample of the general population of the area (population 7,900). The estimated sample size to document the overall prevalence in the total population was approximately 340 persons. Figure 1 shows the distribution of the general population and that of the survey participants by sex and age. Local physicians explained the objectives of the study to participants, and an informed consent agreement was signed by each person or by parents or legal guardians of minors. Each participant had a blood sample drawn and completed a questionnaire that covered personal data, ethnicity, household and workplace characteristics, occupation, domestic sightings of rodents, recreational activities, time of residence in the area, history of travel inside and outside the country, previous disease compatible with HPS, and contact with a confirmed HPS patient.

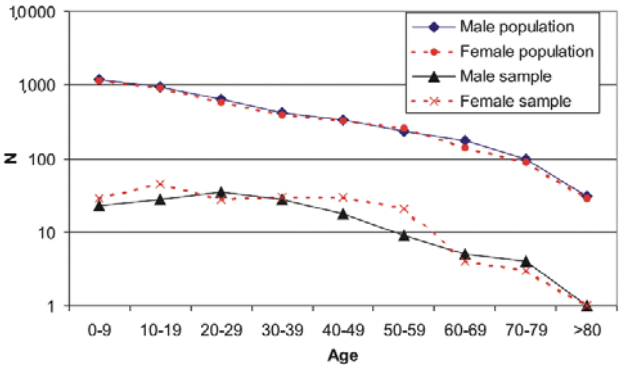

Figure 1. Distribution of general population and survey population by sex and age.

Rodent Study

Trapping Site Selection

Sherman live traps were placed at likely sites of exposure of previously documented HPS cases. Nine sites were selected: four sites in Yuto District (Guaraní [13 lines, 347 traps], Jardín [4 lines, 60 traps], 17 Has [4 lines, 124 traps], and 8 Has [7 lines,168 traps]); one in El Bananal, a small rural village 7 km outside Yuto (11 lines, 214 traps); three on or adjacent to farms (*fincas* [26 lines, 1,100 traps]); and one in a brushwood area (seminatural habitat [8 lines, 500 traps]). One farm was located in Urundel, Salta Province, in the immediate vicinity of Yuto, and the owner, workers, and inhabitants belonged to the Yuto community. Of the 73 capture lines, 19 were inside the household, 25 were peridomestic, 6 were in weeds near grapefruit culture, 5 in a brushwood, 5 at the side of a river or stream, 3 in vegetable gardens, 3 at roadsides, 2 in fruit orchards, 2 at the edge of a canal, and 3 adjacent to wire fences, railroads, or gullies. Outside lines consisted of 25 traps, each separated by 5 m. Lines located inside and outside the houses corresponded both to rural and urban areas. The number of traps inside the houses and in peridomestic urban lines depended on the area available at each site (8–20 traps). Figure 2 shows the location of trapping sites in Yuto and its surroundings.

Trapping and Processing

Trapping was performed from May 30 to June 4, 2000. Small mammals were collected each morning and transferred to a field laboratory for processing. After being anesthetized with Isofluorane (Abbott Laboratories Ltd.,

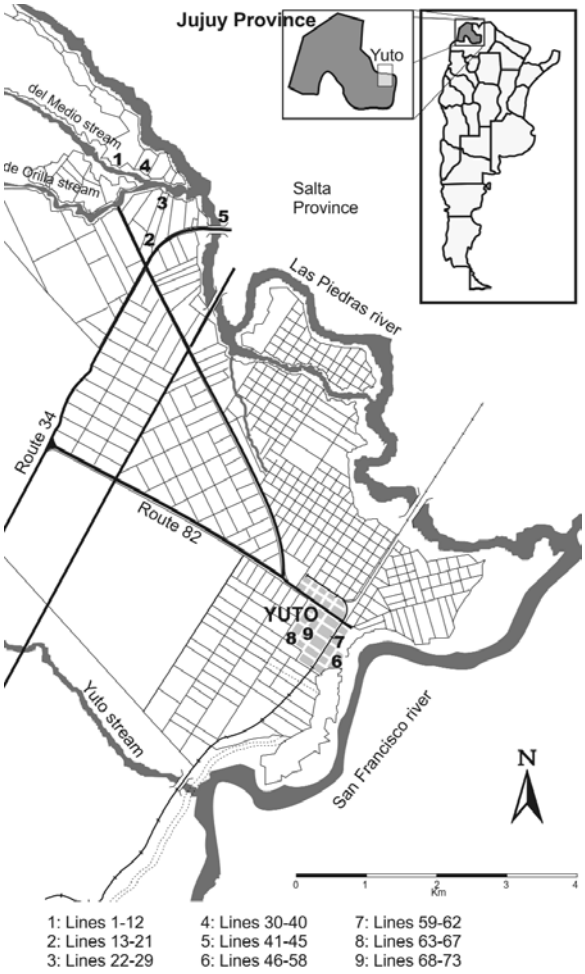

Figure 2. Localization of rodent trapping sites in Yuto and its surroundings.

Queenborough, England), animals were bled from the retroorbital sinus by using heparinized capillary tubes, and then killed by cervical dislocation while still anesthetized. Samples of serum, blood clot, brain, heart, kidney, liver, and lung were placed in cryovials and stored in liquid nitrogen for their subsequent analysis at the Instituto Nacional de Enfermedades Virales Humanas (INEVH). Carcasses were tentatively identified in the field and preserved in 10% formalin and sent to the Natural Sciences Museum "Miguel Lillo" in San Miguel de Tucumán for taxonomic confirmation. Small mammal trapping and processing were performed according to established safety guidelines (7).

Serology

Human blood samples were centrifuged at Yuto Hospital. Serum was separated and placed in cryovials and stored in liquid nitrogen until further testing at INEVH. Rodent samples were centrifuged in the field laboratory and stored as described. Hantavirus antibodies were detected by an ELISA. Briefly, 96-well polyvinyl microplates were coated with SNV recombinant and control antigen overnight; then, serum samples and positive and negative controls were applied, followed by a peroxidase-conjugated antihuman IgG for human serum and a mix of peroxidase-conjugated anti–*Rattus norvegicus* and anti–*Peromyscus maniculatus* IgG for rodent serum. The substrate applied was 2,2′-azino-di (3-etilbentiazolin sulfonate) (ABTS, Kierkegaard & Perry Laboratories, Inc., Gaithersburg, MD). Serum dilutions were considered positive if the optical density was >0.2 after adjusting by subtraction of the corresponding negative-antigen optical density. Serum samples with titers >1:400 were considered positive.

Results

Serologic Survey in the General Population

Hantavirus IgG was found in 22 (6.5%) of 341 serum samples tested. For males, hantavirus antibody prevalence was 10%; females had a prevalence of 3.7%. Among the 341 participants, 56 were <10 years of age, 239 were 11–50 years of age, and 45 were >51 years of age (1 was without age data). Mean age among antibody-positive persons was 41 (range 18–87); 77% of these were in the 11- to 50-year age group. Hantavirus antibody prevalence according to sex and age are shown in Table 1.

Most (292/341, 85.6%) of the population in the survey were local born or native Argentinians. Twenty-five (7.3%) participants were foreigners, including 24 Bolivians and 1 Paraguayan. Twenty-two persons had aboriginal ancestors (6.5%), with 17 belonging to the Guarani community. No information was available for two persons. Only one of the aboriginal participants had IgG antibodies to hantaviruses (1 [4.5%] of 22). Table 2 shows hantavirus antibody prevalence among the study population by ethnicity or nationality.

Dwellings were characterized according to their location as urban (>500 m from an open field), suburban (50–500 m from an open field), and rural (<50 m from an open field). Table 3 shows hantavirus antibody-prevalence

Table 1. Serologic findings for hantavirus antibodies by age, sex

	Females	Males
Age (y)	No. positive/tested (%)	No. positive/tested (%)
0–9	0/29 (0)	0/23 (0)
10–19	0/45 (0)	2/28 (7.1)
20–29	1/28 (3.6)	3/35 (8.6)
30–39	0/30 (0)	4/28 (14.3)
40–49	4/30 (13.3)	3/18 (16.7)
50–59	0/21 (0)	1/9 (11.1)
>60	2/8 (25)	2/10 (20)
Without data		0/1
Total	7/191 (3.7)	15/150 (10)

Table 2. Ethnic demographics of the study population and hantavirus antibody prevalence[a]

Ethnicity or nationality	No positive/tested (%)
Native[b] without specification	13/229 (5.7)
Native with Bolivian lineage	6/56 (10.7)
Native with Paraguayan lineage	1/7 (14.2)
Native total	20/292 (6.8)
Indigenous without specification	0/3 (0)
Indigenous Guaraní	0/17 (0)
Indigenous Chaguanco	0/1 (0)
Indigenous Charagua (Bolivia)	1/1 (100)
Indigenous total	1/22 (4.5)
Bolivians	1/24 (4.2)
Paraguayan	0/1 (0)
Foreigners total	1/25 (4)
Without data	0/2 (0)

[a]By immunoglobulin G enzyme-linked immunosorbent assay.
[b]Native, born in Argentina.

findings in relation to house location and occupation of participants. Forty persons with urban occupations included 10 administrative employees, 13 health workers, 5 housewives, 4 students, and other miscellaneous occupations (technician, gardener, bricklayer, retired). Among suburban study participants, 23 were housewives, 28 were students, and the rest were employed in a variety of occupations (employee, health agent, maid, bricklayer). Among rural participants, 75 were agricultural workers, 64 housewives, 29 students, 10 sawmill workers, and the rest had miscellaneous occupations (employee, trader, bricklayer, retired). All three hantavirus-antibody–positive participants living in urban dwellings worked in rural areas (a bricklayer, a sawmill worker, and an agricultural worker), as did two antibody-positive participants living in suburban houses (agricultural workers). Thirteen (17.1%) of 76 participants with antibodies were agricultural workers (laborers, farmers, *fincas* owners). Antibody prevalences for other occupations included housewives (4 [6.3%] of 64), and sawmill workers (1 [10%] of 10). We found no hantavirus antibodies among 61 students or 20 healthcare workers, including physicians, nurses, health agents, and a dentist.

If occupations are considered as rural (positive IgG, 20 [10%] of 201) or not rural (urban and suburban, positive IgG 1 [1.03%] of 97), hantavirus antibody prevalence is significantly higher in the former (chi square=7.95, p=0.004); among those with rural occupations, those whose employment included agricultural activities had a higher prevalence of hantavirus antibodies (positive IgG 13 [17.1%] of 76; 7 [5.6%] of 125; chi square=6.98, p=0.008).

Table 4 shows clinical and epidemiologic data for the study population. Most (86%) hantavirus antibody–positive participants did not recall previous HPS clinical manifestations. The presence of rodents was reported by 77% of hantavirus antibody–positive and 79% of hantavirus antibody–negative persons, both in peridomestic and workplace settings.

Among persons who had previous contact with known HPS patients, 6 (6.1%) of 98 cases had hantavirus antibodies. Similar antibody prevalence was found in persons who did not have prior contact with a known HPS patient (16 [6.6%] of 242; chi square p>0.05). One hundred five persons (30.8%) reported no trips outside the area, and 58% of the remainder had traveled only to other areas inside the province or to nearby Salta Province; 41.6% reported trips to Bolivia in addition to local trips. Only a small percentage (0.4%) had visited relatively distant areas of the country. Hantavirus antibodies were found in 15 (6.4%) of 233 persons who traveled outside the immediate region, and 7 (6.6%) of 105 who had not traveled outside of the immediate region.

Rodent Study

A total of 361small mammals were captured in 2,427.5 trap-nights (overall trap success 14.8%). Captures represented two rodent families, three subfamilies, and 13 species (Table 5). *Calomys* and *Akodon* were the most frequently trapped genera (38% and 40.2%, respectively) and the sole taxa that were found with hantavirus antibodies. Hantavirus IgG was found in 4 (2.8%) of 140 *Akodon simulator* and 7 (5.1%) of 137 *Calomys callosus*. The genus *Oligoryzomys* has several species previously identified as hantavirus reservoirs in the three HPS-endemic areas of the country. In this study, this genus accounted for 12 (3.3%) of the 361 captures; however, none was positive for hantavirus antibody. Table 6 shows the species distribution according to the different habitats sampled. The specimens of *C. callosus* were trapped inside dwellings located at different *fincas*.

More than half (6 [54.5%] of 11) of the positive rodents were captured in weeds, roadsides, or peridomestic sites at *fincas* (fruit trees and vegetable plantations); four were trapped in brushwood, and the last one at the riverside very near an HPS patient's dwelling at El Bananal. Two lines contributed with two positive rodents on each. These were located in grapefruit plantations (weeds and roadside).

Table 3. Hantavirus antibody prevalence by location of residence site and location of occupational activity[a]

	Urban no. positive/tested (%)	Suburban no. positive/tested (%)	Rural no. positive/tested (%)
Dwellings	3/31 (9.7)	4/121 (3.3)	15/189 (8)
Occupational activity	0/40 (0)	1/57 (1.8)	20/201 (10)

[a]As determined by immunoglobulin G enzyme-linked immunosorbent assay.

Table 4. Hantavirus antibody prevalence by clinical and epidemiologic characteristics

	Yes	No		Without data
	Positive/tested (%)	Positive/tested (%)	Chi square,[a] p value	Positive/tested (%)
Previous clinical HPS[b] symptoms	3/29 (10.3)	19/307 (6.2)	0.75, 0.38	0/5 (0)
Contact with a confirmed HPS case-patient	6[c]/98 (6.1)	16/242 (6.6)	0.03, 0.86	0/1 (0)
Recreational activities (fishing, hunting)	6/77 (7.8)	16/260 (6.2)	0.26, 0.60	0/4 (0)
Sighting of rodents	17/270 (6.3)	5/71 (7.0)	0.05, 0.81	
Trips outside the area	15/233 (6.4)	7/105 (6.7)	0.01, 0.93	0/3 (0)

[a]Chi-square test for comparison of two proportions in two independent samples, p>0.05 no significance. Epi Info version 2000 (Centers for Disease Control, Atlanta, GA).
[b]HPS, hantavirus pulmonary syndrome.
[c]All positive contacts were relatives of a confirmed HPS patient.

Discussion

The differences observed in the South American hantavirus infections relative to the classical SNV-related syndrome in North America have been suggested to reflect approaches to surveillance and differences in the pathogenicity of the viruses, host-reservoirs, and ecologic factors. The particular pattern of mild clinical illnesses and low case-fatality rate found in Yuto determined our selection of the area for detailed studies. In this first investigation, we attempted to determine the prevalence of past infection in the general population by testing for hantavirus IgG antibodies, to identify risk factors, and to identify the rodent species implicated in the transmission of hantaviruses

The hantavirus antibody prevalence found in the human population survey is one of the highest reported in Argentina, with a mean of 6.5% (females 3.7%, males 10%). Previous studies in the other HPS-endemic areas (central and southwestern) of Argentina found antibody prevalence varying from 0.1% to 1.5% (4,8). Males in their 30s and 40s showed antibody prevalences of >14% and 16%, respectively. Most antibody carriers (82%) did not report clinical manifestations consistent with HPS. Thus, the low case-death rate clearly reflects milder clinical illnesses (reported case-fatality rates are 40% to 50% in both Americas) (9,10).

In previous studies of asymptomatic contacts of HPS case-patients from this area, we found a high prevalence of IgG (4 [9.5%] of 42). This finding could be the result of infection from a common source or interhuman transmission (6), as described in the 1996 outbreak in El Bolson-Bariloche, southern Argentina (11–13). In this survey, we did not find differences in the hantavirus antibody prevalence in persons with and without known HPS case contact (6.1% and 6.6%, respectively). No antibodies were found among the healthcare workers studied, and the distribution of clinical cases and antibody carriers by sex showed a predominance of males (in patients from 1997 to 2000, the percentage of males was 76.7%, 23/30). These findings, collectively, do not favor the hypothesis that interhuman spread is playing a large role in the transmission of hantaviruses in this area. These findings also reinforce the view that environmental, occupational, and residential factors create an increased risk for rodent exposure in occupational, domestic, and peridomestic settings. This conclusion is supported by the noticeable observation of rodents and their signs in households and workplaces reported by the study population and patients. The risk factor that showed a significant difference between antibody-positive and -negative persons was a rural occupation, especially one associated with agricultural activities. This finding was also reflected in the high male antibody prevalence

Table 5. Relative abundance and hantavirus antibody prevalence of rodent species in Yuto (Jujuy Province)

Order	Family	Subfamily	Genus/species	Captures (%)	No. positive/tested (%)
Rodentia	*Muridae*	Sigmodontinae	*Akodon simulator*	140 (39.0)	4/140 (2.8)
			A. caenosus	3 (0.8)	0/3
			Akodon sp	2 (0.6)	0/2
			Calomys callosus	137 (38.0)	7/137 (5.1)
			Holochilus chacarius	11 (3.1)	0/11
			Oligoryzomys chacoensis	4 (1.1)	0/4
			O. longicaudatus	6 (1.7)	0/6
			Oligoryzomys sp	2 (0.6)	0/2
			Oxymycterus paramensis	3 (0.8)	0/3
		Murinae	*Mus musculus*	10 (2.8)	0/10
			Rattus rattus	6 (1.7)	0/6
Didelphimorphia	*Didelphidae*	Marmosinae	*Thylamys venustus*	2 (0.6)	0/2
			T. elegans	1 (0.3)	0/1
No identification				34 (9.4)	0/34
Total				361 (100)	11/361(3.0)

Table 6. Distribution of rodent species by capture habitat

	Habitat						
	Districts		Farms				
Species	Domestic[a]	Peridomestic[b]	Domestic[a]	Peridomestic[b]	Cultivation[c]	Brushwood[d]	Total
Akodon simulator		5		7	51	77	140
Akodon sp						2	2
Akodon caenosus						3	3
Calomys callosus		8	5	25	44	55	137
Holochilus chacarius					7	4	11
Mus musculus	7	3					10
Oligoryzomys sp					2		2
Oligoryzomys chacoensis						4	4
O. longicaudatus						6	6
Oxymycterus paramensis		1				2	3
Rattus rattus		1	4	1			6
Thylamys elegans					1		1
T. venustus		1			1		2
No identification		1			17	16	34
Total	7	20	9	33	123	169	361

[a]Inside households.

[b]Immediate vicinity of houses, including yards, outbuildings, vegetable gardens, weeds, fence lines, and railroad.

[c]Includes grapefruit and banana plantations, vegetables cultivation, weeds, and roadside in the same sites.

[d]Seminatural habitat that includes a streamside.

observed in the survey and predominance of males among patients.

A high antibody prevalence has been previously found in indigenous communities of the Gran Chaco of Paraguay (40.4%) and Argentina (17.1%) (in Salta Province). In those studies, the aboriginals evaluated belong to closed communities that still fish, hunt, and gather for their sustenance. Their main ethnic groups are Chorote, Chulupi, and Wichi of the Mataco-Mataguayan linguistic family (5). In the area in our study, aboriginal and foreign people are in the minority (14% in the sample). Indigenous people (22 in this sample) belong to different groups; 77% are Guaranies from Paraguay. Only one person from the Charagua community, which originated in Bolivia, participated in the study. This person had hantavirus antibodies. Among the 25 foreign participants, the only antibody-positive person belonged to the Bolivian majority (14). All nonnative inhabitants, including those with aboriginal ancestors and foreigners, are integrated members of the general population, sharing jobs and household conditions with local people, and therefore sharing similar risk factors.

More than two thirds of the studied group had traveled inside or outside the province, and/or to Bolivia. Such trips are frequent among migrant farm laborers, who follow harvest seasons. No differences were found in the antibody prevalence between persons who traveled and those who did not, probably because the reported trips were primarily within the same ecologic area.

The genetic diversity of sigmodontine rodents in South America is well known (15). Characterization of rodent species and their association with indigenous hantaviruses are currently under study. Putative rodent reservoirs of pathogenic hantaviruses identified in Argentina thus far belong to the *Oligoryzomys* genus (*O. longicaudatus* for Andes and Oran genotypes, *O. chacoensis* for Bermejo genotype, and *O. flavescens* for Lechiguanas genotype). Three previous rodent expeditions were performed in the northwestern Argentine hantavirus-endemic area in relation to HPS studies: two in Salta Province (July 1995 and October 1996) and one in Jujuy Province (May 1998), involving the villages of La Mendieta and Libertador General San Martin. Hantavirus antibody–positive species from Salta included *O. chacoensis* (1 [3.7%] of 27), *A. simulator* (1 [3.8%] of 26), and *O. longicaudatus* (2 [7.7%] of 26) and in Jujuy *O. chacoensis*, (1 [8.3%] of 12) (16).

In the present study, hantavirus antibody prevalence among rodents was similar to that previously reported in the country (2.7% to 12.5 %, varying by area and species) (16), but the species found with hantavirus antibodies were different. The genera of hantavirus antibody–positive rodents corresponded to those with higher relative abundance, *Akodon* and *Calomys*. *Akodon,* associated with the Pergamino virus in central Argentina, has thus far not been reported to be pathogenic for humans (17). Among the species of *Calomys*, *C. laucha* has been identified as the reservoir of Laguna Negra virus in Paraguay, but no previous evidence suggests it circulated in Argentina (14). Sigmodontine rodents were collected in every rural habitat in which we used traps, including inside dwellings, peridomestic sites, weeds close to grapefruit and banana plantations, vegetable fields, and mainly natural habitats such as woodbrush and the sides of rivers and streams. Most posi-

tive rodents were captured in weeds or roadsides inside or close to cultivated citrus or vegetables. Other rodents were captured in peridomestic sites associated with HPS cases and in woodbrush near one of the *fincas*. Focal concentration of positive rodents appeared to occur, with multiple positive rodents often trapped in the same trap line.

Characteristics of household or working habitats included a great deal of available potential food and cover for rodents attributable to substandard housing and sanitation. Sigmodontine rodents were also trapped in peridomestic sites of the urban area (8 Has and 17 Has quarters), where the features of the environment and buildings were similar to suburban or rural areas. *C. callosus* was the only wild rodent species captured inside houses. This observation is in accord with previous descriptions from Bolivia in relation to the Bolivian hemorrhagic fever outbreaks; *C. callosus* is the reservoir of Machupo virus, the arenavirus linked to Bolivian hemorrhagic fever, in the BHF-endemic area of El Beni (18). Control of the large Bolivian hemorrhagic fever outbreaks of the 1960s was achieved through measures directed to prevent infestation of *C. callosus* in towns and villages. These same measures should also be useful in this area to prevent hantavirus transmission, at least from rodents of the genus *Calomys* that are adapted to domestic and peridomestic settings.

Our results favor the hypothesis that less virulent hantaviruses are responsible for the mild and subclinical illnesses circulating in this region. Ongoing investigations that include the genetic characterization of the viruses associated with the different clinical forms will help to clarify this point.

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DNA Vaccine for West Nile Virus Infection in Fish Crows (*Corvus ossifragus*)

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A DNA vaccine for West Nile virus (WNV) was evaluated to determine whether its use could protect fish crows (*Corvus ossifragus*) from fatal WNV infection. Captured adult crows were given 0.5 mg of the DNA vaccine either orally or by intramuscular (IM) inoculation; control crows were inoculated or orally exposed to a placebo. After 6 weeks, crows were challenged subcutaneously with 105 plaque-forming units of WNV (New York 1999 strain). None of the placebo inoculated–placebo challenged birds died. While none of the 9 IM vaccine–inoculated birds died, 5 of 10 placebo-inoculated and 4 of 8 orally vaccinated birds died within 15 days after challenge. Peak viremia titers in birds with fatal WNV infection were substantially higher than those in birds that survived infection. Although oral administration of a single DNA vaccine dose failed to elicit an immune response or protect crows from WNV infection, IM administration of a single dose prevented death and was associated with reduced viremia.

West Nile virus (WNV), a mosquito-borne flavivirus, was recognized for the first time in the Western Hemisphere during summer 1999 in New York City and was associated with human, equine, and avian deaths (1–4). This virus is transmitted by a variety of mosquito species, mostly in the genus *Culex* (5–7). The New York 1999 strain of WNV differed genetically from other known strains of WNV except for an Israeli strain isolated from a dead goose in Israel in 1998 (1). With the exception of a laboratory study in Egypt involving hooded crows (*Corvus corone*) and house sparrows (*Passer domesticus*) (8), only these two nearly identical strains are known to kill birds (9,10). In 2000, WNV was detected in >4,000 bird carcasses in the United States (11), and the overall mortality rate was considered much greater. Several deaths attributed to WNV in the United States have occurred in valuable captive birds in zoologic collections (12). Currently, no treatment or vaccine is available for susceptible birds.

Vaccination may protect birds from lethal WNV infections. Accordingly, we examined a DNA vaccine developed for use in horses (13) for its ability to protect crows, a species known to be highly susceptible to lethal infection with this virus (8,10).

Materials and Methods

Vaccine

The plasmid DNA, pCBWN, codes for the prM and E glycoproteins of WNV. The plasmid was purified from *Escherichia coli* XL-1 blue cells with EndoFree Plasmid Giga Kits (QIAGEN, Inc., Santa Clarita, CA) and suspended in 10 mM Tris buffer, pH 8.5, at a concentration of 10.0 mg/mL. For IM vaccination, the DNA vaccine was formulated in phosphate-buffered saline (PBS), pH 7.5, at a concentration of 1.0 mg/mL. For oral exposure, the dry-microencapsulated DNA was suspended in PBS, pH 7.5, at a concentration of 2.0 mg/mL.

Microencapsulation

The method for microencapsulating DNA was adapted from procedures previously described for virus and subunit vaccines and isolated proteins (14–16). We performed all steps with sterile reagents and aseptic technique. Two 10-mg aliquots of WNV cDNA were transferred to sepa-

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rate test tubes with enough water to make 9-mL volumes. Resulting suspensions were mixed on a clinical rotator until solution was complete; 1 mL of 0.6% w/v aqueous sodium alginate (Fluka Chemical Co., Ronkonkoma, NY) solution was added to each tube, and the contents of each were gently inverted 20 times. Each DNA/alginate solution was pumped at 1.2 mL/min through a 76-µm orifice in a 1-mm internal diameter glass tube against the side of which a 20-KHz laboratory sonicator probe was firmly pressed. The emerging train of droplets was directed into a modified T-tube, through which a recirculated 40 mL of 0.25% w/v neutral aqueous spermine hydrochloride (Sigma-Aldrich Corp., St. Louis, MO) solution was pumped at 10 mL/min. A placebo microcapsule formulation was prepared by using alginate reagent without DNA. Resulting microcapsule suspensions were allowed to equilibrate for 30 min, pelleted at 500 x *g* for 20 min, and washed three times by decanting, suspending, and repelleting. Wash liquids were reserved for measuring the DNA that escaped encapsulation. Placebo and vaccine formulations and washes were frozen at –20°C and lyophilized overnight, then suspended in 5 mL of PBS to produce a final concentration of 2 mg/mL of the encapsulated DNA.

Crows

Fish crows (*C. ossifragus*) were captured with a rocket-propelled net at various locations in Maryland. Birds were transported to a biosafety level 3 laboratory at the U.S. Army Medical Research Institute of Infectious Diseases, allocated into four groups, and placed in stainless steel cages (3–4 birds/cage); blood was collected for evidence of antibodies against flaviviruses. Birds were provided a mixture of cat and dog food ad libitum and water. This diet was supplemented with hardboiled eggs as well as vitamin supplements.

Plaque Assay

Serial 10-fold dilutions of the blood samples from each crow were made in standard diluent (10% heat-inactivated fetal bovine serum in medium 199 with Earle's salts, $NaHCO_3$, and antibiotics). These samples were tested for infectious virus by plaque assay on Vero cells in 6-well plates (Costar, Inc., Cambridge, MA) as previously described (17), except that the second overlay, containing neutral red stain, was added 2 or 3 days after the first overlay.

Plaque-Reduction Neutralization Assay

Serum samples were assayed for WNV-specific antibodies by using the plaque-reduction neutralization test (PRNT), as previously described (18). Briefly, each serum sample was diluted 1:10 in standard diluent (as above) and mixed with an equal volume of BA1 (composed of Hanks' M-199 salts, 1% bovine serum albumin, 350 mg/L of sodium bicarbonate, 100 U/mL of penicillin, 100 mg/L of streptomycin, and 1 mg/L of fungizone in 0.05 M Tris, pH 7.6) containing a suspension of WNV (NY99-4132 strain) at a concentration of approximately 200 plaque-forming units (PFU)/0.1 mL, such that the final serum dilution was 1:20 and the final concentration of WNV (the challenge dose) was approximately 100 PFU/0.1 mL. After 1-h ncubation at 37°C, we added the serum/virus mixtures onto Vero monolayers in 6-well plates, 0.1 mL per well in duplicate. We determined the mean percentage of neutralization for each specimen by comparing the number of plaques that developed (see Plaque Assay section) relative to the number of plaques in the challenge dose, as determined by back titration. Preliminary samples were screened for antibodies to WNV in the same manner, as well as for neutralizing antibodies to St. Louis encephalitis virus, a closely related flavivirus that may cross-react serologically with WNV (19) and may partially protect against WNV infection (20).

Experimental Design

The crows were placed in four groups: 1) those inoculated IM with vaccine, 2) those that had oral vaccine, 3) positive controls (i.e., those that received placebo inoculation and viral challenge), and 4) room controls (i.e., those that received placebo inoculation and placebo challenge). After an acclimatization period of approximately 1 month, the 10 crows in group 1 (9 fish crows and 1 American crow [*C. brachyrhynchos*]) were inoculated IM with 0.5 mg of the DNA vaccine in a total volume of 0.5 mL (0.25 mL in each breast). The 9 crows in group 2 (8 fish crows and 1 American crow) were given 0.5 mg of the encapsulated DNA vaccine orally in 0.25 mL of PBS, and 20 fish crows (groups 3 and 4) were each inoculated and orally exposed as above except that a placebo was used in place of the vaccine. Blood was collected weekly from the jugular vein and the serum tested for neutralizing antibodies to WNV. Six weeks after vaccination, all birds in groups 1, 2, and 3 were inoculated subcutaneously with 0.1 mL of a suspension containing 10^5 PFU (10^6 PFU/mL) of the 397-99 strain of WNV, which had been isolated from the brain of an American crow that died in New York City during the fall of 1999 and passaged once in Vero cells before use in this study. The crows in group 4 were inoculated with 0.1 mL of diluent. Three or four crows in each group were bled (0.1 mL) from the jugular vein each day; each bird was bled every third day. Blood samples were added to 0.9 mL of diluent + 10 U of heparin/mL. Blood samples were frozen at –70°C until tested for infectious virus by plaque assay.

Results

Serologic Response

While neutralizing antibodies developed in 5 of the 9 fish crows that received the vaccine by the IM route at the 80% neutralization level for WNV by 14 days after vaccination, neutralizing antibodies to WNV did not develop in any of the remaining fish crows (8 orally exposed to vaccine and 20 placebo-exposed) in the same time period (Table 1). An antibody response at the 78% level developed in one of the remaining IM-vaccinated fish crows. Thus, a serologic response developed in six (67%) of the nine fish crows that received the vaccine by the IM route. However, by day 42 after vaccination, none of these crows retained a response at the 80% neutralization level.

Viremia Profiles and Survival

All of the mock-challenged crows survived. Similarly, all nine fish crows that received the IM vaccine survived (Table 1). However, 5 of 10 fish crows that received the placebo vaccine and 4 of 8 fish crows that received the oral vaccine died when challenged with virulent WNV. The difference in survival rates between the fish crows that received the IM vaccine and either of the other two groups was significant (Fisher exact test, $p \leq 0.03$). A veterinary pathologist examined all crows that died during these studies, and signs typical of WNV infection in avian hosts (i.e., heart necrosis) were observed in all of these birds. (These data will be described in a separate article on WNV viral pathogenesis in fish crows.) Viremias were detected in all 10 crows that received the placebo inoculation, 7 of 8 fish crows that received the oral vaccine, and 6 of 9 fish crows that received the vaccine by the IM route (Table 1). Virus was not detected in any of the crows that received the placebo challenge. Mean logarithm_{10} peak viremia titers were significantly lower ($T \geq 2.75$, $df \geq 15$, $p \leq 0.017$) in the fish crows that received the vaccine by the IM route (mean $\pm$ S.E. = 2.9 $\pm$ 0.4) than in fish crows that received the placebo vaccine (mean $\pm$ S.E. = 4.3 $\pm$ 0.3) or fish crows that received vaccine by the oral route (mean $\pm$ S.E. = 5.2 $\pm$ 0.8). The mean peak viremia titers for fish crows that received the placebo vaccine or the DNA vaccine by the oral route were not significantly different ($T=1.1$, $df=16$, $p=0.287$). In both the oral vaccine and placebo groups, fish crows that died had higher viremia than those that survived their infection with WNV (Table 2). Because birds were bled only every third day, accurately determining the duration of viremia in individual fish crows was not possible. Viremias were detected on days 1 to 6 after infection, and 9 of 10 birds that were viremic on day 1 were still viremic on day 4. However, only five of eight birds that were viremic on day 2 were still viremic on day 5, and only three of six birds that were viremic on day 3 were still viremic on day 6. No birds were viremic 7 days after infection. Thus, most viremias apparently lasted approximately 5 days during this study.

Discussion

Although the DNA vaccine failed to induce a long-lasting immune response, fish crows vaccinated with this vaccine by the IM route all survived challenge with virulent WNV. In contrast, oral administration of this vaccine failed to elicit an immune response, nor did it protect fish crows from a lethal challenge with WNV. The death rate in these crows (4 [50%] of 8), was identical to that observed in the placebo-vaccinated group (5 [50%] of 10) and in a second group of unvaccinated fish crows (4 [50%] of 8) tested later (M.J. Turell and M. Bunning, unpub. data). Although no deaths occurred in the IM-vaccinated fish crows, low-level viremia, consistent with that observed in the birds that survived their WNV infection in the other groups, did develop in six of the nine crows. Therefore, a single dose of the DNA vaccine did not elicit complete protection and sterile immunity to WNV infection. Additional studies need to be conducted with multiple doses of vaccination both by the IM as well as by the oral route to determine whether multiple doses might provide greater protection against WNV infection.

During the course of these studies, we determined that we had two American crows mixed in with the fish crows,

Table 1. Effect of route of administration of a DNA West Nile virus vaccine on the protection of fish crows from challenge with virulent West Nile virus

Treatment[a,b]	No. tested	% seropositive[c]	% viremic	Peak viremia[d]	% survival
Room control	10	0	0	<1.7 (0.0)	100
IM	9	56	67	2.9a (0.4)	100
Oral	8	0	88	5.2b (0.8)	50
Placebo	10	0	100	4.3b (0.3)	50

[a]IM, intramuscularly. Crows were inoculated IM with 0.5 mg of the DNA vaccine. Oral, crows were given 0.5 mg of the DNA vaccine orally. Placebo, crows were inoculated IM with 0.5 mg of nonspecific DNA and given 0.5 mg of nonspecific DNA orally.

[b]Room controls were placebo inoculated and then challenged with diluent.

[c]Percentage of crows whose serum produced ≥80% neutralization at 1:20 dilution.

[d]Logarithm_{10} mean peak viremia in crows bled every third day after challenge (S.E.). No virus was detected in any of the room control birds, and a value of 1.7 was assigned to birds from which no virus was detected for calculation of mean and S.E. Means followed by the same letter are not significantly different at $\alpha = 0.05$ by Student t test.

Table 2. Viremia levels in fish crows that survived or died after challenge with virulent West Nile virus

	Survived		Died	
Treatment[a]	No.	Peak viremia[b]	No.	Peak viremia[b]
IM	9	2.9 (0.4)	0	n/a
Oral	4	3.6 (0.7)	4	6.9 (1.0)
Placebo	5	3.8 (0.4)	5	4.8 (0.4)

[a]IM, intramuscularly. Crows were inoculated IM with 0.5 mg of the DNA vaccine. Oral, crows were given 0.5 mg of the DNA vaccine orally. Placebo, crows were inoculated IM with 0.5 mg of nonspecific DNA and given 0.5 mg of nonspecific DNA orally.

[b]Logarithm$_{10}$ mean peak viremia in crows bled every third day after challenge (S.E.). A value of 1.7 was assigned to birds from which no virus was detected for calculatio of mean and S.E.

one in the oral and one in the IM-vaccinated groups. High viremias (>10^6 PFU/mL of blood) developed in both of these crows, and they died after challenge with virulent WNV. These data, based on a single bird in each group, were not included in the data presented in this report. Both hooded crows (8) and American crows (10) are highly susceptible to infection with WNV with nearly 100% case-fatality rates. In contrast, fish crows appear to be less likely to succumb to the infection.

The continued spread of WNV infection across the United States and reported deaths in raptors and rare captive birds in zoologic parks indicate the need to develop an effective avian vaccine for WNV. To break the transmission cycle, the vaccine must be able to substantially reduce the level of viremia below the level needed to infect a feeding mosquito, which is about 10^5 PFU/mL of blood (21). By this standard, the vaccine performed reasonably well, with no vaccinated fish crow having a recorded viremia $\geq 10^{4.7}$. In contrast, 3 of 10 placebo-vaccinated fish crows had viremias $\geq 10^5$ PFU/mL of blood, and 5 of 10 had a peak viremia $\geq 10^{4.8}$ PFU/mL of blood. However, because the crows were bled only every third day, determining the actual peak viremias in these birds was not possible. If the goal of the vaccine is to protect rare and endangered avian species from death, rather than to prevent transmission, then the occurrence of a low-level viremia is not critical.

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Aseptic Meningitis Epidemic during a West Nile Virus Avian Epizootic

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While enteroviruses have been the most commonly identified cause of aseptic meningitis in the United States, the role of the emerging, neurotropic West Nile virus (WNV) is not clear. In summer 2001, an aseptic meningitis epidemic occurring in an area of a WNV epizootic in Baltimore, Maryland, was investigated to determine the relative contributions of WNV and enteroviruses. A total of 113 aseptic meningitis cases with onsets from June 1 to September 30, 2001, were identified at six hospitals. WNV immunoglobulin M tests were negative for 69 patients with available specimens; however, 43 (61%) of 70 patients tested enterovirus-positive by viral culture or polymerase chain reaction. Most (76%) of the serotyped enteroviruses were echoviruses 13 and 18. Enteroviruses, including previously rarely detected echoviruses, likely caused most aseptic meningitis cases in this epidemic. No WNV meningitis cases were identified. Even in areas of WNV epizootics, enteroviruses continue to be important causative agents of aseptic meningitis.

When national surveillance for aseptic meningitis was conducted in the United States, the Centers for Disease Control and Prevention (CDC) received reports of 7,000 to 14,000 cases of aseptic meningitis per year from 1984 to 1994 (1). Enteroviruses are the leading identifiable cause of aseptic meningitis in children and adults, particularly in summer and autumn (2). In smaller proportions, mumps virus (primarily in studies before 1980), herpesviruses, lymphocytic choriomeningitis virus, arboviruses, *Leptospira,* and many other viral and nonviral agents have been identified in etiologic studies of aseptic meningitis in the United States (3,4). However, the epidemiology of aseptic meningitis is not static and, with the appearance of emerging infectious agents such as West Nile virus (WNV), warrants periodic reevaluation.

WNV infection is usually asymptomatic but may cause a wide range of syndromes including nonspecific febrile illness, meningitis, and encephalitis. In recent WNV epidemics in which neurologic manifestations were prominent (Romania, 1996 [5]; United States, 1999–2000 [6,7]; and Israel, 2000 [8]), meningitis was the primary manifestation in 16% to 40% of hospitalized patients with WNV disease. However, because WNV meningitis has nonspecific clinical manifestations and requires laboratory testing for a definitive diagnosis, case ascertainment and testing practices can affect the number of cases diagnosed. Because WNV testing in U.S. surveillance programs has focused on patients with encephalitis of undetermined cause (9), the role of WNV as a cause of aseptic meningitis in the United States is not clear.

A 2001 investigation in Baltimore provided an opportunity to evaluate the role of WNV in the epidemiology of aseptic meningitis and to assess WNV surveillance. From Baltimore City and County, 118 cases of aseptic meningitis with onsets from June 1 to September 30, 2001, were reported to Maryland's Department of Health and Mental Hygiene (DHMH), compared to an average of 39 cases during the same summer season in 1997–2000. Approximately 95% of these 2001 cases were reported without known cause. Simultaneously, an intense WNV epizootic among birds was detected in the Baltimore area (288 WNV-infected dead birds and 14 WNV-infected mosquito pools were collected before September 30, 2001). Early in the summer, nearly 100% of dead crows collected from some sections of Baltimore City tested positive for WNV. When the investigation of aseptic meningitis was initiated in mid-September, one case of human WNV encephalitis had been reported from Baltimore. The investigation's objectives included 1) identification of the predominant cause(s) of the aseptic meningitis epidemic, emphasizing the relative contribu-

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tions of WNV and enteroviruses and 2) evaluation of hospital-based–WNV surveillance of patients with aseptic meningitis, including strategies used for diagnostic testing.

Methods

Discharge Diagnoses Code Review

To confirm an increase in aseptic meningitis cases by a method independent of case reporting to DHMH, a discharge diagnosis code review was conducted at the six investigation hospitals. Included were patients with aseptic meningitis (including International Classification of Diseases [ICD]-9-CM codes 047.0, 047.1, 047.8, 047.9, 049.0, 049.1, 053.0, 054.72, 072.1) who were evaluated from June 1 to September 30, 1998–2001.

Case Definitions

The investigation was conducted at six hospitals that collectively reported to DHMH 76% of the 118 aseptic meningitis cases from Baltimore City and County. A case of aseptic meningitis was defined as an illness with onset from June 1 to September 30, 2001; cerebrospinal fluid (CSF) cell count of >5 leukocytes per milliliter; negative CSF bacterial cultures; and no physician or laboratory documentation of bacterial, fungal, or parasitic central nervous system (CNS) disease, cerebral hemorrhage, carcinomatous meningitis, or cerebral vasculitis. Neonates who developed CSF abnormalities before first hospital discharge were excluded, as were persons with a physician-documented diagnosis of encephalitis, confusion, or obtundation. A case of enteroviral meningitis was defined as an illness meeting the criteria for aseptic meningitis and, in addition, a positive enterovirus culture of a CSF, rectal swab, or nasopharyngeal specimen, or a positive enterovirus polymerase chain reaction (PCR) test of a CSF specimen. A case of WNV meningitis was defined as an illness meeting the criteria for aseptic meningitis and, in addition, WNV immunoglobulin (Ig) M detected in a CSF specimen by enzyme-linked immunosorbent assay (ELISA), a greater than-four-fold rise of WNV-neutralizing antibodies in acute- and convalescent-phase serum specimens, or WNV nucleic acid detected in a CSF specimen by PCR. The investigation was limited to persons living in Baltimore City or County who were evaluated at one of the six investigation hospitals.

Case Ascertainment

Cases reported to DHMH as the code "viral meningitis" were reviewed. Depending on the resources of each hospital, additional cases were identified by computerized queries for test results of >5–10 leukocytes per milliliter in CSF, and by review of hospital discharge diagnoses codes. For each case, a standardized form was used to abstract clinical information from the medical record.

Acute-Phase and Convalescent-Phase Specimens and Interviews

Acute-phase (<8 days after illness onset) CSF, serum, rectal swab, and nasopharyngeal specimens were collected if ordered by the patients' physicians. Specimens were stored at hospital, DHMH, or private reference laboratories at temperatures ranging from 4°C to –70°C. During home visits to consenting patients >12 years of age who had had aseptic meningitis of unknown cause, convalescent-phase (>7 days after illness onset) blood specimens were collected and a standardized questionnaire was administered to characterize symptoms and duration of illness.

Diagnostic Testing

Hospitals performed routine cell counts, chemistries, and bacterial cultures of CSF for patients with sufficient specimen quantity. Some patients' physicians ordered additional tests. These tests commonly included, for CSF specimens, latex agglutination screening tests for bacterial antigens, culture or PCR tests for enteroviruses or herpesviruses, and culture for fungi; for CSF or serum specimens, *Borrelia burgdorferi* antibody, Venereal Disease Research Laboratory test, and cryptococcal antigen test; and for nasopharyngeal and rectal swab specimens, culture for enteroviruses.

For available specimens from patients with aseptic meningitis of unknown cause, arbovirus serologic testing was performed at DHMH laboratories, and WNV PCR tests were completed at DHMH laboratories or the Division of Vector-Borne Infectious Diseases, CDC. CSF specimens were tested by ELISA for IgM antibodies to WNV and by TaqMan reverse transcriptase (RT-) PCR tests for WNV (10,11). Serum specimens were tested at DHMH laboratories by ELISA for IgM antibodies to WNV, and by immunofluorescence assay (IFA) for IgM and IgG antibodies to La Crosse, St. Louis encephalitis, eastern equine encephalomyelitis, and western equine encephalomyelitis viruses.

Available CSF and rectal swab specimens from patients with aseptic meningitis of unknown cause were tested by enterovirus culture at the Respiratory and Enteric Viruses Branch, CDC. Three cell lines were used at CDC for enterovirus culture: RD (human rhabdomyosarcoma), HELF (human embryonic lung fibroblast), and LLC-MK2 (monkey kidney). Isolates were serotyped by RT-PCR amplification and subsequent sequencing of an approximately 320-nt segment of the VP1 enterovirus gene (12). When available, enteroviruses already isolated by hospital laboratories from CSF, nasopharyngeal, or rectal swab specimens were serotyped by CDC.

Evaluation of Aseptic Meningitis and WNV Surveillance

To evaluate strategies used to diagnose WNV meningitis in Maryland, reporting and testing policies were reviewed. Information was obtained from DHMH case-reports, surveillance plans and summaries, and laboratory tests of investigation patients.

Results

Confirmation of an Epidemic by Discharge Diagnosis Review

Each summer season (June 1–September 30) of 1998–2000, an average of 67 Baltimore residents were evaluated at the six investigation hospitals and assigned an aseptic meningitis ICD-9-CM code; in the 2001 season, 133 persons were evaluated, a 99% increase above the 1998–2000 seasonal average (Figure 1). At one of the investigation hospitals, the specificity of ICD-9-CM codes was assessed. A medical record review showed that 23 (96%) of 24 cases identified by ICD-9-CM codes met the investigation's case definition of aseptic meningitis.

Cases

At the six investigation hospitals, 113 aseptic meningitis patients were identified with illness onsets from June 1 to September 30, 2001. By the week of illness onset, the number of cases peaked in late August and early September (Figure 2). The median patient age was 18 years (range 1 week–74 years of age), and 56% of patients were male. Seventy-eight percent of patients were medically evaluated within the first 3 days after illness onset. Of the 110 patients with available information, the median duration of hospitalization was 2 days (range 0–11 days). No fatalities occurred during hospitalization nor were any subsequently reported to DHMH.

The median CSF leukocyte count was 135/mL (range 7–1,083/mL). Of the 110 patients with available data, 45 (41%) had >50% polymorphonuclear cells in CSF leukocyte count, including 9 (43%) of 21 patients who had CSF collected 4–10 days after illness onset. CSF glucose was normal (≥40 mg/dL) in 99% of patients (n=110), and CSF protein was elevated (>50 mg/dL) in 52% (n=111: median 53 mg/dL; range 10–215 mg/dL) (Table 1).

On the basis of standardized interviews of 33 patients (median age, 31 years; range 13–55 years of age) at the time convalescent-phase blood specimens were obtained, the most commonly reported acute-phase symptoms were headache (100%), fever (85%), and eye pain or sensitivity to light (85%). Illness was sometimes prolonged by persistent fatigue and headaches. The median duration of illness was 18 days (n=32; range 5–47 days). Twelve of the patients had not fully recovered by the time of interview; for these patients, the duration of illness was defined as

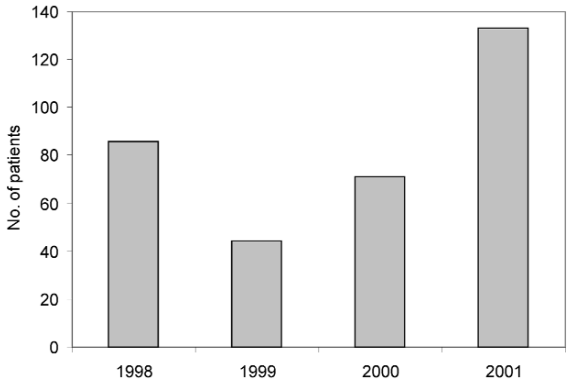

Figure 1. Residents of Baltimore City and County evaluated at six hospitals and assigned aseptic meningitis ICD-9-CM discharge diagnosis codes[a] during June 1–September 30, 1998–2001. [a]If during one season a patient had >1 discharge diagnosis codes for aseptic meningitis, the patient was only counted once.

(date of interview) – (date of illness onset). Patients ≥18 years of age reported longer duration of symptoms (n=24; median duration 22 days) than patients 13-17 years of age (n=8; median duration 7 days) (p=0.001, Kruskal-Wallis test of significance in Epi-Info 6 software).

WNV Test Results

Of the 69 patients for whom at least one ELISA WNV IgM test was performed on CSF or serum, none tested positive. Of these 69, ELISA WNV IgM testing was performed on both acute- and convalescent-phase specimens for 23 patients, on only acute-phase specimens for 36 patients, and on only convalescent-phase specimens for 10 patients. Arboviral IgM IFAs completed on acute- or convalescent-phase serum specimens from 39 patients were all negative. WNV PCR tests completed on acute-phase CSF specimens

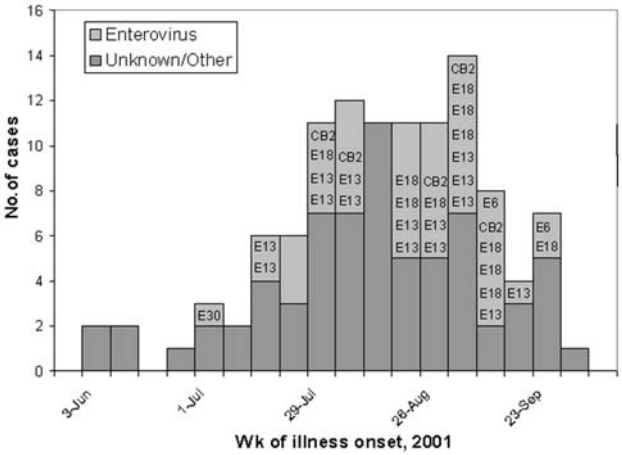

Figure 2. Aseptic meningitis cases* by week of illness onset, June 1–September 30, 2001, identified at six hospitals, Baltimore, Maryland. *N=112 (illness onset date missing for one patient); Coxsackievirus B2 = "CB2"; Echovirus 6 = "E6", Echovirus 13 = "E13"; Echovirus 18 = "E18"; Echovirus 30 = "E30".

Table 1. Descriptive summary of aseptic meningitis cases with onsets from June 1 to September 30, 2001, identified at six hospitals, Baltimore, Maryland[a]

Cause	Cases	Median age (range)	% <18 y of age	CSF		
				Median leukocyte count/mL (range)	% PMN predominant	Median protein mg/dL (range)
All enterovirus meningitis cases	43	9 y (1 wk–49 y)	70	178 (10–850)	49	48 (10–215)
Echovirus 13	15	7 y (1 mo–49 y)	87	132 (11–650)	80	37 (18–97)
Echovirus 18	11	17 y (2 mo–35 y)	55	173 (12–409)	27	44 (16–215)
Coxsackievirus B2	5	19 y (1 wk–31 y)	40	250 (45–850)	0	87 (57–120)
Echovirus 6	2	33 y (31–34 y)	0	330 (130–530)	50	56 (52–59)
Echovirus 30	1	10 y	1	10	0	18
Untyped enterovirus	9	9 y (1 mo–20 y)	89	178 (43–850)	56	48 (10–153)
Herpes simplex virus meningitis cases	2	39 y (29–49 y)	0	246 (136–355)	0	128 (77–179)
Lyme meningitis case	1	74 y	0	227	0	143
Cases of undetermined cause	67	25 y (2 wk–67 y)	39	100 (7–1083)	38[b]	53[c] (19–209)
Total cases	113	18 y (1 wk–74 y)	50	135 (7–1083)	41[d]	53[e](10-215)

[a]CSF, cerebrospinal fluid; PMN, polymorphonuclear cells.
[b]N=64.
[c]N=65.
[d]N=110.
[e]N=111.

from 27 patients were also negative. Acute-phase specimens were collected <8 days after illness onset, and convalescent-phase specimens were collected a median of 40 days after illness onset (range 12–111 days); exact dates were not available for two patients.

Enterovirus Test Results

Of 70 patients who had at least one test (viral culture or PCR test) performed for enteroviruses, 43 (61%) patients were confirmed to have enteroviral meningitis. Among patients who had at least one enterovirus test performed, the percentage enterovirus-positive was highest in infants and children; however, of 30 tested patients ≥18 years of age, 13 (43%) were enterovirus-positive (Table 2). Of the 34 cases in which enterovirus serotyping was completed, five serotypes were identified. Echovirus 13 (15 cases) and echovirus 18 (11 cases) together accounted for 76% of the serotyped isolates (Table 1).

Other Diagnoses

Two patients were diagnosed with herpes simplex virus meningitis by culture-positive CSF specimens, and one patient was diagnosed with Lyme meningitis on the basis of clinical signs and symptoms of acute facial palsy and meningitis, and positive serum *B. burgdorferi* antibody by ELISA and Western blot tests.

Sixty-seven (59%) of the 113 patients in the investigation remained undiagnosed; for many, sufficient specimens did not exist for further testing. The median age of these undiagnosed patients was 25 years, and 61% were male. Duration of hospitalization was similar to the cases with known cause. Five of the undiagnosed patients had HIV infection, and another four had a history of prior meningitis. Twenty-seven (40%) of the undiagnosed patients had at least one enterovirus test (culture or PCR) performed. Forty-six (68%) of the undiagnosed patients, including 24 with convalescent-phase specimens collected a median of 44 days after illness onset (exact dates not available for two patients) (range 12–111 days), had at least one WNV IgM ELISA performed. To estimate the number of WNV meningitis cases that could have been missed, we assumed that these 24 patients represented a random sample of the 67 undiagnosed patients, and that WNV infection was ruled out in patients with a negative result from a WNV IgM ELISA performed on a convalescent-phase serum. On the basis of these assumptions, 0% (95% confidence interval 0% to 10%) WNV IgM positivity among the sample suggests that fewer than seven cases of WNV meningitis were missed among the investigation patients.

Evaluation of Aseptic Meningitis and WNV Surveillance

Human WNV surveillance in Maryland focused on patients with two reportable CNS infections, encephalitis or aseptic meningitis, of unknown cause. Of the 113 aseptic meningitis cases identified at the six investigation hospitals, 76 (67%) had been reported to DHMH. Of these 76 aseptic meningitis cases, 71 (93%) were reported without a cause. Because of the urgency to detect WNV epidemics, WNV serologic testing was the first-line test conducted by DHMH laboratories for patients with aseptic CNS infections of unknown cause. WNV testing was first prioritized for patients with encephalitis, and secondarily for hospitalized patients with aseptic meningitis who were ≥17 years of age (late in the season, this last criteria was expanded to ≥5 years of age). During 2001, DHMH laboratories conducted WNV testing for 440 patients statewide (including approximately 230 patients reported with aseptic meningitis); 6 patients were diagnosed with WNV disease.

Table 2. Enterovirus meningitis cases by age group, onsets June 1 to September 30, 2001 identified at six hospitals, Baltimore, Maryland

Age group (y)	Aseptic meningitis cases	% test-positive[a] for enterovirus (no. test-positive/no. tested for enterovirus)
<1	12	80 (8/10)
1–10	24	94 (15/16)
11–20	29	50 (11/22)
21–30	11	75 (3/4)
31–40	26	38 (5/13)
41–50	5	33 (1/3)
>50	6	0 (0/2)
All	113	61 (43/70)

[a]Thirty-four (79%) of the 43 enterovirus meningitis cases had a positive viral culture or polymerase chain reaction test result of cerebrospinal fluid (CSF) specimens. Seven cases had negative CSF tests and were only diagnosed by positive viral culture of nasopharyngeal or rectal swab specimens. Two additional cases did not have sufficient CSF available for testing and were diagnosed by positive culture of nasopharyngeal or rectal swab specimens.

DHMH requested CSF, acute-, and convalescent-phase serum specimens for the diagnosis of WNV infection. However, before the investigation, essentially only acute-phase specimens (CSF more frequently than serum) from Baltimore patients were tested by WNV serologic tests; routine collection of convalescent-phase serum specimens was not feasible.

Enterovirus testing was not a component of DHMH aseptic meningitis surveillance. If enterovirus testing was initiated by the hospital, positive results were often not communicated to DHMH laboratories. Of 69 patients for whom at least one WNV IgM test was performed by DHMH, enteroviral meningitis was subsequently diagnosed in 23 (50% of 46 for whom at least one enterovirus culture or PCR test was performed).

Discussion

Although enhanced WNV surveillance among persons with aseptic meningitis could have been partially responsible for the tripling of Baltimore case-reports of aseptic meningitis during the summer of 2001, trends in discharge diagnosis codes suggest that a true increase in aseptic meningitis cases occurred. Despite a concurrent, intense WNV epizootic among birds, no evidence existed that WNV substantially contributed to the aseptic meningitis epidemic. By routine surveillance, five WNV encephalitis cases but no WNV meningitis cases were ultimately detected in Baltimore in 2001. However, in this setting, the five recognized WNV encephalitis cases did not appear to represent large numbers of undiagnosed WNV meningitis cases. Surveillance conducted in other states has also suggested that intense WNV epizootic activity does not necessarily correlate with large numbers of human WNV CNS infections (13).

Instead, multiple enterovirus serotypes likely caused most of the Baltimore aseptic meningitis cases. This finding is consistent with population-based studies (14–16) and large outbreak investigations (17,18) of aseptic meningitis occurring during the summer and fall in the United States. However, unlike outbreak investigations of the past few decades, echovirus 13 and echovirus 18 were the most commonly isolated agents in this investigation and might have accounted for the increased number of aseptic meningitis cases in Baltimore. Echovirus 13 was previously rarely detected in the United States. From 1970 to 2000, only 65 of 45,000 enterovirus isolates reported to CDC were echovirus 13 (19). Echovirus 18 had been relatively quiescent for over a decade; from 1988 to 2000, only 200 isolates were reported to CDC (20). In a study conducted from 1986 to 1990 in Baltimore hospitals among infants <2 years of age who were hospitalized with aseptic meningitis, only one case of echovirus 13 and two cases of echovirus 18 (among 167 serotyped enterovirus isolates) meningitis were identified (14).

In 2001, the previously rarely detected echoviruses 13 and 18 were the two enteroviruses most commonly reported to CDC (20). Multiple states reported echovirus 13 in association with aseptic meningitis outbreaks, and Tennessee reported an aseptic meningitis outbreak attributed to both echovirus 13 and 18 (19). Where previously rarely detected, echovirus 13 was isolated in association with aseptic meningitis outbreaks in Australia during 2001 (21) and in the United Kingdom (22) and Germany (23) during 2000. Surveillance data have previously demonstrated that in one or two seasons a usually quiescent serotype may cause an outburst of clinical disease superimposed on background, area-endemic enteroviruses (24). The worldwide spread of epidemics of clinical enteroviral disease has been documented with other serotypes, including echovirus 9 and enterovirus 70 (25).

Limitations of this study should be acknowledged. Physicians may have diagnosed more aseptic meningitis in response to WNV publicity; however, physicians more likely recognized that the risk and discomfort of a lumbar puncture required to diagnose meningitis outweighed the public health interest in identifying an untreatable condition, WNV meningitis. Regarding the investigation, only aseptic meningitis cases that could be rapidly identified at the six Baltimore hospitals were included. Not all patients underwent the same tests in the same laboratories, and the quality of enterovirus testing differed because of variable conditions of specimens. Additional results of other tests

performed at reference laboratories might have become available only after the investigation ended. As a result, although any positive WNV test result would likely have been reported, the percentage of enterovirus test-positive cases could be inaccurate. Finally, similar to previous studies of the epidemiology of aseptic meningitis (2), 59% of cases remain undiagnosed; another, untested agent may have caused the increased number of aseptic meningitis cases in Baltimore.

The consistent predominance of enteroviruses among the known causes of aseptic meningitis in children and adults versus the relative infrequency of WNV meningitis (even during an intense WNV epizootic) warrants reconsideration of WNV surveillance testing strategies. Most cases of aseptic meningitis were reported to DHMH without a known cause. For these patients, WNV testing was the first priority to enable early detection of WNV epidemics that would warrant additional vector control interventions. By contrast, enterovirus testing was not a component of surveillance among patients with aseptic meningitis. Although no WNV meningitis was identified, >30% of the patients who underwent WNV testing were later determined to have had enteroviral meningitis. Many patients with aseptic meningitis were tested for an apparently rare virus, WNV, before being tested for the common agents, enteroviruses. During nonepidemic years, WNV IgM ELISA may be low yield when performed as a first-line test for aseptic meningitis. By contrast, enterovirus testing likely can identify the cause of a large fraction of aseptic meningitis cases.

The complexities and resource requirements of WNV serologic testing suggest that other testing strategies need to be developed. During 2001, DHMH laboratories conducted WNV testing for 440 patients statewide, often performing multiple tests for each patient; 6 patients were diagnosed with WNV disease. WNV ELISAs require at least 2–3 days to complete. PCR tests for WNV in CSF specimens are more rapid but have poor sensitivity (26). Because patients often seek medical care early after illness onset when WNV antibodies might not be detectable, serologic tests of specimens collected at the time of first symptoms may also have poor sensitivity. Serologic testing of convalescent-phase serum specimens may be the most sensitive method to detect WNV infection. Yet, collecting convalescent-phase specimens can be logistically difficult and, as in Baltimore's WNV surveillance program, may not be routinely feasible. When collected through primary care physicians, billing issues can be problematic, and each home visit for collection of blood specimens may require several hours of staff time.

An improved laboratory-based surveillance strategy might include a two-stage testing algorithm at hospital or public health laboratories to evaluate patients with aseptic meningitis. The goals would be 1) to judiciously use specimens of limited quantity (especially CSF) to rapidly identify common or treatable causes of aseptic meningitis and 2) to improve the yield of the more complex testing required to diagnose arboviral disease.

As a first stage of testing, multiplex PCR tests have been used to detect enteroviruses, herpes simplex 1 and 2, and varicella zoster (27,28). Several studies indicate that PCR tests for enteroviruses (29) and herpes simplex virus (30) are highly specific, can be completed more rapidly (<6 hours required), require less quantity of CSF, and potentially are more sensitive than traditional cell culture. Using PCR tests to identify enterovirus infections in patients with aseptic meningitis can reduce unnecessary ancillary tests and antibiotic or antiviral therapy and allows shortened hospitalizations (31). In addition, treatment for enteroviral infection may become available in the near future (32).

If no diagnosis is made after completion of screening tests for common or treatable agents and evidence of regional WNV or other arbovirus activity exists, a second stage of testing might include arbovirus IgM ELISA of acute- and convalescent-phase specimens. To rule out WNV disease, WNV IgM and WNV IgG ELISAs may need to be conducted approximately 8–45 days after illness onset (WNV IgG ELISAs may be complicated by cross-reactions that can only be differentiated by logistically difficult plaque reduction neutralization tests) (10). The importance of the timing of specimen collection should be clearly communicated to healthcare providers.

The epidemiology of aseptic meningitis and other CNS infections is not fixed and may vary by location; in the same location, the epidemiology may vary by different seasons. For example, while relatively quiescent in most years, WNV has the potential to cause large epidemics in humans. Because WNV disease does not have unique clinical manifestations, adequate laboratory testing is the only way to identify human WNV epidemics. Refinement of laboratory testing strategies for WNV surveillance may help build broader public health laboratory capacity for the diagnosis of arboviral and other CNS infections.

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Aggregated Antibiograms and Monitoring of Drug-Resistant *Streptococcus pneumoniae*

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Community-specific antimicrobial susceptibility data may help monitor trends among drug-resistant *Streptococcus pneumoniae* and guide empiric therapy. Because active, population-based surveillance for invasive pneumococcal disease is accurate but resource intensive, we compared the proportion of penicillin-nonsusceptible isolates obtained from existing antibiograms, a less expensive system, to that obtained from 1 year of active surveillance for Georgia, Tennessee, California, Minnesota, Oregon, Maryland, Connecticut, and New York. For all sites, proportions of penicillin-nonsusceptible isolates from antibiograms were within 10 percentage points (median 3.65) of those from invasive-only isolates obtained through active surveillance. Only 23% of antibiograms distinguished between isolates intermediate and resistant to penicillin; 63% and 57% included susceptibility results for erythromycin and extended-spectrum cephalosporins, respectively. Aggregating existing hospital antibiograms is a simple and relatively accurate way to estimate local prevalence of penicillin-nonsusceptible pneumococcus; however, antibiograms offer limited data on isolates with intermediate and high-level penicillin resistance and isolates resistant to other agents.

Infections from *Streptococcus pneumoniae* tax the healthcare system in the United States and other countries. Scientific advances have been made in the treatment and prevention of pneumococcal infections through antibiotics and licensure of vaccines for both adults and children; however, the last few decades have witnessed the emergence of *S. pneumoniae* resistance to antibiotics (1). In a multistate, population-based surveillance system that follows invasive disease from pneumococcus and other bacterial pathogens, the percent of isolates resistant to penicillin reached 24% in 1998; concurrent increases in resistance to other antimicrobial drugs have also been noted among penicillin-resistant pneumococci (1,2). Implications of drug-resistance extend beyond the laboratory and into clinical practice as treatment failures from drug resistance have been reported with meningitis (3–5) and otitis media (6,7). In some studies, increased death and disease in patients hospitalized with pneumonia caused by high-level β-lactam-resistant pneumococci have been reported (8,9).

Measuring pneumococcal resistance to penicillin and other antibiotics enables epidemiologists and healthcare providers to monitor trends, develop guidelines for optimal empiric therapy, and provide impetus for and ascertain the success of educational efforts promoting the judicious use of antibiotics. Antimicrobial resistance is not uniform across the United States (10). Nonsusceptibility to penicillin among invasive pneumococcal isolates has been shown to range from 15% to 35% among populations in the Centers for Disease Control and Prevention's (CDC) national surveillance system (1).

The ideal method for accurate tracking of antimicrobial-resistance patterns in a community may be active, laboratory-based surveillance systems that collect strains for susceptibility testing in a reference laboratory. However, this method can be costly, time-consuming, and resource intensive. Alternative methods of measuring local drug-resistant pneumococci that are less expensive and more timely are needed. One alternative is to use aggregated antibiograms. A study conducted by epidemiologists at the Oregon Health Division found that aggregating existing hospital antibiograms, also known as cumulative suscepti-

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bility data, provided relatively accurate, community-specific, drug-resistant *S. pneumoniae* data when compared with active-, laboratory-based surveillance, the standard criterion for invasive disease. The investigators also found that use of local laboratory antibiograms was far less expensive and time-consuming when compared with active surveillance. Whether Oregon's results can be generalized is unknown; however, only 12 hospitals in one city (Portland) were surveyed, and the percent of *S. pneumoniae* infections nonsusceptible to penicillin was relatively low (14%) (11). We compared the two methods of surveillance in a larger study that involved sites in geographically disparate areas and represented a larger fraction of the national population and varying degrees of drug resistance across the United States. Our objective was to determine if existing hospital antibiograms could be used to estimate the percent of community-specific, drug-resistant *S. pneumoniae* in multiple sites.

Methods

We compared the proportions of drug-resistant *S. pneumoniae* isolates reported by participating clinical laboratories from the Active Bacterial Core Surveillance (ABCs) sites to proportions obtained by aggregation of existing antibiograms produced by the same ABCs laboratories.

Active Laboratory-Based Surveillance

ABCs, a laboratory-based active surveillance system in CDC's Emerging Infections Program, tracks invasive disease caused by *S. pneumoniae* and other bacterial pathogens of public health importance (12). Surveillance areas included in this analysis were: California (CA) (San Francisco County), Connecticut (CT) (entire state), Georgia (GA) (20-county area, including Atlanta), Maryland (MD) (6-county area including Baltimore), Minnesota (MN) (7 counties), New York (NY) (7 counties), Oregon (OR) (3-county area including Portland), and Tennessee (TN) (5 counties). The total population under surveillance was 17 million. A case of invasive pneumococcal disease was defined as the isolation of *S. pneumoniae* from a normally sterile site (e.g., blood, cerebrospinal fluid) from a surveillance area resident. Surveillance personnel routinely contacted all clinical microbiology laboratories in their site to identify cases and conducted audits every 6 months to ensure complete reporting.

Pneumococcal isolates collected through ABCs were sent to reference laboratories for susceptibility testing by broth microdilution according to the methods of the National Committee for Clinical Laboratory Standards (NCCLS) (13). Isolates were defined as susceptible, having intermediate resistance, or resistant to agents tested according to NCCLS definitions (14).

Antibiograms

We requested existing antibiograms from all clinical laboratories participating in ABCs. The antibiograms were to cover the most recent 12-month period for which completed data were available at the time of the inquiry (1997 for GA, TN, CA, MN, OR, MD, and CT; 1998 for NY). Any identifying information (e.g., hospital name) obtained during collection of antibiogram data was removed before the data were forwarded to study investigators at CDC. Surveillance personnel also used a standardized questionnaire to query each hospital's infection control practitioner or microbiology supervisor regarding the production and distribution of local antibiograms and whether antibiogram data included sterile isolates, nonsterile site isolates, or duplicates isolates from a single patient.

We compiled total numbers of *S. pneumoniae* isolates identified from the ABCs sites along with the percent of intermediate and resistant isolates, focusing on nonsusceptibility to penicillin, macrolides, and extended-spectrum cephalosporins (e.g., cefotaxime, ceftriaxone). We defined nonsusceptible isolates as those that were of intermediate and high-level resistance or that were simply described as not susceptible to the antibiotic tested. We aggregated data obtained from the participating hospitals within each ABCs site to produce summary antimicrobial susceptibility percentages. When generating tables comparing percent of nonsusceptible pneumococcal isolates estimated by antibiograms and by ABCs, we used only antibiogram data for the year in question (1997 for all sites excluding New York [1998]); antibiograms covering other periods were excluded from this portion of the analysis. Also, we used only antibiograms that included both the total number of isolates tested and the percent nonsusceptible for each of the antibiotics evaluated; this system allowed for aggregation of the laboratory's data with those from other laboratories. If only a subset of isolates were tested against erythromycin and extended-spectrum cephalosporins, we excluded these results from the aggregated total for erythromycin, cephalosporins, or both. We also calculated the percent of laboratories that included *S. pneumoniae* susceptibility testing to a variety of other antimicrobial agents and the percent of laboratories generating antibiograms that included susceptibility testing of gram-negative bacteria.

To compare the proportions of resistant and susceptible *S. pneumoniae* isolates detected by the two surveillance methods, we examined the proportion of hospitals whose aggregated antibiogram data fell within a range of ± 5% and ± 10% compared with that detected through active surveillance.

Results

Generation of Antibiograms

One hundred and forty-five ABCs laboratories completed the surveys; these laboratories conducted antibiotic susceptibility and other testing for a total of 170 (85%) of the 199 hospital laboratories participating in ABCs at the time the study was undertaken. Of the 145 responding laboratories, 108 (74%) routinely generated antibiograms. The 108 antibiograms created include pneumococcal susceptibility testing results for 140 (70%) of the 199 ABCs hospital laboratories. In-house microbiologists typically generated the antibiograms (83%), while infection control practitioners (7%) and pharmacists (10%) created the remaining. Nearly all laboratories included both sterile site (98%) and nonsterile site (92%) isolates in the antibiograms. Ninety-five percent included inpatient, and 79% included outpatient isolates. Forty-six laboratories (43%) included duplicate isolates from individual patients in their antibiograms.

When asked how pneumococcal isolates with intermediate susceptibility were categorized, survey responders stated that their laboratory characterized these isolates as intermediate (37%), resistant (32%), susceptible (5%), and nonsusceptible (22%). This question did not specify the antibiotic tested. Only 25 (23%) laboratories generated antibiograms that included data distinguishing isolates intermediate and resistant to penicillin; 77% only indicated whether the isolates were susceptible or nonsusceptible.

The average number of isolates included in the summary antibiograms was nearly double the number collected through active surveillance; the mean number of pneumococcal isolates (per site) tested for penicillin susceptibility was 417 (range 69–850) for ABCs and 826 (range 383–1,291) for summary antibiograms. Hospitals (n=40) that excluded duplicate isolates from antibiograms averaged similar numbers of isolates (mean 89 isolates) tested for penicillin susceptibility as did hospitals (n=34) whose antibiograms included multiple isolates from a single patient (mean number of isolates tested 88).

Of the 140 hospital laboratories whose pneumococcal antibiotic susceptibility testing results were summarized in antibiograms, 96 (70%) created antibiograms with penicillin-susceptibility results in a format that could be aggregated for the year in question. The proportion of laboratories in each site that generated usable penicillin susceptibility data ranged from 70% (MD) to 100% (NY and MN). Antibiograms included susceptibility-testing results for macrolides (63%) and third-generation cephalosporins (57%). The proportion of laboratories for which this susceptibility information was in a format that could be aggregated, however, was smaller for macrolides (44%) and third-generation cephalosporins (39%). For the eight sites, the proportion of penicillin-nonsusceptible isolates from ABCs ranged from 14.5% (NY) to 38.4% (TN), whereas antibiograms yielded a range of 18.5% (CA) to 41.7% (TN) (Table 1). For all sites the overall proportion of isolates nonsusceptible to penicillin according to antibiograms was within 10 percentage points of the population- and laboratory-based surveillance (ABCs); for six sites it was within 5 percentage points. The proportion of penicillin-nonsusceptible isolates for each site identified by antibiograms was higher than that generated by ABCs (median difference: 3.65%; range 8.6% to 1.8%). No correlation existed between site-specific levels of penicillin resistance and the magnitude of difference between site-specific penicillin resistance identified by the two methods.

The proportions of pneumococcal isolates nonsusceptible to a third-generation cephalosporin and to erythromycin were lower than the proportion of penicillin-nonsusceptible isolates, regardless of the method used (Tables 2 and 3). Similar to the results for penicillin, the percentage of strains nonsusceptible to third-generation cephalosporins or erythromycin as detected by antibiograms tended to be

Table 1. Comparison of percent of *Streptococcus pneumoniae* isolates nonsusceptible to penicillin by site: active surveillance (ABCs) versus antibiogram

	Antibiogram				ABCs				Difference in % nonsusceptible (antibiograms vs. ABCs)
Site	No. laboratories[a]	Non-susceptible isolates	Total no. isolates tested	% non-susceptible	No. laboratories	Non-susceptible isolates	Total no. isolates tested	% non-susceptible	
Connecticut	16	168	845	19.9	32	113	624	18.1	1.8
California	9	107	577	18.5	10	30	184	16.3	2.2
Oregon	9	115	550	20.9	15	32	178	18.0	2.9
Tennessee	10	432	1,037	41.7	31	169	440	38.4	3.3
Maryland	10	171	886	19.3	27	85	557	15.3	4.0
Georgia	14	505	1,291	39.1	39	292	850	34.4	4.7
New York	9	85	383	22.2	20	10	69	14.5	7.7
Minnesota	19	315	1,037	30.4	25	95	435	21.8	8.6
Total	96	1,898	6,606	28.7	199	826	3,337	24.8	Median: 3.65

[a]Only laboratories whose antibiograms covered the calendar year in question (1997 for all sites except New York [1998]) were compared to ABCs.

Table 2. Comparison of percent of *Streptococcus pneumoniae* isolates nonsusceptible to third-generation cephalosporins by site: active surveillance (ABCs) versus antibiograms

Site	Antibiogram				ABCs				Difference in % nonsusceptible (antibiograms vs. ABCs)
	No. laboratories[a]	Non-susceptible isolates	Total no. isolates tested	% non-susceptible	No. laboratories	Non-susceptible isolates	Total no. isolates tested	% non-susceptible	
Tennessee	10	54	357	15.1	31	114	440	25.9	–10.8
New York	3	2	84	2.4	20	5	69	7.2	–4.8
California	4	14	412	3.4	10	15	184	8.1	–4.7
Connecticut	5	19	267	7.1	32	73	624	11.7	–4.6
Oregon	6	34	419	8.1	15	14	178	7.9	0.2
Maryland	5	53	476	11.1	27	48	557	8.6	2.5
Minnesota	7	104	543	19.2	25	60	435	13.8	5.4
Georgia	14	222	1,272	17.5	39	102	850	12.0	5.5
Total	54	502	3,830	13.1	199	431	3,337	12.9	Median: –2.25

[a]Only laboratories whose antibiograms covered the calendar year in question (1997 for all sites except New York [1998]) were compared to ABCs.

greater than the percent nonsusceptible detected by ABCs. In contrast, the range of the differences for third-generation cephalosporins and erythromycin detected by the two surveillance methods was larger than the range of differences for penicillin as measured for each ABCs site. The magnitude of the difference in overall susceptibility to third-generation cephalosporins determined by the two surveillance methods was <10% for seven of eight sites and <5% for five sites. The magnitude of the difference in susceptibility to erythromycin as determined by the two surveillance methods was <10% for all sites and <5% for only four sites.

In addition to penicillin, cephalosporins, and macrolides, submitted antibiograms included susceptibility testing results for a variety of other antibiotics that included the following: trimethoprim/sulfamethoxazole (35%), vancomycin (59%), clindamycin (47%), gentamycin (3.9%), and one or more fluoroquinolones (14%). Thirty-eight percent of antibiograms returned for analysis also included antimicrobial susceptibility testing results for various gram-negative bacteria.

Discussion

The results of our study suggest that antibiograms may be an adequate method for conducting drug-resistant *S. pneumoniae* surveillance for many health departments, illustrating the comparability of aggregated antibiograms that include both sterile and nonsterile site isolates to active, laboratory- and population-based surveillance for invasive isolates. For more than half the comparisons between the two methods, the difference in antibiotic resistance detected was <5 percentage points, and for 23 (96%) of the 24 comparisons the difference was <10 percentage points. No significant differences in comparability of the two methods were noted between high- and low-resistance areas. This study indicates that antibiograms may be an alternative tool for evaluating penicillin nonsusceptibility in a region and validates the earlier findings of the Oregon study, conducted in an area of relatively low antibiotic resistance (11).

Although the estimates of level of resistance obtained from antibiograms approximated that from ABCs, aggregated antibiogram data tended to show a higher proportion of nonsusceptible isolates within each site and for each

Table 3. Comparison of percent of *Streptococcus pneumoniae* isolates nonsusceptible to erythromycin by site: active surveillance (ABCs) versus antibiogram

Site	Antibiogram				ABCs				Difference in % nonsusceptible (antibiogram vs. ABCs)
	No. laboratories[a]	Non-susceptible isolates	Total. isolates tested	% non-susceptible	No. laboratories	Non-susceptible isolates	Total isolates tested	% non-susceptible	
Georgia	10	178	805	22.1	39	207	850	24.4	-2.3
Tennessee	8	133	460	28.9	31	113	440	25.7	3.2
Oregon	6	57	405	14.1	15	18	178	10.1	4.0
Maryland	7	64	596	10.7	27	35	557	6.3	4.4
New York	4	23	128	11.7	20	4	69	5.8	5.9
California	9	92	577	15.9	10	15	184	8.2	7.7
Minnesota	10	140	684	20.5	25	55	435	12.6	7.9
Connecticut	7	58	287	20.2	32	65	624	10.4	9.8
Total	61	737	3,942	18.7	199	512	3,337	15.3	Median: 5.15

[a]Only laboratories whose antibiograms covered the calendar year in question (1997 for all sites except New York [1998]) were compared to ABCs.

antibiotic evaluated. This trend is likely due to the inclusion of nonsterile (noninvasive) site isolates. In studies from centers that include both sterile and nonsterile isolates, nonsterile site isolates have been found to be equally or more resistant (15–17). The reason for this difference is unclear but may reflect differences in serotype distribution between strains causing invasive and noninvasive disease. Disparity in results between clinical and reference laboratories could also contribute to this trend; use of the E test (AB Diodisk, Solna, Sweden) by clinical laboratories might vary from the referent method (broth microdilution) by one half or one dilution (18). In this study, we were unable to examine the role of laboratory error or differences in susceptibility-testing methods as a reason for differences in results from antibiograms compared with those from active surveillance.

Compared to penicillin, differences between the two surveillance methods were greater for extended-spectrum cephalosporins and erythromycin. This finding may be because of smaller numbers of isolates included in the antibiograms, fewer laboratories that included susceptibility testing of *S. pneumoniae* to these antibiotics, or greater disagreement between clinical and reference laboratory results. We could not include antibiogram-susceptibility testing results for some hospital laboratories because only a subset of the pneumococcal isolates that were tested for penicillin nonsusceptibility were also tested for susceptibility against third-generation cephalosporins (20 laboratories) and erythromycin (13 laboratories). The potential explanations for why these laboratories tested only a subset of pneumococcal isolates (i.e., only penicillin-nonsusceptible isolates were tested) against the same antibiotics were not indicated on the antibiograms.

We chose to evaluate the comparability of the two surveillance methods by observing how often the percent nonsusceptible isolates estimated by aggregated antibiograms differed by <5 and 10 percentage points from that estimated by ABCs active surveillance. As there exists no standardized or absolute level of antimicrobial drug resistance that would dictate a change in empiric treatment of pneumococcal infections, we chose a priori two conservative thresholds of difference. A healthcare provider may not modify empiric therapy based on the differences found in our study, and the magnitude of differences reported here are likely not relevant from a public health perspective. Trends of pneumococcal antibiotic resistance over time may be of more clinical and epidemiologic relevance than an absolute level. Knowledge of local trends may help communities assess regional antibiotic use and evaluate the effects of local educational measures promoting the judicious use of antibiotics. As this study did not span multiple years, we could not document the ability of antibiograms to detect trends. However, given that sentinel surveillance conducted in ABCs sites has been shown to detect pneumococcal resistance trends over time (19) and that in our study antibiograms provided site-specific point estimates of antibiotic resistance similar to those measured by active surveillance, antibiograms may be able to follow trends in pneumococcal antimicrobial resistance at the local level.

Drawbacks to this surveillance method include the inability to evaluate resistance to multiple drugs. Relatively few drugs can be evaluated because of laboratory variations in antibiotics selected for susceptibility testing by antibiograms. Health departments that wish to monitor emerging resistance patterns to antibiotics, such as vancomycin or fluoroquinolones, might consider a method other than aggregated susceptibility tables, or they might encourage hospital laboratories within a defined community to standardize their susceptibility panels to facilitate aggregation of results. Another limitation of antibiograms is the inability to distinguish between intermediate- and high-level resistance to penicillin; 77% of antibiograms in our study expressed resistance as percent nonsusceptible rather than distinguishing between intermediate and resistant isolates. This distinction has become relevant for treatment of some infections. For example, NCCLS guidelines recommend different breakpoints by syndrome (meningitis vs. nonmeningitis) for some agents (20). Aggregating antibiograms is useful for infections that are generally community-acquired, but antimicrobial resistance in hospital-acquired infections should be evaluated based on the knowledge of the particular institution's flora. Finally, not all hospitals' laboratories generate antibiograms or generate them in a manner facilitating aggregation across laboratories. However, we demonstrated the comparability of the two surveillance methods despite the fact that the penicillin-nonsusceptibility results, as measured by antibiograms, was known for only 96 (48%) of the 199 ABCs hospital laboratories.

This study should help clinicians and public health personnel in state or local health departments determine which surveillance tool for obtaining estimates of antibiotic-resistant *S. pneumoniae* is best suited to their specific region or community by providing background information on two alternative systems; the benefits and limitations of each system may be reviewed to determine the most useful and practical surveillance tool for a particular region. Antibiograms are relatively inexpensive and easy to use. Although not measured in our study, epidemiologists in Oregon found that the cost of active surveillance was approximately 70 times that of aggregating antibiograms for the three-county study area (11); the high cost of this type of surveillance, however, is partially due to the fact that ABCs is an integrated system that accomplishes multiple objectives in addition to susceptibility

testing of pneumococcal isolates (12). Most hospitals and laboratories routinely generate antibiograms; therefore, obtaining this information is relatively easy and within the capacity of local health departments. Active surveillance, on the other hand, excludes duplicate isolates for a single patient or infection and is able to provide extensive additional information such as risk factors for resistant infections, outcome data, and other laboratory testing such as serotype determination. Active surveillance also limits case and isolate collection to persons who are residents of the defined surveillance area, allowing for calculation of rates of disease. Furthermore, active surveillance provides individual patient-level data, allowing assessment of the impact of specific interventions such as pneumococcal conjugate vaccination of infants and young children. Attainment of patient-level data through active surveillance also permits detection of possible changes in the incidence of resistant pneumococcal infections (e.g., because of a general decrease in cases of pneumococcal infection among children receiving pneumococcal conjugate vaccine) that might go unnoticed if only the proportion of resistant isolates were tracked (i.e., as done by antibiograms).

Increasing antibiotic drug resistance is a problem that is global in scale and that has practical implications for the treatment and outcome of invasive infections from *S. pneumoniae* and other bacteria of public health importance. Clinicians and researchers are now acknowledging the importance of preventing resistant infections through appropriate use of antibiotics and vaccines. Surveillance data are needed to monitor the success of these campaigns and to raise awareness of the problem. Because most local laboratories generate antibiograms routinely, collecting aggregating antibiogram data is an inexpensive and readily available method of measuring local antibiotic resistance levels. Although providing less information than active surveillance, aggregated antibiogram data are a generally accurate way for health departments to generate needed community-specific estimates of pneumococcal resistance.

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Antibiotic Use in Hispanic Households, New York City

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Trained interviewers visited 631 inner city households to determine community prevalence and predictors of antibiotic use. Infectious disease symptoms were reported in 911 (33.2%) of 2,743 household members in the previous 30 days: medical attention was sought by 441 (48.4%) of 911 persons, and 354 (38.9%) of 911 took antibiotics for symptoms. Reported symptoms were respiratory (68.9%), gastrointestinal (15.3%), fever (12.8%), and skin infection (2.8%). Medical attention was sought significantly more often among those with chronic illness, those born in the United States, and those with fever, runny nose, or skin infections (all $p<0.05$). Antibiotics were taken significantly more often among those with poor health, those who spent more time at home, and those with fever and respiratory symptoms. Interventions to promote judicious use of antibiotics must include clinicians and the public, and for the Hispanic population such interventions must also be culturally relevant and provided in Spanish.

Evidence is overwhelming that antibiotic use is linked with increasing antimicrobial resistance in the community (1–7), even when biases and confounding are controlled for (8). Nevertheless, inappropriate prescribing and use of antimicrobial agents continue to be global problems. The reasons include public expectations and demand for medication and lack of understanding about the ineffectiveness of antibiotics against viral illness, the worldwide ease of access to antibiotics, the clinician's wish to satisfy the patient, and pressure to weigh the interests of individual patients against the overall public health benefit (9–11). Data regarding antibiotic use have been gathered primarily from prescription information from primary care practices, despite the fact that antibiotics may be obtained in many communities by inter- and intra-household sharing or by other informal means. Thus, the use of antibiotics in the community may be underreported. In 1994, one community prevalence survey was conducted in Mexico in which homemakers were interviewed regarding the occurrence of diarrhea and use of antibiotics within the previous 2 weeks (12). However, we found no such studies which tracked antibiotic use among households in the United States. Therefore, we conducted a survey among 631 inner city households to determine self-reported prevalence and correlates of antibiotic use in Hispanic households.

Methods

Sample and Setting

The study was conducted in an upper Manhattan neighborhood with a >90% Hispanic population, who lived primarily in apartment complexes. The neighborhood was selected by convenience as part of a larger clinical trial to examine the role of home hygiene practices on the incidence of infectious disease symptoms because the neighborhood is densely populated with many households, including several generations, often with young children, living in close proximity. Participants were recruited by posters placed on local bulletin boards, brochures, and word-of-mouth in local pediatric clinics, community centers, and churches. To qualify for the study, households had to include at least three persons with at least one preschool child; study participants had to be able to converse in either Spanish or English and willing to allow the research staff to make a home visit. An initial group (n=398 households with 1,645 individual participants) was interviewed in a one-time home visit and prevalence survey from January 1999 to April 1999 (13), and a second group of households (n=238 with 1,177 participants) was surveyed between October 2000 and February 2001. Of those surveyed, 2,743 (97.2%) of 2,822 members of 631 households were Hispanic, and only those persons were included in this study. Fourteen households (2.2%) participated in both surveys.

Procedures

The study was approved by the Institutional Review Board of Columbia Presbyterian Medical Center. Written informed consent was obtained from the primary caretaker in each household, usually the mother, by a member of the

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research team. This person served as the informant for all members of the household. Participants were extensively interviewed on a single occasion during a home visit of approximately 1 hour. We used a standardized interview form to obtain detailed demographic and health information about each household member, including whether one or more of seven symptoms of infection (vomiting, diarrhea, fever, runny nose, cough, sore throat, skin infections) had been present within the previous 30 days, and, if symptoms were present, whether medical treatment was sought, antibiotics were taken, or both, by any household member. Data regarding sore throat were obtained for persons ≥3 years of age only.

The questionnaire used in the home interview underwent extensive psychometric assessment and pilot testing (14). In the pilot work, we found high levels (>90%) of test-retest reliability and agreement between informant response and direct observation on a number of verifiable factors. On the basis of the pilot testing, a few items in the questionnaire were modified between the first and second survey. For example, in the first survey the specific country of origin was recorded, but since the majority of those born outside the United States were from a single country, the Dominican Republic (1,391/1,520, 91.5%), the second survey asked only whether each participant was born outside or within the United States.

All interviews and home visits were conducted by one of four trained interviewers: three were physicians trained in the Dominican Republic and the fourth was a community health interviewer from Guatemala. All were native Spanish speakers. Interviewers underwent an extensive orientation process and were monitored throughout the entire study by the project director, who accompanied the interviewers on a random sample of 10% of home visits. No information about socioeconomic status, household income, or insurance status was requested, but the population was quite homogeneous because the study was conducted in a single neighborhood of predominantly working poor and middle-class immigrants. Participants were given the option of having the interview in Spanish or English; all chose Spanish.

As part of the larger study, we attempted to verify the validity and reliability of the self-reported symptoms in the following manner. For the first 100 reports of any symptom during the second round of interviews, one of the physician interviewers made an additional home visit for the purpose of directly observing the symptom (e.g., if fever was reported, the temperature was taken, if vomiting or diarrhea was reported, the output was examined; if sore throat was reported, the throat was examined). In 93% of cases, directly verifying the presence of the reported symptoms was possible. Further, in 3% of visits a symptom was identified by the interviewer that had not been reported. Hence, the sensitivity and specificity of the symptom self-reports were 93% and 97%, respectively. To verify the accuracy of reporting of antibiotic usage, participants were asked to give the name of the antibiotic and, when available, the labeled bottle or envelope containing the antibiotic was examined by the interviewer. Interviewers only recorded that an antibiotic was taken when the informant was able to give the specific name or when the package label was examined and confirmed to be an antibiotic.

Data Analysis

Analyses were conducted only on Hispanic participants at the level of the individual household member. Chi square and Student t tests were used to compare demographic and illness variables between the two survey periods. Then, for all participants combined, logistic regression models were fit to identify predictors of seeking medical attention and taking an antibiotic among those with an infectious disease symptom. Independent variables used in the models included age, sex, health status (excellent/good vs. fair/poor), presence of chronic illness, household (since symptoms and practices were likely to be interdependent among members of the same household), type of symptom, time spent outside the home, and whether born outside the United States. A two-sided p value of <0.05 was considered statistically significant.

Results

Characteristics of the participants (1,586 persons in survey 1; 1,157 in survey 2) are summarized on Table 1. Approximately one third of household members were 5 years of age or younger (27.4%), 56.3% were women, 55.5% born outside the United States, and most reported excellent or good health (80.6%) with no chronic illnesses (85.9%). About one third spent ≥40 hours/week outside the household (35.3%), and another 35.1% spent ≥20 hours/week outside the household. No significant differences were found between survey 1 and survey 2 with regard to participants' sex (p=0.09), mean ages (20.6 years in survey 1 and 20.2 years in survey 2; p=0.51), or proportion born outside the United States (p=0.16). Significantly more persons in survey 1 had fair/poor health ($p<0.001$), chronic disease (p=0.006), and spent ≥40 hours/week outside the home ($p<0.001$) than in survey 2.

Prevalence of Symptoms and Antibiotic Use

Among the 2,743 household members, 911 (33.2%) reported 1,983 symptoms in the previous 30 days, the most common being respiratory (runny nose, cough, and sore throat, 68.9%), followed by gastrointestinal (vomiting, diarrhea, or both, 15.3%), fever (12.8%), and skin infection (2.8%). Approximately half of persons with infectious disease symptoms (441/911, 48.4%) sought medical atten-

Table 1. Characteristics of 2,743 Hispanic household members

Characteristic	Survey 1, Jan. 4, 1999 (%)	Survey 2, Oct. 2000–Feb. 2001 (%)	Total (%)
Sex			
Female	914 (57.6)	629 (54.4)	1,543 (56.3)
Male	672 (42.4)	528 (45.6)	1,200 (43.7)
Unknown			0
Age (y)			
0–5	405 (25.6)	347 (30.0)	752 (27.4)
6–10	228 (14.4)	130 (11.2)	358 (13.1)
11–19	208 (13.1)	119 (10.3)	327 (11.9)
20–35	414 (26.1)	335 (29.0)	749 (17.3)
36–45	196 (12.4)	137 (11.8)	333 (12.1)
46–60	111 (7.0)	69 (6.0)	180 (6.6)
>60	23 (1.7)	20 (1.7)	43 (1.6)
Unknown	1 (0.06)	0	1 (0.04)
Country of birth			
United States	687(43.3)	530 (46.0)	1,217 (44.5)
Outside United States	899 (56.7)	621 (54.0)	1,520 (55.5)
Unknown			6 (0.2)
Health status[a]			
Excellent or good	1,259 (79.4)	953 (83.2)	2,212 (80.6)
Fair or poor	326 (20.6)	193 (16.8)	519 (18.9)
Unknown			12 (0.4)
Chronic illness[a]			
Yes	248 (15.6%)	133 (11.9%)	381 (14.1)
No	1,338 (84.4%)	984 (88.8%)	2,322 (85.9)
Unknown			0
Time spent outside the home/week [a]			
40+ h/week	469 (29.6)	497 (43.1)	955 (35.3)
20–40 h/week	508 (32.1)	302 (26.2)	810 (29.5)
<20 h/week	607 (38.3)	355 (30.8)	962 (35.1)
Unknown			16 (0.6)
Prevalence of infectious disease symptoms in previous 30 days			
Cough[a]	371 (24.6)	150 (31.6)	521 (26.3)
Runny nose[a]	369 (24.5)	142 (29.9)	511 (25.8)
Sore throat (only in age ≥3 y)[a]	273 (18.1)	61 (12.8)	334 (16.8)
Fever[a]	205 (13.6)	49 (10.3)	254 (12.8)
Vomiting[a]	134 (8.9)	39 (8.2)	173 (8.7)
Diarrhea[a]	102 (6.8)	32 (6.7)	134 (6.6)
Skin infection[a]	54 (3.6)	2 (0.4)	56 (2.8)
Total symptoms	1,508	475	1,983
Persons with at least one symptom[a]	662/1,586 (41.7)	249/1,157 (21.5)	911/2,743 (33.2)
If symptoms present, sought medical attention for symptoms[a]	353 (53.3)	88 (35.3)	441 (48.4)
Received some treatment[a]	345 (52.1)	41 (16.5)	387 (42.5)
Took antibiotics[a]	257 (38.8)	97 (49.5)	354 (38.9)

[a]Significantly different between two survey periods, chi square, all p<0.01.

tion for the symptoms, 42.5% received some type of treatment, and 38.9% took an antibiotic. Respondents in survey 1 reported significantly more symptoms than those in survey 2 (p<0.001) (Table 1).

Predictors of Seeking Medical Attention and Taking Antibiotics

Among those with one or more infectious disease symptom, we found no significant differences in either seeking medical attention or taking antibiotics by age (p=0.07 and 0.56, respectively), gender (p=0.25 and 0.44, respectively), or by presence of gastrointestinal symptoms (vomiting or diarrhea, all p>0.20). As expected, household number was significantly associated with both seeking medical attention and taking an antibiotic. Those born in the United States were significantly more likely to seek medical attention (odds ratio [OR] 2.1; 95% confidence limits [CL] 1.53 to 2.80; p<0.001) but not to take an antibiotic. Chronic illness or poor health status was a significant predictor of seeking medical attention and of taking an

antibiotic. Finally, those who spent less time at home were significantly less likely to report symptoms of infection (28.6% of those outside the home ≥40 hours/week compared with 35.3% outside the home <20 hours/week had symptoms; p=0.006) and less likely to receive antibiotics (OR 0.63; 95% CL 0.43 to 0.92; p=0.02).

Several symptom complexes were predictive of seeking medical attention, taking an antibiotic, or both. Those with skin infections were significantly more likely to seek medical attention (OR 2.70; 95% CL 1.53 to 4.78; p=0.001) but not to take antibiotics. Those with runny nose and fever were significantly more likely to seek medical attention and take antibiotics; those with cough and sore throat were also significantly more likely to take antibiotics. The regression models with the best fit for seeking medical attention and taking an antibiotic are summarized in Table 2.

Table 2. Significant predictors of seeking medical attention and taking antibiotics among those with symptoms[a]

Predictor	Odds ratio (95% confidence limits)	p value[b]
Seeking medical attention		
Household	0.995 (0.994 to 0.996)	<0.001
Chronic illness	2.01 (1.43 to 2.85)	<0.001
Born outside United States	2.07 (1.53 to 2.80)	<0.001
Skin infection	3.06 (1.65 to 5.69)	<0.001
Fever	2.55 (1.82 to 3.57)	<0.001
Runny nose	1.44 (1.06 to 1.96)	0.02
Taking an antibiotic		
Household	0.997 (0.996 to 0.998)	<0.001
Excellent/good health	0.44 (0.28 to 0.70)	<0.001
Outside household ≥40 h/wk	0.59 (0.41 to 0.86)	0.007
Fever	2.39 (1.72 to 3.32)	<0.001
Runny nose	1.38 (1.01 to 1.88)	0.04
Sore throat	2.44 (1.80 to 3.31)	<0.001
Cough	1.42 (1.05 to 1.94)	0.03

[a]n=911.
[b]Logistic regression.

Discussion

Prevalence of Antibiotic Use

The impact of illness with infectious disease symptoms in this community seemed high with about one third of household members reporting at least one symptom within the previous 30 days. However, determining how this compares with the prevalence of such symptoms in other generally healthy community populations is not possible since surveillance systems for infections are either hospital-based or physician-based (15), and most infections in the community never come to the attention of a healthcare provider. Respondents in survey 1 reported more symptoms than respondents in survey 2, perhaps because significantly more persons reported chronic diseases and poor health during survey 1. However, this difference may reflect some seasonal variation; survey 1 was carried out primarily through the winter and early spring; survey 2 was conducted primarily during the fall and early winter.

Of those who reported symptoms, about half sought medical attention and over one third took an antibiotic. In fact, antibiotic usage was higher in this New York City neighborhood than in a periurban community in Mexico City over a decade ago, in which 5% of 8,279 persons reported using an antibiotic during the previous 2 weeks (16). The prevalence of antibiotic use in our study likely represents overuse, since most symptoms reported were indicative of acute respiratory viral illness (runny nose, cough, sore throat), and antibiotics are inappropriate for such infections (17,18). This finding is disappointing in light of the increased attention among primary care providers and in the public media to the problem of antibiotic resistance and the need for prudent use of antimicrobial agents (4,7,19). Although antibiotic resistance among agents that cause community-acquired respiratory tract infections seems to be increasing in the Americas, and particularly in Mexico (20), a recent national survey of generalist physicians found that the issue of contributing to antibiotic resistance was the lowest determinant of their choice regarding antibiotic prescribing for patients with community-acquired pneumonia (9).

As with all self-reported data, this survey contains the potential for recall bias and underreporting or overreporting. Although the answer would be useful to know, we chose not to ask respondents whether the antibiotics they took were obtained by prescription to discourage underreporting. One of the weaknesses of this study is that some participants were reluctant or unable to show the antibiotic container and label to the interviewer, perhaps because antibiotics were not always obtained by written prescription or were borrowed from others. In the study neighborhood, we verified that antibiotics were available over the counter in local *bodegas* (small stores). Households also informally reported sharing antibiotics between family members or among friends. Although the interviewers did not record the specific name of antibiotic after verifying that the medication was, in fact, an antibiotic, clearly, the antibiotics taken were frequently obtained without prescription. Another limitation of the study was that we were unable to examine differences between subgroups of the Hispanic population, since most respondents in this study were from a single country.

Our findings have several important implications. First, estimates of antibiotic usage that are based on prescription data are likely to result in considerable underreporting of antibiotic usage in the community. Second, this generally unaccounted-for usage may be a major contributor to the ongoing problem of antibiotic resistance in the community. Few studies have investigated prevalence of use of

unprescribed antibiotics. Among a Mexican immigrant population in Los Angeles, 28% reported obtaining medication, frequently antibiotics, in Mexico (21). Antibiotic use without prescription is certainly not limited, however, to immigrant or Hispanic populations. Among 1,363 primarily Anglo-American and college-educated adults seen in a New Jersey emergency department, 22% reported that their physicians routinely prescribed antibiotics for cold symptoms, and 17% reported taking antibiotics left over from previous prescriptions, primarily for respiratory symptoms (22). Further, parents who lived in a suburban site were more likely than urban parents to have misused antibiotics, but urban parents were more likely to have been discharged from one health facility and gone to another office or emergency department to obtain antibiotics for their children (23).

To be effective within populations such as the one in this study, media and public health efforts to educate the public on the prudent use of antibiotics must be circulated widely in Spanish and other languages appropriate for recent immigrants, particularly those from countries where antibiotics are widely available without prescription. Further, the messages conveyed must be culturally meaningful for such targeted subgroups. The households in this study all spoke Spanish as their primary language. Language barriers, particularly among Hispanics, have been found to be associated with higher costs of care in the emergency department (24) and Spanish-speaking Hispanics in the United States have a larger gap in immunization rates than do English-speaking Hispanics (25,26).

Correlates of Antibiotic Use

Persons with poor health or a known chronic condition were more likely to receive medical attention and antibiotics. The fact that more U.S.-born persons sought medical attention may relate to insurance coverage or other access issues. Although we did not ask respondents about their insurance or immigration status in this study, in a previous survey of this community, Hispanics reported particularly low participation in insurance plans; 47% who had lived in the United States <5 years were uninsured, and the major reported reason for not seeking care was financial concern (27). In one study, conducted in Colorado and Utah, neither Hispanic ethnicity nor payment source was associated with antibiotic prescription rates (28), but other studies have shown lower insurance rates among children of immigrant parents than among those with U.S.-born parents (29). Several surveys have shown that Hispanics have fewer encounters with the healthcare system and are less likely to receive preventive care than Anglo-Americans (30,31).

On the one hand, this study showed a possible improvement in specific antibiotic-taking practices. A study conducted almost 10 years ago reported that 60% of patients seeing a physician for the common cold filled a prescription for an antibiotic (10). In our study, a younger age of patients, when other factors were controlled for in the regression analyses, was not associated with increased likelihood of taking antibiotics. These findings are consistent with a recent report from the National Ambulatory Medical Care Survey that demonstrated a reduction in population-based and visit-based antimicrobial prescriptions for persons younger than 15 years of age 40% and 29%, respectively (32).

On the other hand, since a large proportion of persons in this study took an antibiotic for symptoms that were likely to be viral in origin, antibiotic overuse seems likely. A number of factors may contribute to a continued overuse of antibiotics. First, even though antibiotic prescribing for children with upper respiratory infection improved during the decade of the 1990s (32), 20% to 50% of antibiotic prescriptions in the community are considered unnecessary (10,33,34). Antibiotics are still prescribed by primary care physicians for most patients seen with respiratory illnesses such as a sore throat, bronchiolitis, or "upper respiratory tract illness" (35,36).

Inappropriate use of antibiotics in this Hispanic population may also relate to practices in their country of origin. McKee et al. reported that persons from countries where antibiotics are readily available over the counter are more likely to use antibiotics not prescribed by clinicians; about one fourth of 192 persons surveyed in an ethnically diverse urban community in New York City obtained antibiotics from sources other than prescription (11). Further, a summary from the SENTRY Antimicrobial Surveillance Program reported that gram-negative isolates from Latin America were uniformly more resistant to all classes of antimicrobial agents than isolates from North America (37). Not surprisingly, persons in this study who spent less time at home were less likely to be ill and to take antibiotics. Additionally, a recent study has confirmed that most cases of influenza in households in which one member was infected resulted from secondary transmission within the household rather than acquisition from community sources (38). Thus, those who spend more time at home are at higher risk for secondary transmission.

Interventions directed at providers and patients to promote more judicious use of antibiotics have met with variable success. Some trials have included both clinicians and patients or the patient's parents (39,40), and others have focused primarily on clinicians (41,42). After considering these studies, we note that interventions involving only the patients or the public are not successful; providers must also be included. Clinicians are resistant to changing their behavior (43), and antibiotic-prescribing patterns are a particular challenge because of pressure from patients or their

family members for antibiotics. In fact, one of the most successful interventions reported to date was a comprehensive, communitywide educational endeavor directed to all three audiences—providers, parents, and patients. In that study, an 11% decrease in prescription rates (which was greatest among prescriptions for children 1 to 5 years of age) could be attributed to an intervention (44).

In summary, about one third of members of this Hispanic community reported infectious disease symptoms over a 30-day period, and antibiotics were taken by more than one third when a symptom was present. When controlled for other factors in the logistic regression analyses, age was not a predictor of seeking medical attention or of taking antibiotics. Persons with fever, runny nose, or skin infection; persons born in the United States; and those with chronic disease were significantly more likely to seek medical attention. Those with respiratory symptoms and poor health and those who spent more time at home were significantly more likely to take antibiotics. Continued interventions to promote judicious use of antibiotics must include both clinicians and the public; for the Hispanic population, such interventions must also be culturally relevant and provided in Spanish.

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Dyspepsia Symptoms and *Helicobacter pylori* Infection, Nakuru, Kenya

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The prevalence of *Helicobacter pylori* infection was studied in 138 patients with dyspepsia in a hospital in Nakuru, Kenya, and in 138 asymptomatic sex- and age-matched controls from the same population. Anti–*H. pylori* immunoglobulin (Ig) G was more prevalent in dyspeptic than asymptomatic persons (71% vs. 51%), particularly those <30 years old (71% vs. 38%). *H. pylori* seropositivity was associated with dyspepsia after adjusting for age, sex, and residence (urban or rural). Among adults, the association between *H. pylori* infection and dyspepsia remained after adjusting for the above factors and for educational attainment, family size, and manual occupation. *H. pylori* infection in asymptomatic residents of Nakuru, Kenya, was more prevalent in older persons, with a rate of 68%, than in those 31–40 years of age. However, young persons with dyspepsia had an unexpectedly high prevalence of *H. pylori* infection. *H. pylori* test-and-treat strategy should be considered in Kenyan patients with dyspepsia, particularly in persons <30 years of age.

Dyspepsia is a complex set of symptoms, rather than an indication of a specific disease, and defies simple categorization. Many causes of dyspepsia exist, including *Helicobacter pylori*. *H. pylori* may also produce different symptoms in different people. Moreover, what is known about variations in host susceptibility and *H. pylori* virulence has not been correlated with specific symptoms (1).

Many patients with upper gastrointestinal symptoms who seek health care do not have follow up treatment. In 60% of the investigated patients, results of tests to rule out peptic ulcer disease, gastro-esophageal reflux disease, and gastric cancer are normal, and the diagnosis is functional dyspepsia (2). The benefit of treatment to eradicate *H. pylori* in functional dyspepsia remains controversial (3,4). To manage uninvestigated dyspepsia in developed countries, some authors recommend screening patients <50 years of age without severe symptoms with a noninvasive test for *H. pylori,* and then treat those with positive results with *H. pylori*–eradicating drugs (5). However, in Africa, a disparity exists between the high prevalence of *H. pylori* infection (>90% in many areas) (6) and the occurrence of clinically important disease ("the African enigma"). This finding has led researchers to postulate that *H. pylori* does not play a major role in the etiologic findings of upper gastrointestinal system pathology apart, from gastritis (7,8). Thus, a noninvasive *H. pylori* test-and-treat strategy in a primary care setting in an economically depressed area, such as Africa, should be based on data that show an association between dyspepsia and *H. pylori* infection. The aim of our case-control study was to investigate the association between *H. pylori* infection and dyspepsia in Nakuru, Kenya.

Materials and Methods

Selection of Patients

Patients who arrived at the outpatient Gastroenterology Clinic of the Rift Valley Hospital in Nakuru, Kenya, with uninvestigated symptoms of dyspepsia for at least the previous 3 months were included in the study. Inclusion criteria were 1) presence of at least two of the following symptoms; upper abdominal pain or discomfort, bloating, nausea, vomiting, or early satiety; 2) persistent or recurrent symptoms occurring at least three times per week during >6 months in the year or years preceding the study; 3) absence of nocturnal or postprandial symptoms of gastroesophageal reflux; 4) no previous abdominal surgery except for uncomplicated appendectomy, cholecystectomy, or hernia repair.

For every dyspeptic patient, a sex- and age-matched control was recruited from a convenience sample of asymptomatic persons from the local community of Nakuru by public advertisement. Dyspepsia in the control group was excluded by clinical interview and a structured screening questionnaire.

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Gastrointestinal Symptom Questionnaire

All participants (patients and asymptomatic participants) were interviewed by one of the authors (S.O.), a local Kenyan physician, to assess symptoms. A bowel disease questionnaire formulated on the basis of a previously validated instrument (the Bowel Disease Questionnaire) was used, modified, and shortened to accommodate local Kenyan needs (9).

Demographic and Socioeconomic Status

Participants were questioned about demographic data and current and childhood socioeconomic status. Age was coded into five categories (0–20, 21–30, 31–40, 41–50, and >50 years of age); among adults ≥21 years of age, occupation was classified as manual versus nonmanual (clerical, professional, homemaker); educational attainment as less than or at least eighth grade; number of siblings as less than or at least 7; residence as urban or rural; tobacco use as ever or never smoked cigarettes; alcohol use as less than or at least 1 L of beer or 0.5 L of wine (average 50 g ethanol) per week.

Determination of *H. pylori* status

Whole blood was obtained from all participants. Anti-*H. pylori* immunoglobulin (Ig) G was determined with the Helisal Rapid Blood Test kit (Cortecs Diagnostics, UK). This test achieved 89% sensitivity and 91% specificity versus histologic examination and urease testing in Australian adults (10).

Kits were stored at 4°C and equilibrated to room temperature before use. The tests were performed according to the manufacturer's instructions. All results were read by one of the authors (S.O.). Our laboratory recently evaluated the Helisal test in 20 Israeli adults (20–70 years of age, median 42 years of age), and demonstrated the test to be 100% sensitive and 90% specific (11).

Statistical Analysis

Bivariate analyses were performed by using the Fisher exact test for categorical variables and the Student t test or Kruskal-Wallis two-sample test for integer and continuous variables. Multivariate analyses were performed by applying backwards-elimination logistic regression to all demographic and socioeconomic variables evaluated in the bivariate analyses; parsimonious models were developed, which included only age and those variables associated with a mutually adjusted p value of <0.10. Only participants >21 years of age were included in the models investigating the role of education, occupation, and family size on the *H. pylori*–dyspepsia relationship. All p values were two-tailed.

Results

Seropositivity for *H. pylori* was found in 98 (71%) of 138 symptomatic patients and 70 (51%) of 138 asymptomatic participants (odds ratio [OR], 2.4; 95% confidence interval [CI], 1.4 to 4.0; p<0.001). In the asymptomatic participants, the prevalence of *H. pylori* infection increased with age, from 18% in the 0- to 10-year age group to 48% in the 11- to 20-year age group, peaking (68%) in the 31- to 40-year age group. In the dyspeptic patients, the prevalence of *H. pylori* infection was 60% to 73% in all age groups (Table 1). Among persons ≤21 years old, *H. pylori* infection was more prevalent in those with symptoms than those without (17 [71%] of 24 vs.12 [38%] of 31; OR, 4.1; 95% CI, 1.1 to 14.9; p=0.02). Similarly, *H. pylori* seropositivity showed a significant association with dyspepsia among persons 21–30 years of age (35 [73%] of 48 vs. 36 [48%] of 74; OR, 2.6; 95% CI 1.2 to 6.7; p=0.01), but not among persons >30 years of age (46 [70%] of 66 vs. 20 [63%] of 32; OR, 1.4; 95% CI, 0.4 to 2.8; p=0.8).

On bivariate analysis, infection with *H. pylori*, older age, female sex, working as a manual laborer (≥21 years of age), less education, and larger family size (>7 siblings) were associated with dyspepsia in adults (Table 2). *H. pylori* infection was associated with dyspepsia after adjusting for age, sex, and urban residence (OR, 2.0; CI, 1.1 to 3.3; p=0.02), and among adults, after adjusting for these factors and for education, family size, and occupation (OR, 2.4; 95% CI, 1.1 to 4.9; p=0.02) (Table 3).

Table 1. Risk (prevalence odds ratios) of upper gastrointestinal symptoms associated with *Helicobacter pylori* infection by age, among 276 residents of Nakuru, Kenya

Age (y)	Cases[a] (N=138) (%)	Controls[b] (N=138) (%)	OR (95% CI)[c]	p value
0–20	17/24 (71)	12/32 (38)	4.1 (1.1 to 14.9)	0.02
21–30	35/48 (73)	36/74 (48)	2.6 (1.2 to 6.7)	0.01
31–40	27/38 (71)	15/22 (68)	1.2 (0.3 to 4.1)	1.0
41–50	13/18 (72)	3/6 (50)	2.6 (0.5 to 26.3)	0.4
>50	6/10 (60)	2/4 (50)	1.3 (0.1 to 20.2)	1.0
0–30	52/72 (72)	48/108 (44)	3.3 (1.6 to 6.5)	<0.001
>30	46/66 (70)	20/32 (63)	1.0 (0.4 to 2.8)	0.8

[a]Persons with upper gastrointestinal symptoms.
[b]Persons without upper gastrointestinal symptoms.
[c]OR, odds ratio; CI, confidence interval.

Table 2. Risk factors for upper gastrointestinal symptoms among 276 residents of Nakuru, Kenya

Risk factor	Cases[a] N=138 (%)	Controls[b] N=138 (%)	OR (95% CI)[c]	p value
Helicobacter pylori infection	98/138 (71)	70/138 (51)	2.4 (1.5 to 3.9)	<0.001
Less education[d,e]	42/114 (37)	16/106 (15)	3.3 (1.71 to 6.27)	<0.001
>7 siblings[d]	77/114 (68)	44/105 (42)	2.9 (1.7 to 5.0)	<0.001
Manual laborer[d,f]	32/91 (35)	9/73 (12)	3.9 (1.7 to 8.6)	<0.001
Female gender	81/138 (59)	56/138 (41)	2.1 (1.3 to 3.4)	0.003
Alcohol use[d]	8/113 (7)	21/104 (20)	0.3 (0.1 to 0.7)	0.005
Ever smoked[d]	6/115 (7)	3/105 (3)	1.9 (0.5 to 7.0)	0.4
Urban residence[g]	86/138 (62)	80/138 (58)	1.2 (0.7 to 1.9)	0.5
Age, median (range)	30 y (1–62)	23 y (2–74)		0.001

[a]Persons with upper gastrointestinal symptoms.
[b]Persons without upper gastrointestinal symptoms.
[c]OR, odds ratio; CI, confidence interval.
[d]Adults >21 years of age.
[e]Up to 8th grade.
[f]Manual laborers versus persons in clerical or professional fields or housewives.
[g]City or town versus rural.

Discussion

This *H. pylori* serologic study in residents of the Nakuru District of Kenya found the expected relationship between *H. pylori* prevalence and age among asymptomatic participants. However, among persons with dyspepsia, the prevalence was consistently high for all ages, which yielded an unequivocal association between *H. pylori* infection and dyspepsia among persons ≤30 years of age.

Other studies of Africans with dyspepsia have yielded a mean prevalence of 65% (range 60% to 71%), which is consistent with our results (12,13). Our recruitment strategy was very similar to the strategy of a study conducted in Cape Town, South Africa (13). In that 1993 study, *H. pylori* prevalence among a subset of Africans of non-Caucasian descent with nonulcer dyspepsia attending a gastroenterology clinic was 71%, the same as in our study. However, since the South African study did not include healthy controls, no generalizations can be made about the association between *H. pylori* and dyspepsia in different part of the continent. Recently, healthy Nigerian adults and dyspeptic patients were found to have similar prevalence of *H. pylori* infection (80% vs. 88%), but the sample size (50 persons) may have been too small to detect the moderate effects found in our and other's studies, particularly in the subgroup of persons <30 years of age (14).

The role of *H. pylori* in dyspepsia is poorly understood (15,16). Dyspeptic symptoms are common in sub-Saharan Africa (17); in some regions, they may account for up to 10% of hospital admissions (18). Because healthcare resources in Kenya are limited, physicians direct diagnostic tests for patients in whom a definitive diagnosis is important for treatment (e.g., those with peptic ulcer or gastric cancer). Since a large fraction of the dyspepsia in younger Africans is attributable to *H. pylori,* and since dyspepsia in this age group is likely to represent a benign process, a test-and-treat strategy may be appropriate in this age group. This approach involves *H. pylori* testing of uninvestigated dyspeptic patients without severe symptoms or signs suggestive of underlying malignancy (unexplained

Table 3. Risk factors for upper gastrointestinal symptoms among 276 residents of Nakuru, Kenya

	All ages		Adults >21 y	
Risk factor	OR (95% CI)[a]	p value	OR (95% CI)	p value
Helicobacter pylori infection	2.2 (1.3 to 3.8)	0.003	2.2 (1.1 to 4.8)	0.03
Age (y)				
0–20	1.0[b]	—[b]	—[c]	—[c]
21–30	0.7 (0.4 to 1.4)	0.3	1.0[b]	—[c]
31–40	2.1 (1.0 to 4.7)	0.06	1.5 (0.7 to 3.4)	0.3
41–50	(1.2 to 11.4)	0.02	3.5 (1.1 to 11.1)	0.03
>50	(0.9 to 13.2)	0.06	2.0 (1.4 to 9.0)	0.4
Female gender	2.2 (1.3 to 3.7)	0.003	—[d]	—
>7 siblings	—[c]	—	3.2 (1.5 to 7.0)	0.003
Manual laborer	—[c]	—	3.5 (1.4 to 9.3)	0.003
Ever smoked	—[c]		19.4 (1.5 to 256.7)	0.02
Alcohol use	—[c]		0.3 (0.1 to 1.0)	0.05

[a]OR, odds ration; CI, confidence interval; —, not applicable.
[b]Reference group.
[c]Variable not included in all-ages model.
[d]Removed by backwards-elimination logistic regression.

recent weight loss, dysphagia, hematemesis or melena, anemia, previous gastric surgery, and palpable mass). Those with positive results would undergo *H. pylori* eradication therapy before endoscopy is considered. Those testing negative would undergo endoscopy only if dietary and behavioral maneuvers do not ameliorate the complaints.

Our study has several limitations. We did not investigate the underlying causes of dyspepsia, so we may have included an unknown number of participants with peptic ulcer disease or other organic pathology. Additional limitations include the use of convenience (self-selected) controls as a proxy for population controls and the use of prevalence ORs (to make the crude and adjusted risks commensurate) instead of risk ratios. These factors may have led us to overestimate the association between *H. pylori* and dyspepsia. On the other hand, the use of a test with imperfect sensitivity and specificity may have led us to underestimate this association.

In a recent, randomized, placebo-controlled trial in a developed country, eradication therapy proved successful in a subset of patients with nonulcer dyspepsia (19). However, these findings were not confirmed in another trial of similar design (20). This disparity suggests either that the relationship between *H. pylori* and nonulcer dyspepsia is weak or that dyspepsia is a heterogeneous disorder. Thus, the effectiveness of a test-and-treat strategy in the developing world may vary by the population studied or by biological and cultural differences in the definition of dyspepsia.

This study demonstrates that in Nakuru, Kenya, *H. pylori* infection is associated with dyspepsia, particularly in persons ≤30 years of age. Since solid evidence exists that *H. pylori* eradication prevents the development (21) and recurrence (22) of gastric carcinoma and promotes regression of B-cell lymphoma of the mucosa-associated lymphoid tissue (MALT) tissue of the stomach (23), the proposed test-and-treat strategy may be an efficient use of health resources in Kenya and perhaps other African countries.

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Epidemic and Nonepidemic Multidrug-Resistant *Enterococcus faecium*

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The epidemiology of vancomycin-resistant *Enterococcus faecium* (VREF) in Europe is characterized by a large community reservoir. In contrast, nosocomial outbreaks and infections (without a community reservoir) characterize VREF in the United States. Previous studies demonstrated host-specific genogroups and a distinct genetic lineage of VREF associated with hospital outbreaks, characterized by the variant *esp*-gene and a specific allele-type of the *purK* housekeeping gene (*purK*1). We investigated the genetic relatedness of *vanA* VREF (n=108) and vancomycin-susceptible *E. faecium* (VSEF) (n=92) from different epidemiologic sources by genotyping, susceptibility testing for ampicillin, sequencing of *purK*1, and testing for presence of *esp*. Clusters of VSEF fit well into previously described VREF genogroups, and strong associations were found between VSEF and VREF isolates with resistance to ampicillin, presence of *esp*, and *purK*1. Genotypes characterized by presence of *esp*, *purK*1, and ampicillin resistance were most frequent among outbreak-associated isolates and almost absent among community surveillance isolates. Vancomycin-resistance was not specifically linked to genogroups. VREF and VSEF from different epidemiologic sources are genetically related; evidence exists for nosocomial selection of a subtype of *E. faecium*, which has acquired vancomycin-resistance through horizontal transfer.

Enterococcus faecium has become an important nosocomial pathogen, especially in immunocompromised patients, creating serious limitations in treatment options because of cumulative resistance to antimicrobial agents (1). In the United States, the emergence of nosocomial *E. faecium* infections was characterized by increasing resistance to ampicillin in the 1980s and a rapid increase of vancomycin resistance in the next decade (1,2). The emergence of vancomycin-resistant *E. faecium* (VREF) in the United States illustrates the transmission capacities of bacteria and the possibility of a postantibiotic era for nosocomial infections in critically ill patients.

The global epidemiology of VREF is not well understood. In the United States, prevalences of colonization and infection are high among hospitalized patients, but a community reservoir of VREF in healthy persons or animals seems to be absent (3,4). In contrast, in Europe, colonization and infection rates within hospitals remain low, although colonization among healthy persons and animals is prevalent (5–10).

Previous studies suggested host-specificity of VREF genogroups (11), and isolates associated with nosocomial outbreaks seemed to be genetically distinct from nonepidemic VREF isolated from humans and animals (12). The differences between epidemic and nonepidemic isolates were based on genetic relatedness, as determined by amplified fragment length polymorphism analysis (AFLP), and the presence of an identical sequence of the *purK* housekeeping gene in epidemic strains (12). A recently developed multilocus sequence typing scheme for *E. faecium* confirmed that epidemic isolates belonged to a specific genetic lineage (13). Moreover, a variant of the *esp* gene, which has been found to be more prevalent among isolates of *E. faecalis* associated with infections (14), was found in all but one epidemic hospital-derived VREF isolate and not among community-derived VREF (12). Subsequently, other investigators described the variant *esp* gene in vancomycin-susceptible *E. faecium* (VSEF), and this gene appeared to be predominantly present among clinical isolates (15–18).These findings suggest the existence of a specific subpopulation of *E. faecium*, comprising both VREF as well as VSEF, associated with hospital outbreaks and infections.

In this study, we further investigated the genetic relationship between VREF and VSEF isolates, derived from different epidemiologic sources, such as hospital out-

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breaks, infections, and colonization among hospitalized patients and healthy persons. The genetic relatedness was linked to the presence of the variant *esp* gene and antibiotic resistance to ampicillin and vancomycin. On the basis of our findings, we constructed an evolutionary scheme describing the sequential steps in the development and selection of ampicillin- and vancomycin-resistant *E. faecium* strains.

Materials and Methods

Bacterial Strains and Growth Conditions

Isolates of VREF (n=108) were collected from nosocomial epidemics (n=16), clinical infections (n=20), clinical surveys (n=36), and community surveys (n=36) (Table 1). The genotypes of these isolates have been described previously (11,12). Strains were considered epidemic if they were isolated from patients treated in the same hospital, in the same ward and with an overlapping time-relationship, and if AFLP patterns showed at least 90% similarity (12). Epidemic isolates were recovered from clinical sites, blood and urine, as well as from feces. Only one representative isolate from each outbreak was used for analysis. The number of patients involved in each outbreak varied from 4 to >50 (12,19–23). Isolates were considered to be derived from a clinical infection if obtained from a clinical specimen, such as the blood, urine, and wounds. All surveillance isolates, from patients and healthy persons, were isolated from fecal samples. Surveillance isolates were either from the community or from clinical surveillance when obtained from hospitalized patients. The hospital-stay duration of these patients, when cultures were obtained, was not available.

Isolates of VSEF (n=92) were derived from clinical infections (n=73), clinical surveys (n=5), and community surveys (n=14). The isolates from clinical infectious sites were obtained from the SENTRY Antimicrobial Surveillance Program, and originated from different hospitals in several European countries (Portugal, Germany, United Kingdom, France, Spain, Italy, Austria, Turkey, Switzerland, Greece, and Poland). Fifty-seven strains were blood isolates, 5 were isolated from urine, 8 from wounds, and 2 from respiratory tract specimens. Patient information was not available. All VSEF isolates derived from clinical surveys of fecal samples were from the University Hospital Maastricht. All VSEF isolates from community surveys of fecal samples were collected in the Netherlands. All bacterial isolates were collected during the 1990s.

Identification and Susceptibility Testing

Enterococci were identified to the species level and were tested for the presence of the *vanA* gene by using a multiplex PCR described by Dutka-Malen et al. (24). Vancomycin and ampicillin/amoxicillin susceptibilities were determined by standard agar dilution methods, according to the National Committee for Clinical Laboratory Standards (NCCLS) guidelines (25). We considered MICs ≥16 μg/mL for ampicillin or amoxicillin and ≥8 for vancomycin to be resistant.

Esp PCR

All strains were screened for *esp* by PCR, with two different primer sets (esp 11 [5′-TTGCTAATGCTAGTCCACGACC-3′] to esp 12 [5′-GCGTCAACACTTGCATTGCCGAA-3′] and 14F [5′-AGATTTCATCTTTGATTCTTGG-3′] to 12R [5′-AATTGATTCTTTAGCATCTGG-3′]). PCR conditions included an initial denaturation at 95°C for 15 min for activation of the HotStarTaq DNA polymerase (QIAGEN GmbH, Hilden, Germany), followed by 30 cycles of 94°C for 30 sec, 52°C for 30 sec, and 72°C for 1 min, followed by an extension at 72°C for

Table 1. Description of studied *Enterococcus faecium* isolates

Origin	Country	n
Vancomycin-resistant *E. faecium*		
Epidemic	United Kingdom	1
	Netherlands	4
	United States	11
Clinical infections	Austria	1
	United Kingdom	7
	Israel	2
	Italy	1
	Netherlands	8
	United States	1
Clinical surveillance	Germany	1
	France	4
	United Kingdom	1
	Israel	1
	Italy	1
	Netherlands	27
	Slovenia	1
Community surveillance	United Kingdom	1
	Netherlands	35
Vancomycin-susceptible *E. faecium*		
Clinical infections	Austria	6
	Belgium	1
	Switzerland	3
	Germany	14
	Spain	8
	France	8
	United Kingdom	1
	Greece	1
	Italy	16
	Poland	5
	Portugal	5
	Turkey	5
Clinical surveillance	Netherlands	5
Community surveillance	Netherlands	14

7 min. Reactions were performed in 25 µL by using the HotStarTaq Master Mix (QIAGEN GmbH). Strains negative in PCR were checked for the presence of the *esp* gene by Southern hybridization, as described previously (12). For this check, we generated an *esp*-specific probe (956 bp) using primers esp 11 and esp 12 (see previous explanation).

Sequencing *PurK*

The *purK* gene encodes a phosphoribosylaminoimidazole carboxylase ATPase subunit involved in purine biosynthesis and is one of the seven housekeeping genes selected for multilocus sequence typing of *E. faecium* (13). A 492-bp fragment of the *purK* gene of a selection of strains, divided over all genogroups, was sequenced by using primers 5′-GCAGATTGGCACATTGAAAGT-3′ and 5′-TACATAAATCCCGCCTGTTTC/T-3′. PCR conditions included an initial denaturation at 95°C for 3 min, followed by 35 cycles of 94°C for 30 sec, 50°C for 30 sec, and 72°C for 30 sec, followed by an extension at 72°C for 5 min. Reactions were performed in 50 µL by using buffers and Taq polymerase (SphaeroQ, Leiden, Netherlands). The PCR products were purified with a PCR purification kit (QIAGEN GmbH) according to the manufacturer's instructions. Subsequently, purified PCR products were sequenced directly with the ABI PRISM Big Dye Terminators cycle sequencing kit on an ABI PRISM DNA analyzer (Applied Biosystems, Foster City, CA). Sequences were aligned with BioNumerics (v. 2.5, Applied Maths, Kortrijk, Belgium) software.

AFLP

AFLP typing and computer analysis of AFLP-generated patterns of VSEF was done as described previously (11) with minor modifications. Briefly, chromosomal DNA was digested with *Cfo*I and *Eco*RI and ligated to a single adapter with *Cfo*I and *Eco*RI protruding ends in a simultaneous reaction, followed by PCR using adapter-specific primers. The amplification products were separated and detected by using POP6-polymer on an ABI PRISM 3700 DNA Analyzer (Applied Biosystems). For each sample, 1 µL of the PCR reaction mixture (8 x diluted) was added to 9 µL of Hi-Di Formamid containing 12.5 µL/mL of the internal size marker (GeneScan-500–labeled with the red fluorescent dye 6-carboxy-x-rhodamine) in a MicroAmp Optical 96-well reaction plate (Applied Biosystems). The analyses were run in 3 hours. Genescan software (Applied Biosystems) was used for collection of data during the analysis and the data were subsequently exported into BioNumerics (Applied Maths) for further analysis. The Pearson product moment correlation coefficient was calculated, and the unweighted pair group method with arithmetic averages was used for cluster analysis. Using this methodology, we described four genogroups of VREF (11). We analyzed all isolates of VSEF and defined a cluster of isolates as a set of individual strains with AFLP patterns that shared at least 65% of the banding patterns (criterion defined for four genogroups [11]). Subsequently, to determine the matching genogroup, we compared AFLP banding patterns of each individual VSEF isolate with AFLP banding patterns of a library of 404 VREF, representing the four different AFLP genogroups. The library included VREF recovered from pigs (n=108) and nonhospitalized persons (n=28) as representatives of genogroup A, and strains from poultry (n=32), hospitalized patients (n=196), and calves (n=40), representing genogroups B, C, and D, respectively (11,12).

VSEF isolates were identified by using the identification module in BioNumerics. The genetic distance used for further analysis is 100 minus the calculated Pearson product moment correlation similarity coefficient. The degree of matching was expressed by an identification factor, which is the quotient of the average genetic distance between the tested strain and each of the isolates in the genogroup divided by the average genetic distance within a genogroup. If the average distance of the tested strain to each of the genogroup members is almost equal to the average distance among all strains in a genogroup, the identification factor will approach the value of one. So the lower the value of the identification factor, the more likely the test strain belongs to a particular genogroup.

Results

Using our predefined cutoff points for cluster analysis, we identified four clusters of VSEF (Figure 1). VSEF clustering seemed to be source-related. Clusters 1 (n=4) and 2 (n=12) contained surveillance isolates from community sources predominantly. All but one of the clinical infections isolates belonged to clusters 3 (n=66) and 4 (n=10), respectively.

On the basis of our calculations of the identification factors of VSEF and representative isolates of different VREF genogroups, we found that isolates of cluster 1 (n=4) resembled those of genogroup A, previously allocated to nonhospitalized patients and pig-derived VREF (Table 2). Similarly, isolates of cluster 2 (n=12) fitted best in genogroup B. Isolates from cluster 3 (n=66) showed almost equal resemblance to isolates from genogroups B and C, and isolates from cluster 4 (n=10) were also most identical to isolates from genogroup C (Table 2). The relationship between AFLP clusters of VSEF and sources was congruent with the previously described clustering of VREF. Almost all VSEF isolates (99%) derived from clinical infections clustered in clusters 3 and 4, clearly distinct from most VSEF isolates from community surveys (93%), which were found in clusters 1 and 2. A similar distribution was found previously among VREF with most (60%) iso-

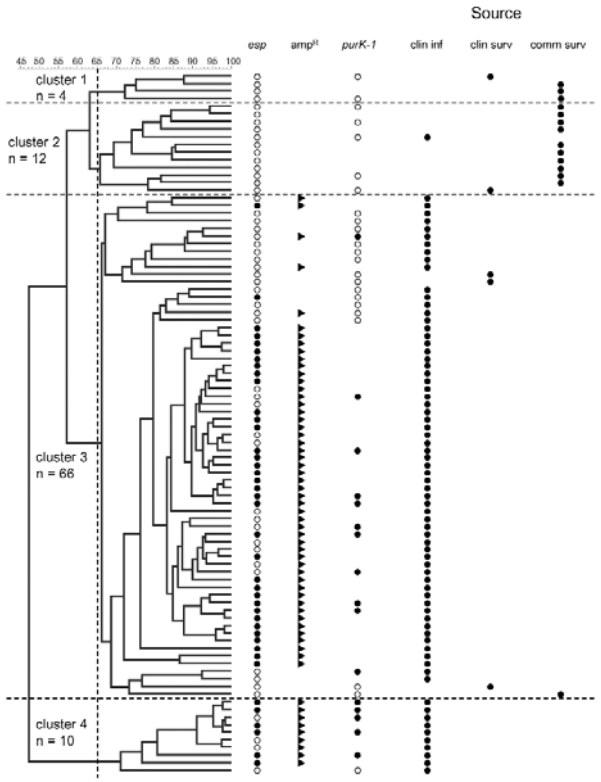

Figure 1. Cluster analysis of vancomycin-susceptible *Enterococcus faecium* (VSEF) isolates originating from clinical infections and clinical and community surveys. VSEF (n=92) were genotyped by amplified fragment length polymorphism (AFLP). Grouping of AFLP patterns showed four different clusters with >65% similarity. Numbers on the horizontal axis indicate percentage similarity. Closed circles indicate presence of the *esp* gene and the *purK*-1 allele and also the source of the isolates. Open circles represent *esp*-negative strains and other *purK* alleles. Closed arrowheads indicate ampicillin resistance. The vertical dashed line indicates the level of 65% similarity. Horizontal dashed lines indicate the boundaries of the four different clusters.

lates from clinical infections in genogroup C and most (89%) isolates from community surveys in genogroup A (11).

The presence of the variant *esp* gene in VREF and VSEF was strongly associated with a specific epidemiologic source because the presence of *esp* is higher in clinical infections and epidemic-associated isolates than in surveillance isolates (Figure 2). VREF isolates associated with nosocomial outbreaks were *esp*-positive, except for one. Prevalences of the variant *esp* gene in clinical infectious isolates were 57% and 40% for VSEF and VREF, respectively (p=ns). Prevalence of the variant *esp* gene in clinical and community survey isolates was low among VREF (6% and 3%, respectively) and completely absent among the 19 VSEF isolates tested.

Associations similar to the variant *esp* gene were found between ampicillin resistance and epidemiologic source for enterococcal isolates (Figure 2). All isolated associated with nosocomial VREF-outbreaks but one were resistant to ampicillin, as were 81% and 65% of infectious isolates of VSEF and VREF, respectively. Thirty-one percent of 36 nosocomial surveillance isolates of VREF were ampicillin resistant, as compared to none of five VSEF isolates obtained by clinical surveillance (p=ns). Finally, all but one isolate of VREF (n=36) and all VSEF isolates (n=14) obtained by surveillance of healthy persons were susceptible to ampicillin. When we combined these data, we found strong associations between the presence of the variant *esp*-gene and ampicillin resistance, both in VREF and VSEF: 98% of *esp*-positive VSEF and 92% of *esp*-positive VREF were resistant to ampicillin, as compared to 37% *esp*-negative VSEF and 20% *esp*-negative VREF isolates ($p<0.0001$).

The *purK* housekeeping gene was sequenced in 103 isolates: 64 VREF and 39 VSEF. The previously described type 1 allele was found in 39 isolates: 23 VREF and 16 VSEF. This specific allele type was associated with the presence of the variant *esp*-gene and ampicillin resistance but not with vancomycin resistance. The variant *esp*-gene was found in 25 (64%) of 39 isolates containing the *purK* type 1 allele and in only 1 (2%) of 64 isolates carrying other *purK* alleles ($p<0.0001$). Similarly, ampicillin resistance was detected in 36 (92%) of 39 isolates with *purK* type 1 allele and in only 5 (8%) of 64 isolates with other allele types ($p<0.0001$). In contrast, the *vanA* transposon was present in 23 (59%) of 39 isolates with *purK* type 1

Table 2. Mean identification factor calculated for 92 vancomycin-susceptible *Enterococcus faecium* (VSEF) in clusters 1, 2, 3, and 4 to the vancomycin-resistant *E. faecium* (VREF) genogroups A–D

		Genogroup A	Genogroup B	Genogroup C	Genogroup D
Cluster	n	IF[a]	IF	IF	IF
1	4	**1.48±0.06**	1.76±0.16	2.5±0.16	3.93±0.31
2	12	2.25±0.20	**1.53±0.10**	2.12±0.15	3.33±0.16
3	66	2.98±0.10	**1.58±0.07**	**1.72±0.09**	3.97±0.06
4	10	3.91±0.45	2.36±0.29	**1.39±0.05**	4.74±0.32

[a]The mean identification factor (IF) represents a measure of how well the entries of the four different VSEF clusters belong to one of the previously described VREF genogroups, taking into consideration the internal spread of the VREF genogroups. Mean identification factor was determined by calculating the arithmetic averages of identification factors of isolates of a given cluster. The lowest identification factor (indicated in boldface type) represents the highest probability that isolates in a given VSEF cluster belong to a certain VREF genogroup. The 95% confidence limits are shown for each identification factor.

allele and in 41 (64%) of 64 isolates with other allele types (p=ns).

Discussion

Our study demonstrates the genetic relatedness of clusters of isolates of vancomycin-resistant and -susceptible *E. faecium* strains from different epidemiologic sources and provides evidence for selection of an *E. faecium* subtype associated with hospital outbreaks. This subtype is characterized by the presence of both ampicillin resistance and the variant *esp* gene. Furthermore, our findings suggest random horizontal spread of the *vanA* transposon to multiple genogroups of *E. faecium*. We hypothesize that the rise in infections caused by VREF resulted from nosocomial selection of a specific ampicillin-resistant *E. faecium* genotype harboring the variant *esp* gene and subsequent horizontal transfer of the *vanA* transposon.

Our study confirms that the previously demonstrated dichotomy between VREF isolated from healthy persons and patients (11) also exists for vancomcyin-susceptible *E. faecium* isolates. In VREF isolates, we could identify four genogroups, which were associated with particular hosts and environments and in which most isolates from healthy persons clustered distinctly from patient isolates. We showed that vancomycin-susceptible isolates clustered into three of these groups and that VSEF isolates from healthy persons also clustered distinctly from patient isolates. The genetic relationship between isolates and the genetic distinction between the four genogroups were based on AFLP analysis. We recently confirmed these findings with multilocus sequence typing (MLST) (13). Other researchers have also demonstrated host specificity of *E. faecium*. Quednau et al. suggested host specificity of isolates from chicken, pork, and humans by comparing restriction endonuclease profiles (26). In contrast, a recent study by Vancanneyt et al. also used AFLP; they did not confirm host-specific clustering of *E. faecium* (27). They described two main genomic groups in a population of 78 *E. faecium* strains isolated from seven European countries, and both groups comprised strains from (healthy) humans, animals, and food. All human clinical strains clustered in the largest genogroup, as did all strains (n=16) containing the *vanA* gene. Our findings that the *vanA* transposon was present in isolates of all genogroups proves that acquisition of this transposon was not influenced by and did not affect the preexisting relationship between the bacterium and host, which is a result of a long-term coevolution and mutual adaptation.

The presence of four genogroups in *E. faecium* seems to parallel the phylogenetic structure in *E. coli*, in which four ancestral groups (A, B1, B2, and D) have been described with some level of host specificity (28). However, in contrast to our finding of a single genetic lineage in *E. faecium* related to clinical symptoms and carrying the *esp* virulence gene, clinical isolates of *E. coli* are more widely distributed among the different ancestral groups (29). Furthermore, MLST of pathogenic *E. coli* strains showed that different ancestral lineages have acquired the same virulence factors (30), indicating that pathogenic potential in *E. coli* is not confined to a single ancestral lineage, which is suggested by our findings for *E. faecium*. Human and animal pathogenic *E. coli* strains share closely related genotypes and carry similar virulence factor profiles, suggesting that certain *E. coli* strains are pathogenic for both animals and humans (31). Whether this holds true for pathogenic *E. faecium* strains is unknown.

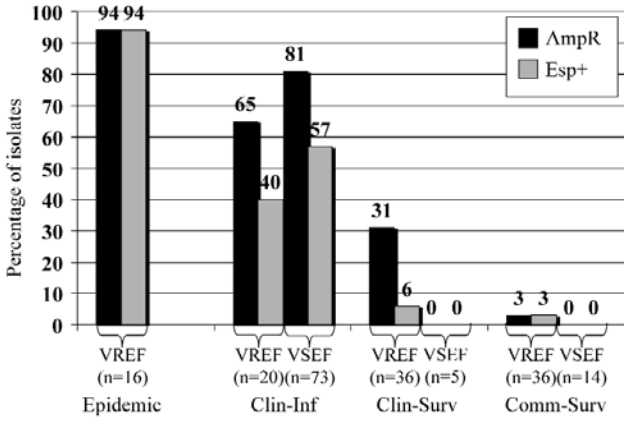

Figure 2. Frequencies of the *esp* gene and ampicillin resistance among vancomycin-susceptible enterococci (VSE) and vancomycin-resistant enterococci (VRE) of different origin. Percentages of *esp*-positive (solid bars) and ampicillin-resistant (dotted bars) VRE and VSE isolates originating from four different sources have been indicated. Clin-Inf, clinical infectious; Clin-Surv, clinical survey; Comm-Surv, community survey.

We recently found that the presence of the variant *esp*-gene is associated with nosocomial outbreaks of VREF in three continents, although this gene was not found in VREF strains isolated from healthy persons or animals (12). The outbreak strains were also characterized by a specific allele type of the *purK* gene, one of the housekeeping genes sequenced in the MLST method (13). Recently, other investigators reported the presence of the variant *esp* gene in clinical isolates of VSEF, demonstrating that this gene is not linked specifically to the *vanA* transposon (15–18). The findings of our study confirm the strong association between the presence of the *esp* gene and the relation with hospital outbreaks and clinical infections among patients with VREF as well as VSEF. Although the *esp* gene is virtually absent among community isolates, the presence of *esp* among VSEF and VREF from clinical infectious sites apparently unrelated to hospital outbreaks implies that this gene is not exclusively relat-

ed to epidemic strains. Excluding the outbreak potential of the *esp*-positive VSEF strains in this and other studies is difficult. Only few outbreaks with VSEF have been documented (32–34). We have investigated and could not demonstrate the presence of the variant *esp* gene in isolates from one hospital outbreak of ampicillin-resistant *E. faecium* in Norway (data not shown).

Little is known about the function of the variant esp-gene, although epidemiologic findings support its role as a virulence factor. In *E. faecalis*, the homologue of this gene encodes for the enterococcal surface protein, and the presence of this gene has been associated with enhanced adherence capacities to uroepithelial surfaces, but not with increased virulence, in a mice model (35). Moreover, in *E. faecalis*, the *esp*-gene was highly associated with biofilm-formation capacity (36). Increased adherence capacities and biofilm formation of *esp*-positive *E. faecium* strains might explain its association with hospital outbreaks.

Like the variant *esp* gene, ampicillin resistance was found more frequently in isolates associated with infections and nosocomial outbreaks, both in VSEF and VREF. This source-relationship is probably caused by selective pressure of β-lactam antibiotics that are used extensively in hospitals. Emergence of ampicillin resistance in *E. faecium* was already demonstrated in the early 1980s and seemed to precede the emergence of vancomycin resistance by 10 years (2). A correlation between high prevalences of the *esp* gene and antibiotic resistance among *E. faecium* isolates from hospitalized patients was also reported recently by Coque et al. (17).

Considering the sources of isolates, presence of ampicillin and vancomycin resistance, the presence of the variant *esp* gene, and the type 1 allele of the *purK* gene, we propose an evolutionary scheme for the specific genogroup of *E. faecium* associated with nosocomial outbreaks (Figure 3). However, our findings might have been biased by the composition of our collection of isolates and the fact that the *purK* was sequenced in a subset of all isolates. The *esp* gene and ampicillin resistance can obviously co-occur in a distinct genetic lineage of *E. faecium* characterized by the type 1 allele of the *purK* gene. We propose that *E. faecium* strains containing the type 1 allele of the *purK* gene have acquired the *esp* virulence gene and that this *E. faecium* genotype (*purK*-1, *esp*-positive) is prominently present among clinical relevant strains and virtually absent among survey isolates. Yet another substantial part of the clinical relevant strains with genotype *purK*-1 do not carry the *esp* gene, which emphasizes that other virulence genes of *E. faecium* apart from *esp* are involved in the development of infections. Ampicillin resistance was predominantly found among the *purK*-1 genotype and is almost absent among other *E. faecium* genotypes. This

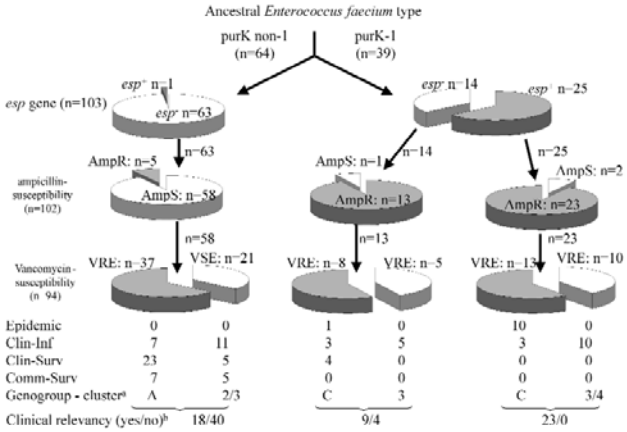

Figure 3. Hypothetical evolutionary scheme for *Enterococcus faecium* genotypes and phenotypes from an ancestral *E. faecium* type. Open slices indicate *esp*-negative, ampicillin-susceptible, and vancomycin-susceptible. Closed slices indicate *esp*-positive, ampicillin-resistant, and vancomycin-resistant. Numbers indicate the number of strains. Arrows indicate the putative evolutionary direction. Clin-Inf, clinical infectious; Clin-Surv, clinical survey; Comm-Surv, community survey. [a], dominant genogroup (A,C) for vancomycin-resistant enterococci and dominant cluster (2,3,4) for vancomycin-susceptible enterococci. [b], clinical relevant strains ("yes") are the total of epidemic and clinical infectious isolates, clinical nonrelevant strains ("no") are the total of clinical and community survey isolates.

occurrence of resistance is, presumably, the result of selective antibiotic pressure. Chromosomal linkage of the *purK*-1 allele, the variant *esp* gene, and ampicillin resistance could have promoted this selection. Finally, glycopeptide usage in and outside hospitals, both in humans and animals, resulted in the selection of vancomycin-resistant strains in both the *purK*-1 genotype and the other genotypes. The presence of similar proportions of vancomycin resistance in all genotypes probably reflects horizontal transfer of the vancomycin-resistance transposon. This hypothesis implies the development of a hospital-adapted genogroup of *E. faecium*, characterized by the type-1 allele of *purK*, the variant *esp*-gene, and ampicillin resistance, which has spread unnoticed, thereby creating a pool of strains with epidemic potential. Only after becoming vancomycin-resistant has this genogroup become recognized as clinically relevant.

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demiology of vancomycin-resistant enterococci and enterococcal pathogenicity mechanisms.

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Reemergence of Epidemic *Vibrio cholerae* O139, Bangladesh

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During March and April 2002, a resurgence of *Vibrio cholerae* O139 occurred in Dhaka and adjoining areas of Bangladesh with an estimated 30,000 cases of cholera. Patients infected with O139 strains were much older than those infected with O1 strains ($p<0.001$). The reemerged O139 strains belong to a single ribotype corresponding to one of two ribotypes that caused the initial O139 outbreak in 1993. Unlike the strains of 1993, the recent strains are susceptible to trimethoprim, sulphamethoxazole, and streptomycin but resistant to nalidixic acid. The new O139 strains carry a copy of the Calcutta type CTX^{Calc} prophage in addition to the CTX^{ET} prophage carried by the previous strains. Thus, the O139 strains continue to evolve, and the adult population continues to be more susceptible to O139 cholera, which suggests a lack of adequate immunity against this serogroup. These findings emphasize the need for continuous monitoring of the new epidemic strains.

Vibrio cholerae O139 Bengal first emerged during 1992 and 1993 and caused large epidemics of cholera in Bangladesh, India, and neighboring countries (1–3). This new strain initially displaced the existing *V. cholerae* O1 strains. During 1994 to the middle of 1995, in most northern and central areas of Bangladesh, the O139 vibrios were replaced by a new clone of *V. cholerae* O1 of the El Tor biotype, whereas in the southern coastal regions the O139 vibrios continued to exist (4–6). During late 1995 and 1996, cases of cholera attributable to both *V. cholerae* O1 and O139 were again detected in various regions of Bangladesh. Since 1996, cholera in Bangladesh has been caused mostly by *V. cholerae* O1 of the El Tor biotype; only a few cases have been attributable to O139 serogroup strains. The epidemiology of cholera in Bangladesh changed again recently, and a large outbreak of cholera caused predominantly by *V. cholerae* O139 occurred in the capital city Dhaka and adjoining areas.

From early March to the end of April 2002, approximately 2,350 cholera patients associated with *V. cholerae* O139 were admitted to the Dhaka Hospital of the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). A preliminary estimate showed that >30,000 cases of cholera occurred in Dhaka and the adjoining areas during this outbreak (A.S.G. Faruque, unpub. data). Since the initial emergence of *V. cholerae* O139 in 1992, we have monitored cholera outbreaks caused by this serogroup in Bangladesh and neighboring regions and have conducted several studies to characterize O139 strains. These studies indicate that strains of the O139 serogroup are undergoing rapid genetic changes, resulting in the origination of new clones; at least seven different ribotypes of O139 vibrios have been documented (6–8). Furthermore, O139 vibrios may have originated from more than one progenitor strain (8). The transient disappearance and reemergence of *V. cholerae* O139 in Bangladesh have raised questions regarding the origin of the reemerged O139 vibrios. In this study, we examined the current epidemiology of cholera in Bangladesh and analyzed *V. cholerae* O139 isolated from the recent outbreak to investigate the origin of the recent epidemic strains as well as to characterize possible genetic changes in O139 vibrios that might have contributed to the recent resurgence of *V. cholerae* O139.

Materials and Methods

Clinical Surveillance

ICDDR,B maintains a 2% surveillance system at its Dhaka Hospital, in which data from every 50th patient treated at the hospital is collected; these data include clinical information and biologic specimens. We used these data to extrapolate the overall numbers of patients with cholera; specimens from these patients were used in the bacteriologic studies described.

V. cholerae Strains

A total of 63 *V. cholerae* O139 isolates obtained from the recent cholera epidemic were analyzed. Seven strains of O139 vibrios isolated in India between 1992 and 1996, 17 strains of *V. cholerae* O139 isolated in Bangladesh

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between 1993 and 1997, and 2 strains isolated in Thailand in 1998 were also included in the study for comparison with the recent epidemic strains. Strains of the recent epidemic were isolated from stools of cholera patients who attended the treatment center of ICDDR,B located in Dhaka during March and April 2002. Stool samples were processed in the laboratory within 2 h of collection for the isolation of *V. cholerae*. Stools were initially streaked on thiosulphate-citrate-bile-sucrose (Becton, Dickinson and Co., Sparks, MD) agar plates for selection and presumptive identification of *V. cholerae*. All strains were subsequently examined by biochemical and serologic tests using standard methods (9). Strains were stored in sealed deep nutrient agar at room temperature until used for this study. Details of the strains are shown in Table.

Polymerase Chain Reaction (PCR) Assays

Presence of *tcpA* genes specific for the classical and El Tor biotypes was determined by using a multiplex PCR assay, as described previously (10). PCR assays for the *tcpI* and *acfB* genes have been described previously (6). Presence of classical, El Tor, and Calcutta type *rstR* genes of CTX phage were also determined with PCR by using specific primers derived from the published sequence of the respective genes. Three different forward primers for $rstR^{class}$, $rstR^{ET}$, and $rstR^{Calc}$ with sequences 5'-CTTCTCATCAGCAAAGCCTCCATC, 5'-GCACCATGATTTAAGATGCTC, and 5'-CTGTAAATCTCTTCAATCCTAGG, respectively, were used with a common reverse primer (5'-TCGAGTTGTAATTCATCAAGAGTG) to amplify the respective rstR genes. Presence of the *rstC* gene was also determined by a PCR assay described previously (11). All primers were synthesized commercially by Oswel DNA Service (University of Edinburgh, Edinburgh, UK). The expected sizes of the amplicons were ascertained by electrophoresis in agarose gels, and the identity of each PCR product was further verified by Southern blot hybridization.

Probes and Hybridization

The gene probes used in this study included a 0.5 kb *Eco*RI fragment of pCVD27 (12) containing part of the *ctxA* gene and a 2.1 kb *Sph*I-*Xba*I fragment of pCTX-Km containing the entire *zot* and *ace* genes and part of *orfU* (13). The *toxR* gene probe was a 2.4-kb *Bam*HI fragment of pVM7 (14). The $rstR^{ET}$ probe was a *Sac*I-*Xba*I fragment of pHK1 (15). The rRNA gene probe was a 7.5-kb *Bam*HI fragment of the *Escherichia coli* rRNA clone pKK3535 described previously (16). The O139-specific DNA probe 2R3 was a 1.3-kb *Eco*RI fragment of pCRII-A3 (17,18), and the SXT probe was a *Not*I fragment of pSXT1 (19). PCR-generated amplicons of the *rstR* genes of classical, El Tor, or Calcutta type CTX prophage were also used as probes whenever appropriate.

For preparation of Southern blots, total cellular DNA was isolated from overnight cultures as described previously (20). Five-microgram aliquots of the DNA were digested with appropriate restriction enzymes (Bethesda Research Laboratories, Gaithersburg, MD), electrophoresed in 0.8% agarose gels, blotted onto nylon membranes (Hybond, Amersham Biosciences, Uppsala, Sweden), and processed by using standard methods (21,22). The probes were labeled by random priming (23) using a DNA labeling kit (Bethesda Research Laboratories) and α-^{32}P-deoxycytidine triphosphate (3,000 Ci/mmol, Amersham Biosciences). Southern blots were hybridized with the labeled probes at 68°C and washed under stringent conditions as described previously (6,8).

Table. Comparative analysis of 63 *Vibrio cholerae* O139 strains isolated from a recent epidemic in Bangladesh versus O139 strains isolated between 1993 and 1998 in different countries

Y of isolation	Country	No. of isolates	Ribotype[a]	Presence of genes[b]				STX genotype[a]	Antibiogram[c]
				$rstR^{ET}$	$rstR^{Clas}$	$rstR^{Cal}$	*rstC*		
1993	Bangladesh	5	B-I	+	-	-	+	A	S^R, SXT^R
1993	Bangladesh	1	B-II	+	-	-	+	B	S^R, SXT^R
1993–1995	Bangladesh	5	B-II	+	-	-	+	A	S^R, SXT^R
1997	Bangladesh	3	B-II	+	-	-	+	C	Susceptible[b]
1997	Bangladesh	3	B-III	+	-	-	+	C	Susceptible[b]
1992	India	3	B-I	+	-	-	+	A	S^R, SXT^R
1993	India	1	B-V	+	-	-	+	A	A^R, S^R, SXT^R
1994	India	1	B-IV	+	-	-	+	A	S^R, SXT^R
1996	India	2	B-II	+	-	-	+	A	A^R, Fz^R, S^R, SXT^R
1998	Thailand	2	B-I	+	-	-	+	A	S^R, SXT^R
2002	Bangladesh	63	B-II	+	-	+	+	C	Nal^R

[a] Ribotypes and SXT genotypes are based on *Bgl*I restriction patterns of the respective genes and their flanking chromosomal sequence.
[b] All strains were positive for *tcpA*, *tcpI*, *acfB*, *toxT*, *ctxA*, *zot*, and *ToxR* genes as well as for the O139-specific genomic DNA in DNA probe or polymerase chain reaction assays.
[c] All strains were susceptible to tetracycline, ampicillin, chloramphenicol, gentamicin, ciprofloxacin, norfloxacin, nalidixic acid, streptomycin, trimethoprim, and sulfamethoxazole.

Autoradiographs were developed from the hybridized filters with Kodak X-Omat AR x-ray film (Eastman Kodak Co., Rochester, NY) at -70°C.

Antimicrobial Resistance

All *V. cholerae* isolates were tested for resistance to antimicrobial drugs by using the method of Bauer et al. (24) with standard antibiotic disks (Oxoid Ltd., Basingstoke, Hampshire, UK) at the following antibiotic concentrations (mg/disc): ampicillin, 10; chloramphenicol, 30; streptomycin, 10; tetracycline, 30; trimethoprim and sulfamethoxazole, 1.25 and 23.75, respectively; kanamycin, 30; gentamicin, 10; ciprofloxcin 5; norfloxacin 10, and nalidixic acid, 30.

Results

Clinical Surveillance

We noted a marked increase in cholera cases associated with *V. cholerae* O139 from March to May 2002 (Figure 1). The highest number of cholera patients admitted to the hospital was in March; 69.8% of these cases were attributed to *V. cholerae* O139, compared to 30.2% of cases caused by the El Tor biotype of *V. cholerae* O1. Cholera attributable to *V. cholerae* O139 occurred with similar frequencies in men and women, similar to those infected with O1 strains. From January 2001 to June 2002, a total of 91 (32%) of 282 of case-patients infected with O1 cholera were <5 years of age, but 15 (15%) of 115 of those infected with O139 were <5 years of age ($p<0.001$). During the same period, 48% of those infected with *V. cholerae* O1 were >15 years of age, while 76% of those infected with O139 were >15 years of age ($p<0.001$).

Genetic Analysis of *V. cholerae* Strains

The rRNA gene restriction patterns using *Bgl*I consisted of 10 to 14 bands between 11 kb and 1.6 kb in size (Figure 2). The 89 analyzed strains belonged to four different ribotypes (B-I to B-IV). All 63 recently isolated O139 strains produced identical restriction patterns of their rRNA genes and belonged to ribotype B-II. Analysis of the *rstR* gene showed that O139 strains isolated from 1992 to 1998 carried El Tor type CTX^{ET} prophage, whereas the recent epidemic strains carry the Calcutta type CTX^{Calc} prophage in addition to the CTX^{ET} prophage (Figure 3). All strains were positive for *tcpA*, *tcpI*, *acfB*, *toxT*, *ctxA*, *zot*, and *ToxR* genes, as well as for the O139-specific genomic DNA in DNA probe or PCR assays.

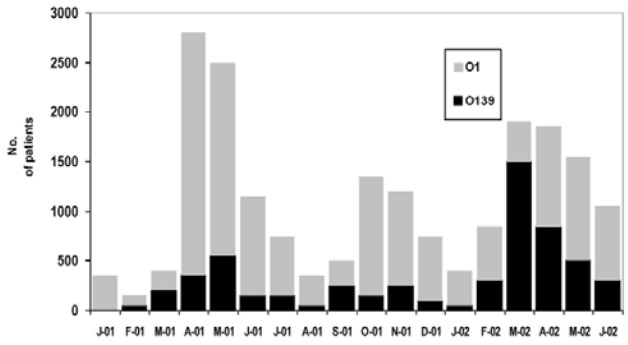

Figure 1. Numbers of diarrhea patients with cholera attributed to *Vibrio cholerae* O1 and O139 from January 2001 to June 2002. Those positive for cholera are extrapolated from a 2% sample of all patients receiving treatment.

Antibiogram

All strains isolated from the recent epidemic were resistant to nalidixic acid and were susceptible to ampicillin, tetracycline, gentamicin, chloramphenicol, ciprofloxacin, norfloxacin, streptomycin, trimethoprim, and sulfamethoxazole. In these strains, the SXT element, which encodes resistance to streptomycin, sulfamethoxazole, and trimethoprim, carried a deletion of an approximately 3.6-kb region.

Discussion

Generally, a seasonality exists in the cholera cases seen at the ICDDR,B hospital, with increased numbers expected before and after the rainy season. Thus, the increase in total number of cases seen during March and April was not unusual (Figure 1). However, the increase in patient numbers during these months of 2002 was associated with a marked increase in cases associated with *V. cholerae* O139, and the numbers of cases infected with serogroup O139 outnumbered those with serogroup O1. The ages of patients infected with O139 strains were significantly higher than those infected with O1 strains ($p<0.001$). Since the onset of O139 cholera in 1992, this organism has tended to infect patients older than those with O1 cholera (1). The more advanced age of this group was explained by a lack of immunity to this new serogroup in adults who were likely partially immune to the O1 serogroup. Thus, after nearly 10 years of endemicity in Bangladesh, *V. cholerae* O139 continues to cause more cases of cholera in older adults.

Ribotype Analysis

The emergence of the O139 serogroup has provided a unique opportunity to witness the epidemiologic changes associated with the displacement of an existing serogroup by a new emerging one and thus provides new insights into the epidemiology of the disease. All 63 recently isolated O139 strains produced the identical restriction pattern of their rRNA genes. This restriction pattern has been previously designated as ribotype pattern B-II (6–8) and was first detected among epidemic *V. cholerae* O139 strains that emerged in 1992 and 1993. Cholera epidemics during

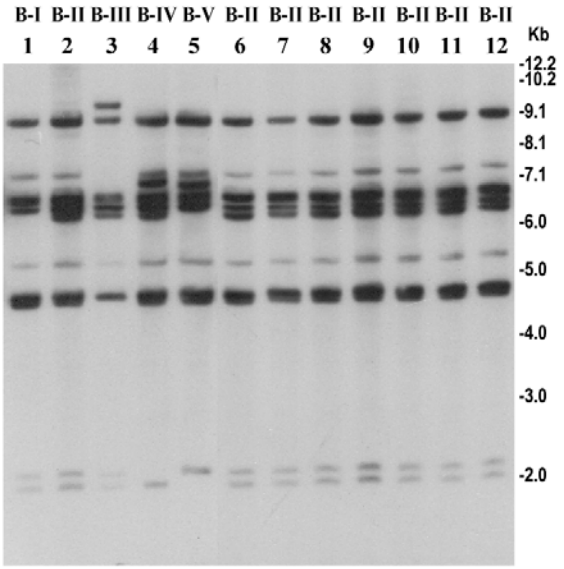

Figure 2. Southern hybridization analysis of rRNA genes in *Vibrio cholerae* O139 strains isolated from the recent epidemic and comparison with representative O139 strains isolated between 1992 and 1998. Genomic DNA was digested with *Bgl*I and probed with a 7.5-kb *Bam*HI fragment of the *Escherichia coli* rRNA clone pKK3535. Lanes 1–6 represent O139 strains isolated from 1992 to 1998; lanes 6–12 represent O139 strains isolated from the recent epidemic in Bangladesh. Designated ribotypes corresponding to each restriction pattern are shown on top of the corresponding lane. Numbers indicating molecular sizes of bands correspond to 1-kb DNA ladder (Bethesda Research Laboratories, Bethesda, MD) used as molecular size markers.

1992 to 1993 in India and Bangladesh that were associated with the first appearance of *V. cholerae* O139 were caused by strains belonging to two different ribotypes, designated as B-I and B-II. Since then, several new ribotypes of O139 vibrios have been detected which were associated with localized outbreaks during 1995 to 1996 or sporadic cases (8). The results suggest that strains of the recent epidemic were clonal and were derived from one of the initial clones of *V. cholerae* O139. We therefore investigated possible genetic changes sustained by this strain during the nearly 9 years since major epidemics were caused by strains of this ribotype.

Analysis of CTX Prophage

In *V. cholerae*, the genes encoding cholera toxin (*ctxAB*) are part of the CTX prophage (25). A typical CTXF genome has two regions: core and the RS2. The 4.5-kb core region comprises several open reading frames including *ctxAB*, *zot*, *ace*, *orfU*, and encodes CT as well as the functions that are required for the virion morphogenesis; by contrast, the 2.5-kb RS2 region encodes the regulation, replication, and integration functions of the CTXF genome (26). Previous studies have described the existence of at least three widely diverse repressor genes (*rstR* genes) carried by different CTX phages (i.e., $CTX^{ET}F$, CTX^{classF}, and CTX^{CalcF}) (27,28). This diversity of *rstR* constitutes the molecular basis for heteroimmunity among CTX phages, which are otherwise genetically similar. We examined the CTX prophage in the recent and previously isolated O139 strains with specific probes. Analysis of the *rstR* gene carried by the recent epidemic strains showed that, unlike the O139 strains of 1993, which carried multiple copies of an El Tor type CTX_{ET} prophage, the new O139 strains carry at least one copy of the Calcutta type CTX^{Calc} prophage in addition to the CTX^{ET} prophage. As a result of heteroimmunity, toxigenic classical strains of *V. cholerae* O1 are known to be infected by CTXF isolated from El Tor biotype strains; toxigenic El Tor strains are resistant to further infection by the same phage. Similarly, strains carrying an El Tor type CTX prophage can be superinfected by the Calcutta type CTX phage (29). Therefore, the new epidemic strains appear to have arisen by acquisition of a Calcutta type CTX phage by strains that originally harbored only El Tor type CTX prophage, since the new strains carry both prophages (Figure 3). What determines the reemergence of particular epidemic strains is not clear, but this study clearly shows changes in the CTX genotype attributed to the acquisition of a new CTX phage by the O139 strains associated with the recent epidemic.

Antibiogram of Reemergent O139 Strains

V. cholerae O139, which emerged during 1992 and 1993, was sensitive to tetracycline and showed a trend of increased resistance to trimethoprim-sulfamethoxazole (SXT) and streptomycin. This resistance was mediated by a ~99-kb self-transmissible transposon-like element (SXT

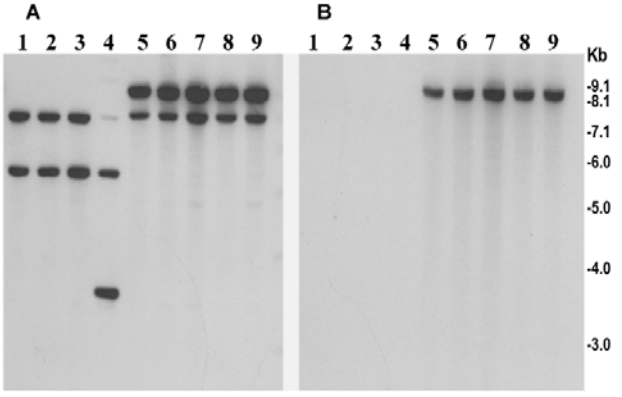

Figure 3. Southern hybridization analysis of *rstR* genes in toxigenic *Vibrio cholerae* O139 strains isolated from the recent epidemic in Bangladesh (lanes 5–9) and in previously isolated O139 strains from 1992 to 1998 (lanes 1–4). Genomic DNA was digested with *Bgl*I and probed with the $rstR^{ET}$ probe (A) and with the $rstR^{Cal}$ probe (B). Numbers indicating molecular sizes of bands correspond to 1-kb DNA ladder (BRL) used as molecular size markers.

constin) encoding resistance to sulfamethoxazole, trimethoprim, and streptomycin, the resistance genes being clustered together in a 9.4-kb region (19). In the present study, all strains isolated from the recent epidemic were found to be susceptible to SXT and streptomycin (Table). To identify the genetic changes associated with the observed SXT sensitivity, we used a cloned SXT gene probe to study restriction fragment length polymorphism in the SXT transposon. Three different *Bgl*I restriction patterns (patterns A–C) of the SXT element were observed among the O139 strains tested (Figure 4). Strains producing pattern A and B were resistant to SXT and streptomycin and included strains isolated between 1992 and 1996, whereas all strains from the recent epidemic produced pattern C and were susceptible to all the three antibiotics. Further analysis of the restriction patterns suggests that the restriction site heterogeneity possibly occurred as a result of a deletion of approximately a 3.6-kb region of the SXT element in strains that were sensitive to SXT and streptomycin. The deletion in the SXT element associated with sensitivity to SXT and streptomycin was first detected in strains of ribotype B-III isolated from an outbreak in Bangladesh in 1997 (6). In keeping with the observation in Bangladesh, comparison of the antibiotic resistance patterns between the O139 strains isolated during 1992 and 1993 and those isolated in 1996 and 1997 in India also showed that the later strains were susceptible to SXT, unlike the O139 strains from 1992 and 1993 (30). However, in contrast to the previously isolated O139 strains, all O139 strains isolated from the recent epidemic were resistant to nalidixic acid.

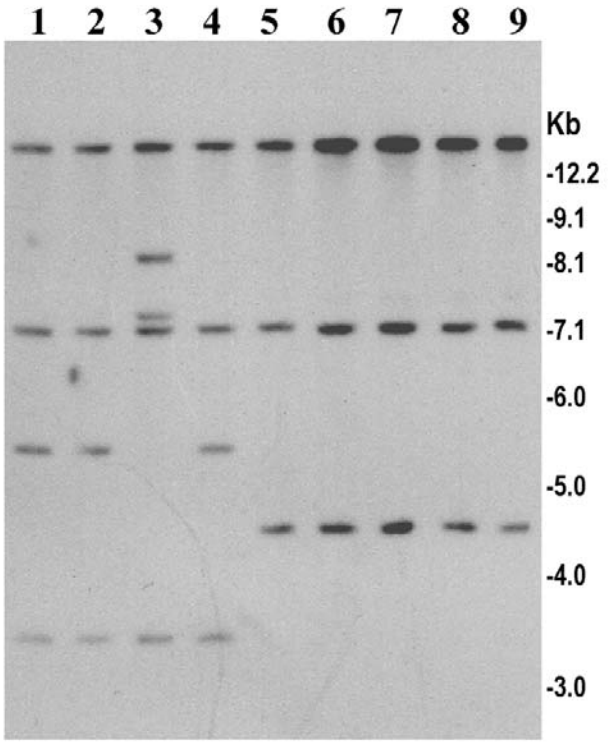

Figure 4. Analysis of SXT element in *V. cholerae* O139 strains isolated isolated from the recent epidemic in Bangladesh (lanes 5–9) and in previously isolated O139 strains between 1992 and 1998 (lanes 1–4). Genomic DNA was digested with *Bgl*I and probed with the SXT gene probe. Lanes 1, 2, and 4 show restriction pattern corresponding to SXT genotype A, lane 3 shows SXT genotype B, and lanes 5–9 represent SXT genotype C. Numbers indicating molecular sizes of bands correspond to 1-kb DNA ladder (BRL).

Epidemiologic Importance of Genetic Changes in *V. cholerae* O139

Several previous studies have shown that the O139 serogroup of *V. cholerae* has been undergoing rapid genetic changes (6–8) since its first emergence. We speculate that the observed changes may have provided increased fitness to strains of this serogroup in some unexplained way to survive in competition with the existing seventh pandemic strain of *V. cholerae* O1 and establish itself as the etiologic agent of a possible eighth pandemic. The transient disappearance of the O139 serogroup in Bangladesh and repeated reemergence associated with somewhat altered genetic or phenotypic properties seem to support this speculation. Our study demonstrated the reemergence of *V. cholerae* O139 strains belonging to a previously described ribotype which has sustained at least three major genetic and phenotypic changes. These changes include the acquisition of a new CTX prophage, deletion in the SXT element associated with reversion of drug resistance phenotype against SXT and streptomycin, and development of nalidixic acid resistance.

The recent epidemic strains were otherwise similar to previously described O139 strains, including possession of the TCP pathogenicity island, as evidenced by the presence of *tcpA*, *tcpI*, and *acfB* genes; the virulence regulatory genes, *toxT* and *toxR*; and the O139–serotype-specific DNA. The role of environmental and host factors that contribute to the emergence of new strains associated with epidemic outbreaks is not clearly known. In the present study, all strains isolated from the recent cholera outbreak belonged to the same ribotype and were genetically and phenotypically identical, suggesting that the recent outbreak in Bangladesh probably started from a point source and may have coincided with the acquisition of one or more critical new properties by a previously existing *V. cholerae* O139 strain. Clearly these properties included the acquisition of the Calcutta Type CTX prophage. Previous studies showed that O139 strains prevailing in Calcutta during 1996 carried this prophage (29,31,32), which might have contributed to the dissimilar incidence of O139 cholera in Calcutta and Dhaka during that period (33). How the initial enrichment of *V. cholerae* occurred before

the initiation of an epidemic is not clear. We speculate that a critical factor for the recent reemergence of O139 vibrios might have been the development of nalidixc acid resistance. Identifying the first index case of the present cholera epidemic is not possible. A spontaneous nalidixic acid–resistant *V. cholerae* O139 strain may have been enriched in a patient undergoing nalidixc acid therapy, leading to the eventual spread of the organism. This is certainly possible in view of the widespread use of nalidixic acid in Bangladesh as a drug to treat other gastroenteritis, including shigellosis. The emergence of *V. cholerae* O139 has received global attention not only as the first non–O1 *V. cholerae* capable of causing epidemic outbreaks but also because of the rapid genetic re-assortment undergone by strains of this new serogroup. Our study shows yet another set of genetic and phenotypic changes in O139 vibrios and their association with an epidemic of cholera in Bangladesh. These results emphasize the need for continuing molecular epidemiologic surveillance of *V. cholerae* in Bangladesh and adjoining areas.

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Dr. Faruque is a scientist at the International Centre for Diarrheal Disease Research, Bangladesh and the head of the Molecular Genetics Unit. His major research interests include microbial evolution, epidemiology and prevention of cholera, and environmental microbiology. Dr. Faruque's current work focuses on understanding the molecular basis for the emergence of epidemic *V. cholerae* strains and developing vaccines against cholera.

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Ehrlichia chaffeensis Infections among HIV-infected Patients in a Human Monocytic Ehrlichiosis–Endemic Area

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Manifestations of human monocytic ehrlichiosis (HME), a tick-borne infection caused by *Ehrlichia chaffeensis*, range from asymptomatic disease to fulminant infection and may be particularly severe in persons infected with HIV. We conducted a serologic study to determine the epidemiology of HME in HIV-positive patients residing in an HME-endemic area. We reviewed charts from a cohort of 133 HIV-positive patients who were seen during the 1999 tick season with symptoms compatible with HME (n=36) or who were asymptomatic (n=97). When available, paired plasma samples obtained before and after the tick season were tested by using an indirect immunofluorescence assay (IFA) to detect antibodies reactive to *E. chaffeensis*. Two symptomatic incident cases were identified by IFA, resulting in a seroincidence of 6.67% among symptomatic HIV-positive participants with paired samples available for testing and 1.64% overall. The baseline seroprevalence of HME was 0%. In contrast to infection in immunocompetent patients, *E. chaffeensis* infection in HIV-positive persons typically causes symptomatic disease.

During the last 25 years, the discovery of a number of newly identified infectious agents, such as *Borrelia burgdorferi, Legionella pneumophila,* and HIV, has raised concern in both the medical and lay communities about novel infectious threats to human populations. Among these emerging pathogens are several species of *Ehrlichia*, small, gram-negative bacteria transmitted by arthropod vectors that can cause human disease, such as human monocytic ehrlichiosis (HME). First described in 1986 (1), HME is caused by *Ehrlichia chaffeensis*, an organism transmitted primarily by the lone star tick (*Amblyomma americanum*) (2). Infection with *E. chaffeensis* can range from being clinically asymptomatic to causing a severe life-threatening illness. HME typically causes systemic symptoms (including fever, headache, malaise, rash, abdominal pain, nausea, and cough) and laboratory signs (leukopenia, thrombocytopenia, and elevated transaminase levels). Rarely, patients have fulminant infection with disseminated intravascular coagulation, sepsis, and adult respiratory distress syndrome, leading to death (2). Asymptomatic infection with *E. chaffeensis* may occur frequently, as suggested in a recent seroepidemiologic study in which 67% of military recruits in an *E. chaffeensis*–endemic area seroconverted without symptoms (3).

The risk for HME in immunocompromised patients is unknown; however, numerous case reports and reviews have described severe ehrlichial infection in immunosuppressed patients (4–6), including several reports of rapidly fatal infection with *E. chaffeensis* in AIDS patients (7–9). Diagnosis of HME in HIV-positive patients is often confounded by the fact that the signs and symptoms of ehrlichial infection mimic typical findings commonly associated with HIV infection, its complications, and the medications commonly used in treating such patients. Delayed consideration and diagnosis of ehrlichial infection may result in additional illness if antibiotic therapy is not instituted promptly.

Studies investigating the epidemiology of *E. chaffeensis* infection have focused on healthy persons living in regions endemic for *E. chaffeensis* or clinical findings among hospitalized case-patients (3,10–12). A systematic evaluation of the seroepidemiology of ehrlichial disease in HIV-infected persons has not been performed. We therefore conducted a descriptive seroepidemiologic study to ascertain the prevalence and incidence of *E. chaffeensis* infections in HIV-infected persons located in an area endemic for HME.

Methods

Selection of Patients

Participants were selected among HIV-positive patients who receive their medical care at the Comprehensive Care Center, an adult HIV-oriented primary care clinic located

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in Nashville that serves middle Tennessee and surrounding regions. Center records were retrospectively analyzed to identify patients seen at the clinic for any reason between March 1, 1999, and October 31, 1999 (the typical period of tick activity in middle Tennessee).

Symptomatic Patient Subset

Those patients discharged with diagnoses indicative of potential ehrlichial infection, according to the International Classification of Diseases, 9th Edition (ICD-9), were identified by means of a blinded database review. Specifically, patients who were assigned the following ICD-9 codes were selected for the study cohort: fever or fever of unknown origin (780.6), viral infection (079.9), upper respiratory infection (465.9) or respiratory disease (478.9) not otherwise specified, Lyme disease (088.81), rickettsial disease (specified, 083.8, or unspecified, 083.9), Rocky Mountain spotted fever (082.0), tick bite (088.89), and myalgias (729.1). To find potential participants who may have been missed in the original search, a second database search identified patients during the study period who were prescribed doxycycline, the therapy of choice in the empiric treatment of febrile illness during the tick season.

Asymptomatic/Other Patient Subgroup

The rest of the study cohort comprised patients who visited the center during the study period and who had plasma banked for serologic investigation (see "Plasma Collection"). To investigate the incidence of asymptomatic *Ehrlichia* infection, patients were selected from a blinded review of the center's plasma sample log. Patients who had banked plasma samples from the pretick season (between September 15, 1998, and March 31, 1999) as well as from the posttick season (after October 31, 1999) and who had visited the center for routine follow-up during the study period were selected for study. Records of patients identified as asymptomatic were reviewed for symptoms suggestive of HME during the study period that were not encoded with an ehrlichiosis-compatible ICD-9 diagnosis.

Chart Review

The center's data charts were analyzed for demographic data (age at start of study period, race, sex, number of clinic visits during the study period), past medical history, medication history (the administration of highly active antiretroviral therapy [HAART] and medication used as prophylaxis against opportunistic infections), and HIV status based on the most recent CD4 count and viral load drawn before the study period began (March 1, 1999). Charts from the symptomatic patients were further analyzed for symptoms suggestive of *Ehrlichia* infection, including the presence or absence of fever, headache, rash, fatigue, malaise, upper respiratory infection symptoms, nausea, vomiting, myalgias, abdominal pain, and mental status changes. Symptom history, laboratory parameters (peripheral leukocyte count, platelet count, aspartate aminotransferase, and alanine aminotransferase) at baseline and during the acute illness, and illness outcomes (including antibiotics prescribed, hospitalization, and death) were also collected. Insufficient data on tick exposure, tick bites, or outdoor activity were available to evaluate exposure risk factors for *Ehrlichia* infection.

Plasma Collection

Since 1998, the center has maintained a repository of plasma samples frozen at –70°C by retaining specimens obtained from patients during routine phlebotomy. All patients who choose to participate in the plasma banking provide written informed consent based on a protocol approved by the Vanderbilt University Institutional Review Board. The plasma log was cross-checked with the study participant list identified from the database review as outlined above. Participants with no banked plasma sample from before the onset of the study period (preseason sample) or at the onset of clinical symptoms (acute sample) were excluded. Samples from at least 4 weeks after the acute clinical illness or after the study period (postseason sample) were also identified for most persons. Persons with no further samples banked after their acute illness or the study period were included only in determination of seroprevalence.

Serologic Testing

All preseason and postseason samples were tested in a blinded fashion by indirect immunofluorescence assay for antibody reactive with *E. chaffeensis* with an assay previously described for human granulocytic ehrlichiosis, which has been widely employed for HME using different antigen substrates (13). A reciprocal antibody titer of $\geq$64 was considered elevated and indicative of infection with *E. chaffeensis*. Seroconversion to *E. chaffeensis* was defined as a fourfold or greater increase in antibody titer between acute-phase or preseason and convalescent-phase samples.

Statistical Analysis

Incidence rates were described as the number of cases of seroconversion divided by the total population of interest. We used 95% confidence intervals determined by using Stata statistical software version 7.0 (Stata Corporation, College Station, TX).

Results

We initially identified a total of 176 patients from the center's records; 43 were excluded because specimens for testing were unavailable, leaving 133 in our study cohort. Thirty-six (27.1%) had symptoms compatible with HME

(29 found by screening of ICD-9 codes and for doxycycline use, 7 found after chart review of initial asymptomatic candidates), and 97 (72.9%) had no symptoms suggestive of this diagnosis. Characteristics of the cohort are shown in the Table. The median CD4 count was 370 cells/mm^3. Symptomatic participants had significantly more visits ($p<0.001$) to the clinic during the study period and were significantly ($p=0.035$) more likely to have received antibiotic therapy (excluding doxycycline; data not shown). As doxycycline was used to select for symptomatic participants, doxycycline therapy was, not included in the analysis of antibiotic use. Other characteristics (specifically age, gender, baseline CD4 count, baseline viral load, use of HAART, use of prophylaxis for opportunistic infection [*Pneumocystis carinii* pneumonia and *Mycobacterium avium* complex], and average number of visits during the study period) between the symptomatic and asymptomatic subgroups did not differ significantly.

None of the patient specimens obtained before the 1999 tick season had serologic evidence of prior *Ehrlichia* infection, resulting in a baseline seroprevalence of 0% for our cohort. Of the 122 patients with paired samples available (92 asymptomatic, 30 symptomatic), 1 patient had a clinical syndrome compatible with HME and had a significant rise in antibody titer to *E. chaffeensis* during the study period (initial titer 64; postseason titer 1,024). Clinically notable disease characterized by fever, myalgias/arthralgias, leukopenia, and thrombocytopenia developed in this patient after tick exposure and required a 4-day hospitalization. During this hospitalization, his diagnosis was confirmed by conducting a polymerase chain reaction assay on his serum, which was positive for *E. chaffeensis*. His symptoms resolved after a course of doxycycline. A second patient with a 10-day history of symptoms compatible with HME (fatigue, cough, and overall malaise), but no documentation of tick exposure or tick bite, had an initial acute-phase titer of 512, drawn when first seen by a clinician (10 days after symptom onset), fulfilling case criteria for probable *Ehrlichia* infection (14). This patient did not have an earlier preseason sample available for analysis but did have a postseason titer of 512 obtained 4 months after clinical illness, suggesting a prolonged elevation in antibody titer. He was thought to have an upper respiratory tract infection by his primary caregiver, and doxycycline was prescribed for his illness. His symptoms resolved without hospitalization.

Table. Baseline characteristics of study cohort of HIV-positive persons residing in Tennessee[a]

Characteristic	N (% or range)
Age (mean, y)	38.8 (21–75)
Sex	
Male	107 (80.5%)
Female	26 (19.5%)
Baseline CD4 count (median, cells/mm^3)	370 (6–1,200)
Baseline viral load (median, copies/dL)	1,003 (<400–>750,000)
On prophylaxis	
HAART	122 (91.7%)
OI prophylaxis	70 (52.6%)
PCP prophylaxis[b]	66 (49.6%)
MAC prophylaxis[c]	31 (23.3%)
Average number of clinic visits[d]	4.75 (1–13)
Treated with antibiotic therapy[d,e]	40/133 (30.1%)
Treated with doxycycline[d]	14/133 (10.5%)
Hospitalized[d]	7/133 (5.3%)

[a]HAART, highly active antiretroviral therapy; OI, opportunistic infection; PCP, *Pneumocystis carinii* pneumonia; MAC, *Mycobacterium avium* complex.
[b]PCP prophylaxis: use of trimethoprim-sulfamethoxazole, dapsone, or aerosolized pentamidine therapy.
[c]MAC prophylaxis: use of azithromycin or clarithromycin therapy.
[d]During the study period.
[e]Antibiotics used to treat the ongoing clinical symptoms; persons taking antibiotic therapy specifically for OI prophylaxis alone were not included.

These two cases resulted in a seroincidence among symptomatic patients of 6.67% (95% confidence interval [CI] 0.82, 22.1) and an overall incidence of 1.64% (95% CI 0.2, 5.8). No asymptomatic cases were identified in our cohort (upper 95% CI for seroconversion in the asymptomatic population, 3.2%).

Discussion

Researchers have conducted various serologic studies to ascertain the epidemiology of *E. chaffeensis* infection in specific populations. Carpenter et al. (10) found a seroincidence of 25.7% in febrile patients in North Carolina with a history of a recent tick bite. In a prospective seroepidemiologic study of residents living in a rural community in California, prevalence rates of 4.6% were reported, and most of the infected participants recalled no recent compatible illness (11). In a comparison of two golf-oriented retirement communities in middle Tennessee, one abutting a wildlife-management area and one 20 miles away from the area used as a control population, Standaert et al. found seroprevalence rates of 12.5% and 3.3%, respectively (12). A study on the seroprevalence in children residing in HME-endemic areas, including Tennessee, found a seroprevalence rate (as defined by *E. chaffeensis* antibody titer >1:80) of nearly 15% among children undergoing phlebotomy in Nashville (15). None of these studies, however, investigated the incidence rates for immunosuppressed persons, such as persons infected with HIV, who may be at increased risk for symptomatic disease after ehrlichial infection.

Our findings indicate that the prevalence and incidence of HME attributable to *E. chaffeensis* infection in an HIV-positive population are quite low in a cohort of HIV-positive patients receiving care at an urban HIV clinic within an HME-endemic region. The incidence rate in our study was similar to those previously reported in a cohort of

healthy military recruits in an area endemic for *E. chaffeensis* (1.3%) (3). However, only 33.3% of seropositive persons in that study had a compatible febrile illness, and none of these symptomatic seroconverters were sufficiently ill to require medical care (3). In contrast, both of our case-patients had symptomatic disease of sufficient severity to require medical care, and one required hospitalization. Furthermore, none of our patients had serologic evidence of asymptomatic infection during the study period. Therefore, while the overall incidence of *Ehrlichia* infection was not increased in our cohort, these results are in agreement with other studies that indicate that HME can cause severe infection in HIV-positive persons.

Our patient with a diagnosis of probable HME had evidence of a sustained antibody response. The acute-phase serum sample and a convalescent-phase sample obtained 4 months later both had titers of 512. This finding suggests that the immune response mounted by HIV-positive persons against *E. chaffeensis* is durable and may persist for several months, similar to the response seen in HIV-negative persons. Because of the low rate of seroconversion in our cohort, we were unable to analyze data on specific risk factors (e.g., CD4 count or use of HAART) that might predispose persons with HIV infection to ehrlichiosis.

Our study has several limitations. The retrospective design placed constraints on the data that could be abstracted, thus introducing possible reporting bias. A prospective study, in contrast, would allow investigators to collect further information on exposure risks, such as level of outdoor activity, and could reduce the variability in symptom reporting found with our study. Our study population could also lead to bias and, as a result, limit generalizability of our results to the HIV-positive population as a whole. The Comprehensive Care Center draws patients from both metropolitan areas (Nashville) and rural communities; however, our cohort may have been more metropolitan and less likely to come into contact with wooded environments. Also, HIV-infected patients who regularly attended the clinic may have had more contact with the healthcare delivery system and thus been more likely to take regular antiretroviral medications that could reduce their viral burden and concomitant immunodeficiency. As a result, those patients who are noncompliant with follow-up (and, by extension, antiviral therapy) may be at greater risk for symptomatic infection and may have been missed in our analysis.

The use of serologic methods to determine actual prevalence and incidence rates for the HIV-infected population may also be problematic. A reduced antibody response to various antigens, including those contained in tetanus and pneumococcal vaccines, in HIV-infected patients has been described in previous studies (16). A potentially decreased ability to mount an immune response to *E. chaffeensis* may have led to false-negative antibody titers and an underestimate of the incidence of ehrlichiosis in this population. Such a finding was highlighted in two previous reports of HIV-positive persons with fatal *E. chaffeensis* infection who did not mount an antibody response during their illnesses (5,7). The serologic response to ehrlichial infection may also be blunted or inhibited by tetracycline therapy, which, when given early in the course of *Ehrlichia* infections, inhibits the development of a serologic response (10,12). Empiric treatment of febrile patients with a clinical picture resembling ehrlichiosis in our population thus could have blunted the antibody response and led to a falsely low seroincidence and prevalence.

In conclusion, we found that levels of HME infection in our HIV-positive cohort were similar to those in normal, healthy persons who received intense exposure to the outdoors in an HME-endemic area. However, both of our case-patients had clinical infections, one requiring hospitalization. Caregivers of HIV-positive patients in regions endemic for *E. chaffeensis* should consider ehrlichiosis as part of the growing list of potential opportunistic infections and maintain a high level of clinical suspicion for this disease. Prospective studies in HIV-positive populations are needed to fully understand the extent of infection with *E. chaffeensis* in these patients.

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Consumer Attitudes and Use of Antibiotics

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Recent antibiotic use is a risk factor for infection or colonization with resistant bacterial pathogens. Demand for antibiotics can be affected by consumers' knowledge, attitudes, and practices. In 1998–1999, the Foodborne Diseases Active Surveillance Network (FoodNet) conducted a population-based, random-digit dialing telephone survey, including questions regarding respondents' knowledge, attitudes, and practices of antibiotic use. Twelve percent had recently taken antibiotics; 27% believed that taking antibiotics when they had a cold made them better more quickly, 32% believed that taking antibiotics when they had a cold prevented more serious illness, and 48% expected a prescription for antibiotics when they were ill enough from a cold to seek medical attention. These misguided beliefs and expectations were associated with a lack of awareness of the dangers of antibiotic use; 58% of patients were not aware of the possible health dangers. National educational efforts are needed to address these issues if patient demand for antibiotics is to be reduced.

Antimicrobial resistance is a rapidly increasing problem in the United States and worldwide. A well-documented risk factor for infection or colonization with resistant bacterial pathogens is recent antibiotic use, particularly within 4 weeks or 1 month before exposure (1–6). As a result, one of the primary strategies to prevent and control the emergence and spread of resistant organisms is to reduce the selective pressure of overuse and misuse of antibiotics in human medicine (7).

Several studies have identified and examined specific causes of the misuse of antibiotics, including unnecessary prescribing (8–14) and patient demand (15–17). Factors contributing to inappropriate prescribing practices have been elucidated. In particular, numerous studies of adults have shown that patients' expectations or physicians' perceptions of those expectations affect the physicians' prescribing behavior (10,13,16–24).

To solve the problem of antibiotic misuse, a more thorough understanding of what influences the development and expression of patients' expectations must be gained. Understanding patients' knowledge, attitude, and practices may facilitate more effective communication between the clinician and patient, as well as aid in the development of strategies to educate patients and the public (25). Several lines of evidence suggest educational interventions directed at patients and clinicians can increase patients' knowledge and awareness, as well as reduce the frequency with which clinicians prescribe antibiotics inappropriately (26–30).

Our investigation, an analysis of data from a national population-based cross-sectional survey, provides a glimpse of the current knowledge, attitudes, and practices regarding antibiotic use among patients. We also attempt to identify demographic characteristics associated with particular knowledge, attitude, and practices and to determine whether a person's attitudes toward and knowledge of risks associated with taking antibiotics are associated with recent antibiotic use. Identifying subgroups of the population with high levels of antibiotic use and with misconceptions about antibiotic use will help public health officials target and track the impact of interventions. Other information obtained from this population-based survey will provide further insight for the development and evaluation of health education and prevention strategies.

Methods

Data Source

From February 2, 1998, through February 15, 1999, the Emerging Infections Program's Foodborne Diseases

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Active Surveillance Network (FoodNet) conducted a telephone-based population survey in Connecticut, Minnesota, and Oregon, and selected counties in California, Georgia, Maryland, and New York (total population 29 million). Each month, approximately 150 residents in each state were interviewed. After screening to remove business and nonworking telephone numbers, an outside contractor contacted respondents by telephone using a random-digit-dialing, single-stage sampling method (31).

These contractors conducted the interviews using methods similar to those used in the Behavioral Risk Factor Surveillance System (32). All interviews were conducted in English. Using a standardized questionnaire, they asked one respondent per household about his or her knowledge, attitudes, and recent practices regarding antibiotic use. All members of the household were eligible for selection. Institutional review boards at the Centers for Disease Control and Prevention and all participating states approved the study.

Interviewers obtained verbal consent from all study participants before administering the questionnaire. They informed participants why the information was being collected, and how it would be used, and read them a statement informing them that their participation was voluntary before the start of the interview. No personal identifiers were included in this dataset.

Survey Questionnaire

Five items (two questions and three statements) addressing participants' knowledge, attitudes, and practices regarding antibiotic use were included in the survey. Recent antibiotic use referred to antibiotic use in the past 4 weeks. Respondents who took an antibiotic were asked whether the antibiotic was prescribed by their physician for a current illness or for a previous illness or if the antibiotic was prescribed for someone else. For the question, "Are you aware of any health dangers to yourself or other people associated with taking antibiotics?" respondents' knowledge of health dangers associated with taking antibiotics was classified into the following categories: emerging drug resistance, allergies/reactions, antibiotics may kill "friendly"/"good" microbes, it is unhealthy to take drugs/chemicals in general, misuse/overuse of antibiotics, multiple reasons, other, don't know, or refused. Answers to survey items 1 and 5 were yes/no. For statements 2, 3, and 4, participants were asked to respond according to the following 5-point Likert scale: 1=strongly agree, 2=agree somewhat, 3=unsure, 4=disagree somewhat, and 5=strongly disagree. We classified those who answered "strongly agree" or "agree somewhat" to the antibiotic knowledge questions as having agreed and those who answered "strongly disagree" or "disagree somewhat" as having disagreed. Those who refused to answer a question were not included in the analysis.

In addition to eliciting participants' responses to these questions, the survey also recorded demographic characteristics of the participants, including their sex, age, income level, education, race, state, and place of residence. Respondents' place of residence was categorized as urban if they reported living in a city or town of ≥50,000 residents. Presence of children in the household (yes/no), month of interview, and medical insurance status were also recorded. Respondents were classified as being "with insurance" if they reported any of the following as their type of insurance: health maintenance organization, preferred provider organization, traditional indemnity insurance, Medicaid, Medicare, or other. If respondents reported their type of insurance as "don't know" or if they refused to answer the question, they were not included in the analysis.

To simplify our analysis, we coded persons indicating Hispanic ethnicity as Hispanic, even if they also identified themselves by race (e.g., a white-Hispanic male would be coded for race as Hispanic). For our multivariable analysis, we grouped persons identified as Asian, Pacific Islander, American Indian, or Alaskan Native into the category called "other." We also added those who responded "don't know" or "unsure" to the attitude questions to the "agree" group to divide respondents into two groups: those who responded correctly (disagree) and those who did not (agree or don't know). For our multivariable logistic regression, we grouped respondents who answered "don't know" to the question, "Are you aware of dangers associated with antibiotics?" with those who answered "no." Persons responding "don't know" to the question, "In the past 4 weeks, have you taken antibiotics?" were not included in the analysis. We evaluated respondents' education and income levels as continuous variables.

Statistical Analysis

To compensate for respondents' unequal probability of selection and allow population estimates to be made, we weighted the data following procedures from the Behavioral Risk Factor Surveillance System (33) and based our weighting on the number of residential phone numbers, the number of people per household, and the 1998 postcensus estimates for the age- and sex-specific population of the FoodNet sites (B. Imhoff, pers. comm.). We did not include race in the poststratification weight since some site-sex-age-race groups contained <10 survey participants.

We analyzed the data using SUDAAN (SUrvey DAta ANalysis, v7.5.2, Research Triangle Institute, Research Triangle Park NC), a specialized statistical procedure for analyzing complex sample survey data, and ran the analysis using SAS (Statistical Analysis Software, v6.12) (SAS Institute, Inc., Cary, NC) This software adjusts for the

complexity of the sampling design (unequal weighting and clustering) and uses Taylor series linearization methods to estimate variances. Because the ratio of sample size to population size was small, we approximated the sample design by a "with-replacement" design for purposes of variance estimation in SUDAAN. Any bias resulting from such replacement sampling will be in the conservative direction.

We examined respondents' attitudes toward, and awareness of, antibiotic use by their age, sex, race, income level, education, state, place of residence, medical insurance status, presence of children in household, and month of the interview. We then tested the relationships between respondents' demographic characteristics and their responses to the questions and statements about antibiotics using chi-square tests for independence. We used the results of the bivariate analyses to develop two multivariable logistic regression models: 1) a model assessing the effects of respondents' awareness of antibiotic dangers on their attitudes toward and expectations of antibiotics; and 2) a model assessing the influence of respondents' attitudes on their recent antibiotic use.

Because of the complexity of the analyses, we used only second-degree product terms to assess interaction effects. Results of the logistic regression models are reported as odds ratios (ORs) with 95% confidence intervals (CIs). The level of significance is p=0.05.

Results

The sample consisted of 12,755 respondents: 7,254 females and 5,501 males. Of these 12,755, a total of 1,975 were <18 years old or of an unknown age and thus were excluded from the analysis (Table 1). Of the remaining 10,780 respondents, 12% reported taking antibiotics within the 4 weeks before the interview (Table 2). Those who took antibiotics within the prior 4 weeks were more likely to be female (13.9% overall, 65% of all who took antibiotics), have medical insurance (12.6%, p<0.01), and live in rural or farm areas (12.9% and 17.6%, respectively, p=0.02). In addition, antibiotic use varied by age group, with the highest use among persons 25–39 years old (13.2%) and those >60 (13.7%) (Figure). We found no significant differences in antibiotic use among groups defined by race, education level, income, state, month of interview, and having children in the household. Of those who took antibiotics (n=1,253), 91% reported using an antibiotic prescribed for a current infection, while 9% reported using an old prescription or someone else's. No demographic variable was significantly associated with whether respondents used antibiotics obtained to treat their own current illness.

Of the 10,780 respondents, 27% believed taking antibiotics when they had a cold prevented more serious illness (survey item 2, Table 2), 32% believed taking antibiotics when they had a cold made them recover more quickly (survey item 3), and 48% expected a prescription for antibiotics when they were ill enough from a cold to seek

Table 1. Demographic characteristics of participants in FoodNet population survey, 1998–1999

Demographic characteristic	N=12,755	%[a]
Sex		
Male	5,501	49.0
Female	7,254	51.0
Age (y)		
<18	1,817	25.4
18–24	1,005	8.9
25–39	3,239	23.5
40–59	4,105	26.1
60 +	2,431	15.4
Unknown	158	0.8
Race		
White	10,278	75.0
Black	1,152	11.2
Hispanic	675	7.6
Asian	339	3.6
American Indian	99	0.9
Other Race	80	0.9
Unknown	132	0.9
Education		
<High school or less	1,792	19.3
High school graduate	3,169	24.7
Some college	3,528	26.4
College graduate	2,556	18.3
Postgraduate	1,595	10.6
Unknown	115	0.8
Income		
≤$15,000	1,536	10.6
>$15,000 but ≤$30,000	2,097	15.5
>$30,000 but ≤$60,000	3,444	26.1
>$60,000 but ≤$100,000	1,969	16.2
>$100,000	947	7.8
Unknown	2,762	23.8
Residence		
City/urban	4,374	34.2
Suburban	4,338	33.3
Town/village	1,807	13.4
Rural (not farm)	1,672	14.4
Farm	493	4.3
Unknown	71	0.5
Insurance		
With medical insurance	10,561	79.6
Without medical insurance	990	8.3
Unknown	1,204	12.2

[a]Percentages are based on weighted population data.

Table 2. Responses of 10,780 persons to survey items, FoodNet population survey, 1998–1999

Survey item	Yes/agree	No/disagree	Unsure	% yes
1. In the past 4 weeks, have you (has he/she) taken any antibiotic medicine?	1,255	9,485	N/A	12.0
2. When I have a cold, I should take antibiotics to prevent getting a more serious illness.	2,544	7,638	538	27.4
3. When I get a cold, antibiotics help me to get better more quickly.	3,053	6,758	896	32.2
4. By the time I am sick enough to talk to or visit a doctor because of a cold, I usually expect a prescription for antibiotics.	4,812	4,954	911	47.6
5. Are you aware of any health dangers to yourself or other people associated with taking antibiotics?	4,860	5,749	164	41.9

[a]Values are numbers of persons who answered the questions or statements. Percentages are based on weighted population data.

medical attention (survey item 4). Respondents agreeing with any one of these statements were significantly more likely ($p<0.01$) to be male, younger (18–24 years), nonwhite, not college educated, and earning <$30,000 per year (Figure). We also found significant differences by place of residence, with respondents living in rural or farm areas being more likely to agree with the statements. Respondents with children were more likely to agree with survey item 2 (28% vs. 26%), item 3 (34% vs. 31%), and item 4 (50% vs. 46%): all differences had p values <0.01. Responses varied among states ($p<0.01$), with residents of Maryland and Georgia consistently having higher levels of agreement than residents of the other study areas. (For item 2: 27% and 38% vs. 22%–26% [other states] item 3: 35% and 41% vs. 26%–31% [other states], and item 4: 50% and 56% vs. 40%–48% [other states]). Agreeing with the statement, "By the time I am sick enough to see a doctor because of a cold, I usually expect a prescription for antibiotics," did not vary significantly by month of interview or health insurance status. However, not having insurance was significantly associated with agreement to the statements, "When I get a cold, antibiotics help me to get better more quickly" (42% vs. 27%, $p<0.01$), and "When I have a cold, I should take antibiotics to prevent getting a more serous illness" (40% vs. 25%, $p<0.01$). Being interviewed from September through January was also associated with agreeing with these statements ($p<0.05$ and $p<0.02$, respectively).

Fifty-eight percent of respondents were not aware of health dangers associated with taking antibiotics (Table 2). Persons not aware of dangers associated with antibiotic use were significantly ($p<0.01$) more likely to be male and younger and to live in rural or farm areas. They were also significantly more likely to have less education, lower income, and no insurance (Figure). We found no association between awareness of the dangers of antibiotic use

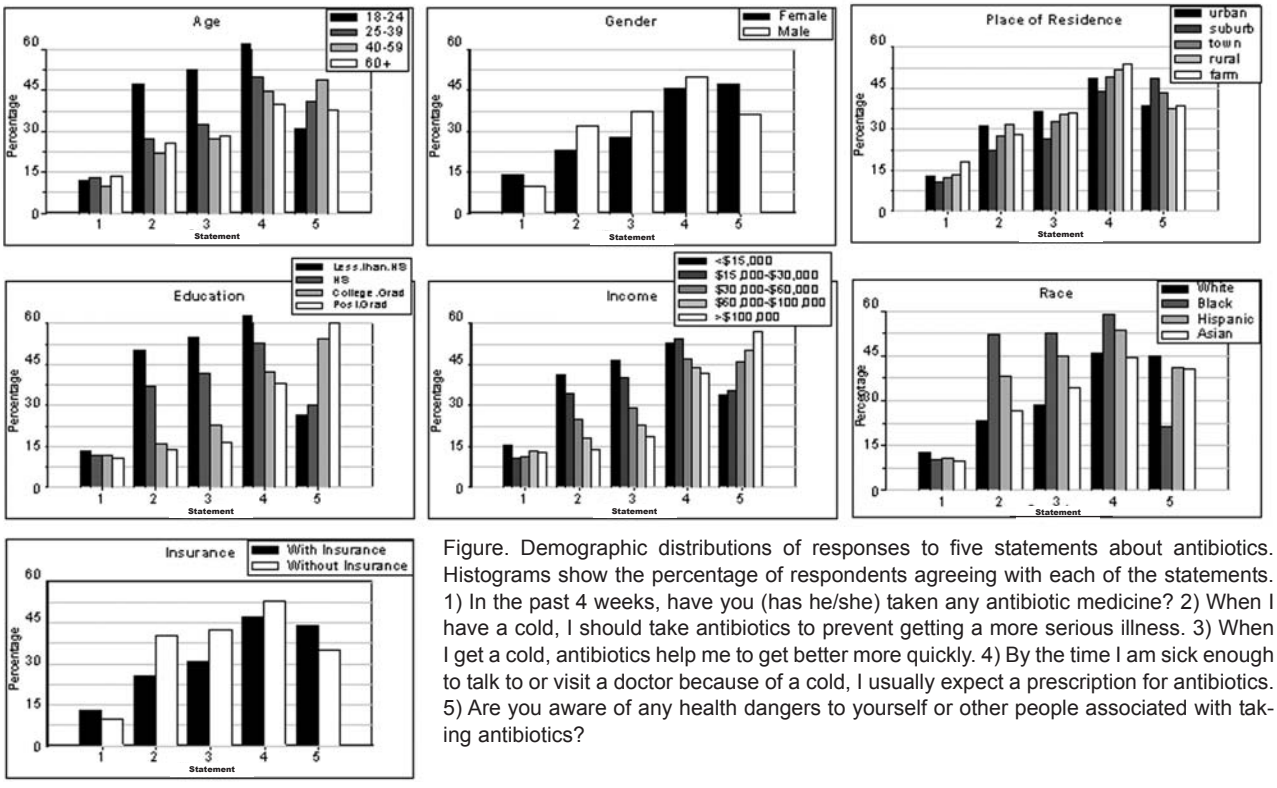

Figure. Demographic distributions of responses to five statements about antibiotics. Histograms show the percentage of respondents agreeing with each of the statements. 1) In the past 4 weeks, have you (has he/she) taken any antibiotic medicine? 2) When I have a cold, I should take antibiotics to prevent getting a more serious illness. 3) When I get a cold, antibiotics help me to get better more quickly. 4) By the time I am sick enough to talk to or visit a doctor because of a cold, I usually expect a prescription for antibiotics. 5) Are you aware of any health dangers to yourself or other people associated with taking antibiotics?

and the month of the interview or having children in the household. Of those aware of health dangers, 58% mentioned factors related to the emergence of drug resistance as a consequence of antibiotic use, 27% mentioned allergies/reactions, 9% recognized that antibiotics kill "good" microbes, and 5% agreed that "it is generally unhealthy to take antibiotics."

Multivariable Analysis

Associations between Attitude Statements and Awareness of Dangers

We constructed three independent models to assess the relationship between participants' knowledge of the dangers of antibiotics (and demographic characteristics) and each of the three different attitude statements as the outcome. Each of these relationships was significant in the univariate and multivariable analyses (Table 3).

Participants not aware of adverse effects of antibiotic use were 2.5 times more likely to agree with the statement, "When I have a cold, I should take antibiotics to prevent getting a more serious illness" (95% CI 2.14 to 2.92). In addition, the demographic variables of age, sex, race, income level, education level, and state were all significant predictors of agreement. We also found significant interactions between the awareness variable and race and education, as well as interactions between age and gender.

We also found a significant association between participants agreeing with the statement, "When I have a cold, antibiotics help me to get better more quickly," and their being aware of health dangers associated with indiscriminate use of antibiotics (OR 2.29, 95% CI 1.99 to 2.65). Those agreeing with this statement were more likely to be older (40–59 years old: OR 2.20, 95% CI 1.32 to 3.66; and >60 years old: OR 2.08, 95% CI 1.22 to 3.25).

Participants not aware of dangers were 1.96 times more likely to agree with the statement, "By the time I am sick enough to talk to or visit a doctor because of a cold, I usually expect a prescription for antibiotics" (95% CI 1.72 to 2.23). The other demographic variables in the model significantly associated with participants' responses to this statement were age, sex, income level, education level, insurance, state, and place of residence.

Association between Antibiotic Use and Attitude Statements and Awareness of Dangers

Using another multivariable model, we examined the association between respondents' taking antibiotics in the prior 4 weeks and their attitudes toward and knowledge of the adverse effects of antibiotic use (Table 4). The overall model was adjusted for participants' sex, age, education, race, household income, state, place of residence, child in the house, and insurance. After adjusting for these demographic variables, we found that only one attitude statement remained a predictor of recent antibiotic use. Participants agreeing with the statement, "When I have a cold, antibiotics help me to get better more quickly," were 1.50 times more likely to have recently taken an antibiotic.

Paradoxically, participants aware of dangers related to antibiotic use were 1.37 times more likely to have taken antibiotics in the previous 4 weeks (95% CI 1.11 to 1.69) even though awareness of these dangers was not a univariate predictor of antibiotic use (OR 0.99, 95% CI 0.49 to 1.98). Of note, only one attitude statement was significant in predicting antibiotic use, suggesting that all of the statements are measuring similar things (Table 4).

Discussion

The results of this FoodNet survey showed that 12% of adult respondents had used antibiotics during the prior month, most (91%) of which were prescribed for a current infection. Extrapolating from the survey data, we estimate that every adult in the United States in 1998 used antibiotics an average of 1.4 times and that approximately 1 in 10 adults who used antibiotics did so without seeing a physician.

The results also suggest that peoples' knowledge and attitudes regarding antibiotic use can be substantially improved and that improved knowledge may be important for efforts to reduce the misconceptions and misguided expectations contributing to inappropriate antibiotic use. Overall, 53% of respondents to this population-based survey reported at least one misconception that may put them at unnecessary risk for infection with resistant bacterial pathogens, and 58% were not aware of the health dangers associated with antibiotic use. Nearly half (48%) of the

Table 3: Effect of knowledge on attitude statements, FoodNet population survey, 1998–1999[a]

Independent models	Adjusted OR[b,c]	95% CI[d] Upper	95% CI[d] Lower
1. Agree that antibiotics prevent serious illness	2.50[d]	2.14	2.92
2. Agree that antibiotics help me get better more quickly	2.29[d]	1.99	2.65
3. Expect a prescription for antibiotics	1.96[d]	1.72	2.23

[a]We constructed three independent models with the three attitude statements as the dependent variables and knowledge of the dangers of antibiotics and selected demographic characteristics as independent variables.
[b]OR, odds ratio; CI, confidence interval.
[c]Adjusted for sex, age, education, race, household income, state, place of residence, and insurance.
[d]Values are significant ($p<0.01$) after adjusting for multiple comparisons.

Table 4: Effect of attitude and awareness on antibiotic use, FoodNet population survey, 1998–1999 [a]

Variable	Adjusted OR[b,c]	95% CI	
		Upper	Lower
Agree that antibiotics prevent serious illness	0.78	0.57	1.06
Agree that antibiotics help me get better more quickly	1.50[d]	1.13	1.99
Expect a prescription for antibiotics	0.96	0.77	1.20
Aware of antibiotic dangers	1.37[d]	1.11	1.69

[a]We constructed a multivariable model to look at the association between respondents taking antibiotics in the previous 4 weeks and their attitudes toward and knowledge about the adverse effects of antibiotic use.
[b]OR, odds ratio; CI, confidence interval.
[c]Adjusted for sex, age, education, race, household income, state, place of residence, child in household, and insurance.
[d]Values are significant ($p<0.01$) after adjusting for multiple comparisons.

respondents indicated that they expected an antibiotic when they visit a doctor.

This survey identified persons in demographic groups who had both higher levels of misconceptions and lower levels of knowledge about the potential adverse impact of antibiotics. These groups included persons of lower socioeconomic status, lower educational status, males, those in younger age groups, and the elderly. Efforts to reach these groups must be a part of any educational efforts to change patient expectations and to reduce the corresponding pressure on providers to prescribe antibiotics inappropriately.

The results of this study did not show a consistent direct link between misguided expectations and higher levels of recent antibiotic use. In part, this lack may have been due to the design of the survey, which focused on collecting frequency data and did not aim to define the reasons for antibiotic use. In addition, in our analysis, we found that the three attitude statements were similar measures of a person's opinions on antibiotic use. The statements have the same demographic predictors and association with the knowledge variable and, in reality, they appear to measure the same thing (Table 3).

We did not find an association between recent antibiotic use and lower knowledge levels. Before the analysis, we assumed that persons lacking knowledge about the dangers associated with antibiotic use would be more likely to take antibiotics. However, we found that study participants aware of these health dangers were actually more likely to have taken antibiotics in the prior 4 weeks. Persons of higher socioeconomic status (higher education and income) have better access to health care and are more likely to use antibiotics in general; we did find that people who took an antibiotic recently were more likely to have medical insurance. Another possible explanation is that those who recently took antibiotics may have learned about the adverse effects of antibiotic use from their physician or pharmacist or from their personal experience with antibiotic side effects. Future epidemiologic studies of antibiotic use in diverse populations should be designed to collect information on why participants use antibiotics to distinguish between appropriate and inappropriate antibiotic use.

This type of study has several other important limitations. A telephone survey creates the possibility of selection bias because it may not reflect the population being surveyed (32). In addition, the survey catchment population did not include persons who refused to participate, did not have a telephone, did not speak English, or could not respond because of physical or mental impairment. However, the weighting process adjusted for age- and sex-based differences in rates.

Another limitation is the cross-sectional nature of this study. Each participant was assessed only once, and the study was not designed to detect recent changes in opinion. Furthermore, the indicators used measured self-reported behavior not actual behavior. We did not attempt to validate responses on the basis of actual observation, and the survey did not determine whether the antibiotic use was appropriate.

Additionally, respondents may have misunderstood the statements about colds and antibiotics. For example, if they had previous experience with what they thought was a cold, and a physician diagnosed a bacterial ear infection, they may have responded that antibiotics help them get better more quickly when they have a cold (17). In addition, several studies have shown that patients often do not have accurate knowledge of antibiotics (15,34). Hong et al., for example, found that patients often could not identify whether a medication was an antibiotic or not and that many patients considered "antibiotics" to be any prescription medication (34).

This study focused only on antibiotic use among adults. Antibiotic use is, however, highest among children, as is the potential for its misuse. In fact, we found that respondents with children in the household were more likely to agree with the attitude statements, demonstrating that it is often parents who influence their children's perceptions of antibiotic use.

The results of this analysis demonstrate that population-based surveys can contribute to efforts to monitor and reduce inappropriate antibiotic use. The magnitude of recent antibiotic use among adults, as well as widespread lack of awareness about and inappropriate attitudes toward such use indicate that continued population-based surveys

could be useful in efforts to monitor trends in antibiotic use. Furthermore, such surveys have the potential to effectively monitor antibiotic knowledge, attitudes, and practices among demographic subgroups of concern. Knowing the magnitude of the problem and the groups who misuse antibiotics most frequently will help public health officials develop and fund intervention efforts, including public information campaigns.

However, our findings also point out some important issues that need to be addressed if this surveillance tool is to be used to full effect. First, additional population-based studies are needed not only to measure antibiotic use but also to determine the reasons that people use them. Such studies should explore the motivations, expectations, and incentives that lead persons to use or not use antibiotics. Second, future studies should include more clearly defined measures of patients' knowledge. Better measures of knowledge may involve asking respondents to differentiate between antibiotics and other types of prescription medicine and to identify types of infections requiring antibiotics. A more thorough evaluation of respondents' attitudes may also be useful. To this end, focus groups may help develop questions that better monitor the general population's attitudes toward antibiotics. Finally, longitudinal tracking of these types of studies will provide important information for the assessment of public health programs.

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Early Identification of Common-Source Foodborne Virus Outbreaks in Europe

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The importance of foodborne viral infections is increasingly recognized. Food handlers can transmit infection during preparation or serving; fruit and vegetables may be contaminated by fecally contaminated water used for growing or washing. And the globalization of the food industry mean that a contaminated food item may not be limited to national distribution. International outbreaks do occur, but little data are available about the incidence of such events and the food items associated with the highest risks. We developed a combined research and surveillance program for enteric viruses involving 12 laboratories in 9 European countries. This project aims to gain insight into the epidemiology of enteric viruses in Europe and the role of food in transmission by harmonizing (i.e., assessing the comparability of data through studies of molecular detection techniques) and enhancing epidemiologic surveillance. We describe the setup and preliminary results of our system, which uses a Web-accessible central database to track viruses and provides the foundation for an early warning system of foodborne and other common-source outbreaks.

Food-related illness is common worldwide, and bacterial pathogens have historically been associated with this mode of transmission. In recent years, however, the cause of most outbreaks of foodborne illness remained unknown, although a significant proportion were presumed to be viral (1). Additional research established the importance of viruses, especially the human caliciviruses belonging to the genus *Norovirus* (NV) (2). Transmission of these viruses is primarily from person to person, but numerous examples illustrate that NV are efficiently transmitted in food, water, or contaminated environmental surfaces. NV similar to, but not identical with, human strains have been found in cattle and in pigs (3,4). Studies in which viruses were molecularly characterized have shown that numerous variants co-circulate in the community but that occasionally shifts occur in which a single variant dominates over a wide geographic region (5). In 1995 to 1996, a worldwide epidemic was observed (6). The mechanism of emergence of these variants is unclear, but one hypothesis is that they represent widespread common-source events.

While it is clear that enteric viral infections are common, far less established is how common the foodborne mode of transmission is and how important it is in the epidemiology of these viruses. The challenge lies not so much in detecting outbreaks related to foodborne contamination at the end of the chain (the food handler in the nursing home or restaurant), because those are likely to be detected by routine outbreak investigation, with or without molecular typing. Linking NV outbreaks to common-source introductions nationally or internationally may be more difficult because of the high secondary attack rate that results from rapid person-to-person transmission. Thus, an initial seeding event will rapidly be masked by the occurrence of new cases or outbreaks, suggesting that person-to-person transmission is the primary mode of spread. The likelihood of detecting such seeding events relies on effective surveillance, which combines epidemi-

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ologic assessment of the outbreak and molecular typing to discover and track potential links between outbreaks. Such molecular tracing, however, requires knowledge on diversity of "resident viruses" in the region under study to be able to recognize unusual increases. Therefore, we established a combined research and surveillance network for foodborne viruses that was granted by the European Commission. This project group combines complementary expertise from the fields of diagnostic virology, molecular virology, epidemiology, and food microbiology to study modes of transmission of NV across Europe. Mapping these pathways allows better founded estimates of the proportion of illness that may be attributed to foodborne transmission and identification of high-risk foods, processing methods, or import and transport routes, which subsequently can be a focus of prevention programs. The data are important for assessing the risks associated with consumption of certain food items. Essential to the success of this project is the establishment of a common, central database, which is now used by all partners to compare data across Europe as soon as they are available. We describe this project and results from its first 18 months of operation.

Materials and Methods

The network is a collaboration among 12 laboratories in 9 countries in Europe to allow more rapid and internationally harmonized assessment of the spread of foodborne viral pathogens. The project is coordinated by the National Institute of Public Health and the Environment in Bilthoven, the Netherlands. Participants are virologists and epidemiologists with active research programs in (foodborne) enteric viruses from Spain (Barcelona, Valencia, Madrid), Italy (Rome), France (Nantes, Dijon), Germany (Berlin), the Netherlands (Bilthoven), the United Kingdom (London), Denmark (Copenhagen), Sweden (Solna), and Finland (Helsinki). In addition, groups from Slovenia and Hungary participate.

The overall objectives for the complete study are as follows: 1) to develop novel, standardized, rapid methods for detection and typing of enteric viruses, particularly NV, to be used in all participating laboratories; 2) to establish the framework for a rapid, prepublication exchange of epidemiologic, virologic, and molecular diagnostic data; 3) to study the importance of enteric viruses as causes of illness across Europe, with a special focus on multinational outbreaks of infection with NV and hepatitis A virus; 4) to provide better estimates for the proportion of NV infections that can be attributed to foodborne infection; 5) to determine high-risk foods and transmission routes of foodborne viral infections in the different countries and between countries; 6) to describe the pattern of diversity of NV within and between countries and identify potential pandemic strains at the onset; and 7) to investigate the mechanisms of emergence of these strains, including the possibility of spillover from animal reservoirs

The central research goal is to better understand the mechanisms of emergence of variant NV strains. We hypothesized that the observed epidemic shifts might be caused by displacement of endemic variants attributable to a large seeding event with a variant that subsequently spread through the population by secondary and tertiary waves of transmission, or possibly by a smaller seeding event of a highly transmissible new variant, generated by genetic mutation or recombination. To address these questions, we built a European surveillance structure for outbreaks of viral gastroenteritis, including food- or waterborne outbreaks. The first phase of the project, described in this report, was designed to review existing surveillance systems for viral gastroenteritis, to design and agree on a minimum dataset for collection during the second phase of the project; to review and evaluate currently used methods for detection and genotyping of NV with the aim of harmonizing methods for virus detection in gastroenteritis outbreaks; and to build a database of combined epidemiologic and virologic data for use by all participants. The overriding aim was to facilitate the early detection of potentially emerging variant strains. Upon completion of this phase, we will begin " enhanced surveillance", i.e., harmonized surveillance for viral gastroenteritis outbreaks to study objectives 4–7.

Results

Review of Current Methods in Europe

From the outset, it was recognized that the best approach in developing an international surveillance scheme for foodborne viruses would not be the standardization of practice, but rather the harmonization of existing practices. To achieve this, a number of surveys were undertaken to determine diagnostic capabilities, genotyping techniques, and the status of surveillance of viral gastroenteritis outbreaks among project participants. The results of these surveys are highlighted below.

Virus Detection and Genotyping

The scale of diagnostic capability in laboratories varies widely, and a range of diagnostic tests (electron microscopy, reverse transcription–polymerase chain reaction [RT-PCR], and enzyme-linked immunosorbent assay) and characterization methods are used (including heteroduplex mobility assay, reverse line blot, microplate hybridization, and sequencing (7–9). Laboratories in all countries now use molecular techniques (RT-PCR) for NV detection (10).

A comparative evaluation of RT-PCR assays was done by analysis of a coded panel of stool samples that had test-

ed positive (81 samples) or negative (9 samples) for NV. Samples provided by four laboratories were included, as well as a samples representing the currently known diversity of NV genotypes. Full details of this study have been published (11). This evaluation showed that no single assay is best, although sensitivities range from 55% to 100%. Most differences were seen when comparing assay sensitivities by genogroup. Based on pre-set scoring criteria (sensitivity, specificity, assay format, length of sequence), one primer combination was ranked as the assay of first choice for laboratories starting surveillance, and protocols and reagents have been made available to all participants on request.

On the basis of the aggregated data from the sequence database, alignments were made of the regions in the viral RNA that contain the primer-binding sites for the set of primers with the highest ranking for the diagnostic evaluation to generate more optimal designs of primers (12). These primers, protocols, and reference reagents have been made available to several groups in the field.

Outbreak Investigations

While all countries in the network now have the diagnostic capability to recognize outbreaks of NV, the structure of their national surveillance differs and therefore, so do the epidemiologic data collected on viral gastroenteritis (10,13). Some countries investigate outbreaks of gastroenteritis irrespective of the size or possible mode of transmission (United Kingdom, the Netherlands); others primarily investigate outbreaks that appear as foodborne from the onset (Denmark, France) (10). Similarly, coverage of the laboratories involved ranges from regional (Italy) to national, although different levels of underreporting are likely to exist (10) These differences, as well as differences in the laboratory test protocols, will be taken into consideration when interpreting aggregated data in the later stages of the project. For the purposes of comparing data across Europe, however, the key finding was that most countries maintain a national database of NV outbreaks (as opposed to individual cases). Although the proportion of the population that these databases effectively survey and the completeness of clinical information collected vary, we recognized that we could network national outbreak surveillance by agreeing on a minimum dataset. That dataset would include the causative organism, mode and place of transmission, diagnostic results, case details, food vehicles, and viral typing information.

Also agreed upon were clinical definitions of a case and an outbreak of viral gastroenteritis based on Kaplan's criteria (14), as follows. A case of gastroenteritis was defined as a person seen with 1) vomiting (two or more episodes of vomiting in a 12-hour period lasting ≥12 hours), or 2) diarrhea (two or more loose stools in a 12-hour period lasting ≥12 hours, or 3) vomiting as defined in 1) and diarrhea as defined in 2). An outbreak was defined as follows: 1) Patients living in more than one private residence or resident or working in an institution at the time of exposure; 2) cases linked by time and place; 3) vomiting in ≥50% of total cases (14); 4) mean or median duration of illness of total cases from 12 to 60 hours; 5) incubation period (if available) of total cases between 15 and 77 hours, usually 24–48 hours (14,15); and 6) testing of stool specimens for bacterial pathogens. (This step is not mandatory;, however, if tested, all specimens should be negative for bacterial pathogens.)

Development of Database

A major goal of the first year was to build a database into which historic information present in the participating institutes would be collected. The rationale behind this was that by combining this existing information, new observations (on seasonality of outbreaks or patterns of emergence of new variants, for example) might be possible. Without harmonization of data collection, the comparative analysis would clearly be limited. The historic database, however, also served as a pilot phase because the definitive format of the database is used in the enhanced surveillance program. Participants who had historic collections of sequences were asked to submit these, along with additional epidemiologic information, as described in Table 1. Data were entered by using the Bionumerics (BN) package (Applied Maths, Ghent, Belgium), which allows storage, comparative analysis, and clustering of combined epidemiologic and biological experimental data (e.g., sequences, reverse line blot results, enzyme immunoassay data). The entries were either uploaded from the public domain or submitted as unpublished sequences from participating laboratories. Publicly available sequences were included to provide a customized report for database searches, e.g., genotype to which the sequence belongs.

Since September 2001, participants have been able to access the database directly through a password-controlled Internet connection. At present, the database contains >2,500 entries, mostly on NV, but including some hepatitis A virus, astrovirus, and Sapovirus (Tables 2 and 3). Upcoming variants will first be subjected to a search of the historic database to determine if the viruses have been seen before in Europe. An automated search tool is available and has been made accessible through the Internet to participants. Partners interested in analyzing the data can obtain the complete dataset, provided they adhere to the confidentiality agreements signed by every partner. Interested parties outside the project group can access the database under certain conditions through the coordinator or one of the participants. This access is not restricted to groups in the participating countries. The limiting factor is

Table 1. Requested data fields for entries of viral sequences in the historic database[a]

Field name	Field type	Mandatory field
Country	A	Yes
Institute	A	Yes
Reference no.	A	Yes
Type of virus (NV, SV, HAV, ASV, HEV	N	Yes
Sequence identifier (e.g., GenBank accession no.)	A	No
Method no.	N	No
Genotype/subtype	N	No
Sequence pol region /VP1/2a	A	Yes
Sequence capsid region	A	
Source of isolate (human, calf, swine, environmental, other)	N	Yes
Specimen	N	No
Epidemiology	N	No
Vehicle (food, water, person-to-person, zoonotic, not known)	N	No
National region	A	No
Date of receipt in reference laboratory	D	Yes
Age	N	No
Sex	N	No
Travel associated	N	No
Travel destination	A	No

[a]NV, Norovirus; SV, Sapovirus; ASV, astrovirus; HAV, hepatitis A virus; HEV, hepatitis E virus; A, letters and numbers; B, numbers; D, data.

the target region used for virus characterization, which has not been standardized globally. A database search will be performed upon request (for groups outside the network). Results are then communicated to them and to the person who submitted any matching sequences. After that initial linking, follow-up discussions and investigations of possible common-source events can be done by the groups involved.

Prospective Enhanced Surveillance

Comparative Evaluation of Diagnostic/Genotyping Methods

The different PCR primers used among the European group all target a highly conserved region within the viral polymerase gene. Sequences of the amplicons from the various diagnostic PCRs overlap and therefore, can be compared to gain inferences on the molecular epidemiology and the spread of NV variants (11). Rapid characterization techniques, notably the reverse line blot (9) and heteroduplex mobility assay (7), are also used within the network; the typing data generated by these techniques can also be accommodated by the database.

Comparative Evaluation of Data

After agreement on a minimum epidemiologic and virologic dataset, we made a standard Web-based questionnaire available to all participants behind a password-protected site (available from: URL: www.eufoodbornevirus-es.net). Using Web-based Active Server Pages (ASP) technology, investigators have full access to the secure outbreak database (Figure). Investigators are asked to enter information that is available as soon as an investigation begins or an event occurs that meets the outbreak definition. A unique reference number is assigned to each outbreak, which is the key used to access records and to update diagnostic or typing data, as an investigation continues.

The database also collects information on the level of evidence (i.e., microbiologic, epidemiologic, circumstantial) implicating food or water as a mode of transmission. Pop-up windows are used to define these criteria, since a range of public health scientists use the system. Other features of the ASP technology, including drop-down menus,

Table 2. Number of entries in the database by year of detection for selected viruses, as determined by reverse line blot typing (RLB)[a]

	N	HAV	SLV	ASV	NLV	NLV capsid	NLV pol	NLV cap and pol	RLB	Animal
Not dated	364	69	4	7	284	152	119	9	1	2
1989 and before	102	68	2	-	32	15	23	7		
1990	43	31	1	-	11	7	10	6		
1991	5	-	-	-	5	2	5	2		
1992	11	-	2	-	9	3	7	3		
1993	21	-	2	-	19	14	26	11		
1994	45	-	1	-	44	20	38	14		
1995	49	-	-	2	47	15	41	9		
1996	122	-	1	4	117	18	100	2		
1997	122	-	-	1	121	8	117	5	3	6
1998	158	-	2	6	152	19	130	11	6	13
1999	531	44	2	26	485	39	366	32	129	7
2000	446	40	2	-	404	61	380	41	26	6
2001	419	5	2	2	410	52	396	52	-	-
2002	155	-	-	-	745	22	742	15	-	

[a]HAV, hepatitis A virus; SLV, Sapporo-like virus; ASV, astrovirus; NLV, Norwalk-like viruses.

Table 3. Number of entries in the database by country of submission[a]

Country	No. of entries	HAV	ASV	NLV	SLV
Argentina	9			10	
Australia	17			17	
Canada	20			20	
Switzerland	23			23	
Czech Republic	1	1			
Germany	167	1		432	
Hungary	93			93	
Denmark	67			67	
Spain	33	18		102	
Finland	90			130	
France	216		24	203	7
Great Britain	509	1		566	3
Ghana	2			2	
Hong Kong	7			7	
Japan	363			388	3
Korea	1			1	
Mexico	1			1	
Netherlands	743	87	24	707	
Norway	13			13	
New Zealand	5			5	
Russia	8			8	
South Africa	9			5	4
Sweden	101			100	1
Turkey	1	1			
United States	67	6		59	2

[a]HAV, hepatitis A virus; ASV, astrovirus; NLV, Norwalk-like viruses; SLV, Sapporo-like virus.

are used to standardize the data collected. Descriptive information from outbreaks (number of people exposed, number of people ill, number of controls infected, symptoms) is collected when possible, to allow comparisons of the clinical characteristics of different NV genotypes. Preliminary data suggest that such differences exist.

One of our main scientific objectives is to explain the mechanism behind the emergence of new variant strains. Essential for the early detection of such emerging variants is a rapid reporting network. The initial suspicion of "something strange" may be from clinicians who investigate outbreaks (e.g., a sudden increase in the number of reports), or from one of the laboratories (e.g., finding the same variant in several outbreaks). The central database is used to facilitate both types of reports. The real power in this format of data exchange is that immediately after entry or update of information, the data are in the database and can be accessed by other collaborators. The database can be searched for common virologic (sequence) or epidemiologic (e.g., a food vehicle) characteristics that would trigger further investigation of links between outbreaks.

Recognition of International Outbreaks

This model has proved successful in recognizing a number of internationally linked events. Clusters of cases in Denmark, Finland, and the Netherlands were all linked to oysters imported from France. Another foodborne outbreak traced in part through the network followed the concluding dinner of an international conference in Finland. Symptoms began the day after the conference, when many attendees had returned to their home countries. Approximately 40 persons were affected, and the same NV variant was detected from cases in Finland, Sweden, and the Netherlands. A dessert item was implicated by cohort study. Importations of hepatitis A from Peru into Spain and from Ibiza, Spain, to Germany have also been recognized through the network. Full details of these outbreaks will be published elsewhere.

Discussion

Microbial food safety is considered an important public heath issue but historically has focused on control of bacterial contamination. Several recent publications, however, show that outbreaks of foodborne infection attributable to viruses are common and may in fact be an important public health concern for several reasons: most clinical laboratories involved in outbreak investigations do not have access to routine diagnostic methods for detecting NV, user-friendly methods for use in these laboratories are only now becoming available and need to be validated, foodborne transmission of NV is quite common, and food microbial quality control largely relies on indicators for the presence of fecal bacteria, which may not correlate with the presence of enteric viruses (2,16). Although foodborne viruses are increasingly studied, no validated methods yet exist for reliably detecting them in food items. In all, these facts indicate that through foodborne transmission an

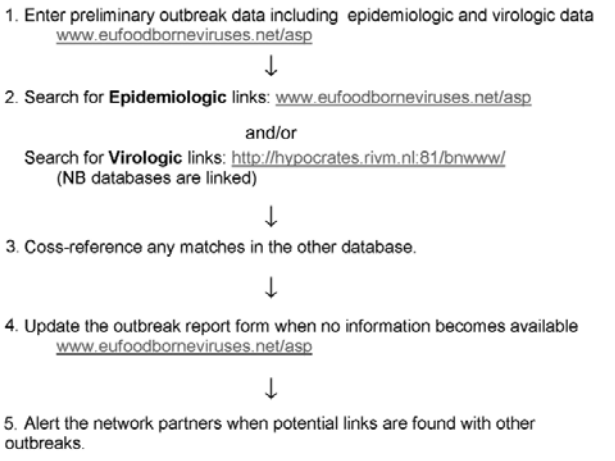

Figure. Timeline of Web-based reporting of epidemiologic and virologic data and interrogation of the database for the Foodborne Viruses in Europe Group.

enteric viral pathogen (NV) can escape detection, possibly resulting in large epidemics.

In the United States, molecular detection techniques are being implemented in state public health laboratories under the guidance of the Centers for Disease Control and Prevention (CDC), which is building an infrastructure for reporting of outbreaks of food-related illness attributable to enteric viruses (Calicinet). In Europe, no central institute yet exists with the authority to do this, so the best efforts to date are the voluntary disease-specific surveillance networks, such as Enternet (which monitors trends in foodborne bacterial pathogens), and the European Influenza Surveillance Scheme (designed to monitor influenza virus activity across Europe) (17,18). We have built such a surveillance network for enteric viruses, using NV as a target organism. NV was an obvious choice: an increasing number of publications illustrate that it is one of the most important causes of outbreaks of gastroenteritis, including food- and water-related outbreaks (reviewed in 2). CDC estimates that up to 66% of all food-related illness in the United States may be due to NV (19). From a community-based case-control study in the Netherlands, risk-factor analysis for NV, based on information collected throughout a 1-year cohort study, suggested an association between NV and a complex score that was used as a proxy for food-handling hygiene. On the basis of this approach, an estimated 12% to 15% of community-acquired illness may be due to food- or waterborne modes of transmission (with 85% attributed to contact with a symptomatic person in or outside the household) (de Wit et al., unpub. data). The proportion of foodborne outbreaks reported in the countries participating in our network ranges from 7% to 100%, but that range merely reflects the differences in the selections used in the different surveillance systems and cannot be used to estimate the true impact of foodborne illness caused by NV (10). While definitive data still need to be collected, the consensus is that NV is an important cause of food-related infection and disease.

Foodborne transmission of viral gastroenteritis has not historically been acknowledged as a public health priority, which means that our surveillance system is inevitably restricted to groups that already have an active program in the field. Ideally, we would like to build a network of national institutes represented by both epidemiologists and microbiologists involved in outbreaks of viral gastroenteritis; however, at present this ideal is not possible for all of Europe. By networking the existing information, assessing comparability of data through studies of primers and protocols used, and examining data from current surveillance, we hope to be able to paint a bigger picture from the fragmented information that is available.

The standardized outbreak questionnaire, accessible through the Internet, is designed to collect a minimum dataset about all outbreaks. However, participants who perform more detailed epidemiologic or virologic investigations can also submit additional data. The minimum dataset will suffice to answer the basic questions for the surveillance, i.e., what is the reported incidence of NV outbreaks across Europe, and which proportion is considered to be due to food- or waterborne transmission.

A key feature of any disease surveillance system is its use as an early-warning tool, in this case for international common-source outbreaks. To facilitate this, several features were included in our database setup. Information on outbreaks can be updated with new information as it comes in, to avoid piling up information until the outbreak investigation has been completed (which may be months later). Both the epidemiologic data and laboratory data (mostly sequences) can be searched easily. Thus, participants can be alerted to similarities in disease profiles (e.g., outbreaks with imported fruits) or in sequences. Either signal can lead to contacts between participants to discuss possible indications for a joint investigation. Crucial in this discussion was the issue of confidentiality, both for patient and product information, and for data from investigations. The present modus operandi is that each participant signs a confidentiality agreement, which states that data submitted to the database are owned by the person submitting them (subject to each participant's national regulations on patient and laboratory data); specific patient and product information is not entered into the database. If necessary for outbreak investigations, the groups involved will decide on a case-by-case basis what information may or may not be used by the consortium. Participants can obtain the complete information from the database for their own analysis, or choose to use it as a search tool and rely on the analysis done by a scientist employed on this aspect of the database, who is stationed in Bilthoven, the Netherlands. So far, five international outbreaks have been detected because of the network.

The food distribution chain in Europe is complex, and therefore the transmission of viruses across borders can occur by means of contaminated food. The surveillance network described here allows early detection of international common-source outbreaks of foodborne viruses. Most of the work to date has involved harmonization of methods for investigating outbreaks and detecting the viruses causing these outbreaks, as well as the development of a database system that facilitates the exchange of information between laboratories and institutes involved in viral gastroenteritis research and surveillance. The system's strength is that it combines basic epidemiologic and laboratory data into a searchable repository. This network has demonstrated its potential to recognize transnational outbreaks. However, the network is inherently limited by the quality of data available at the national level, which is

a reflection of the priority given to foodborne viruses. At present, we are undertaking a 2-year enhanced surveillance project to study the frequency and modes of transmission of viral gastroenteritis outbreaks across Europe.

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Skunk and Raccoon Rabies in the Eastern United States: Temporal and Spatial Analysis

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Since 1981, an epizootic of raccoon rabies has spread throughout the eastern United States. A concomitant increase in reported rabies cases in skunks has raised concerns that an independent maintenance cycle of rabies virus in skunks could become established, affecting current strategies of wildlife rabies control programs. Rabies surveillance data from 1981 through 2000 obtained from the health departments of 11 eastern states were used to analyze temporal and spatial characteristics of rabies epizootics in each species. Spatial analysis indicated that epizootics in raccoons and skunks moved in a similar direction from 1990 to 2000. Temporal regression analysis showed that the number of rabid raccoons predicted the number of rabid skunks through time, with a 1-month lag. In areas where the raccoon rabies virus variant is enzootic, spatiotemporal analysis does not provide evidence that this rabies virus variant is currently cycling independently among skunks.

In North America, variants of rabies virus are maintained in the wild by several terrestrial carnivore species, including raccoons, skunks, and a number of bat species. Each antigenically and genetically distinct variant of the virus in mammalian species occurs in geographically discrete areas and is strongly associated with its reservoir species (1). Within each area, a spillover of rabies into other species occurs, especially during epizootics (2). As a result of spillover, a variant may eventually adapt to a secondary species, which may begin to serve as an alternative reservoir species. This phenomenon of spillover and cross-species adaptation has been inferred from historical relationships (2) but is poorly understood and not routinely investigated.

In the late 1970s, an epizootic of raccoon rabies was reported on the Virginia/West Virginia border attributed to the translocation of raccoons from the southeastern United States (3). This epizootic has spread northward and southward throughout the eastern United States (Figure 1a,b). The establishment of rabies in this species has raised public health concerns about an increased risk for rabies transmission to the human population because the raccoons are well adapted to living at unusually high densities in urban and suburban environments (4,5). As a novel potential control method, several states have initiated raccoon vaccination programs using an oral rabies vaccine (6–9).

Beginning in 1990, a concomitant increase in the number of cases of skunks infected with the raccoon rabies virus variant has occurred in these states (Figure 1c,d). Additionally, these cases appeared to be preceded by cases in raccoons, both temporally and spatially. Moreover, in a growing number of counties in Massachusetts and Rhode Island, the number of rabid skunks has surpassed the number of rabid raccoons. Whether the increasing number of cases in skunks is a result of spillover from raccoons or the raccoon rabies virus variant has begun to circulate inde-

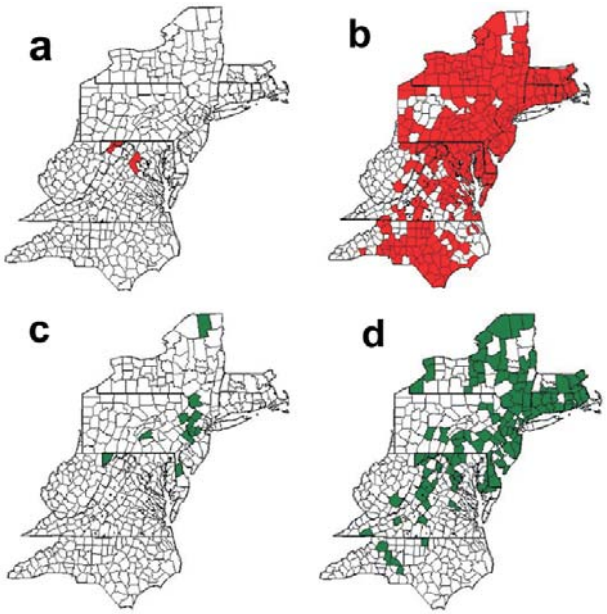

Figure 1. Counties with at least one rabies epizootic among raccoons, 1981(a) through 2000 (b); and among skunks, 1990 (c) through 2000 (d), in the mid-Atlantic states, 1981–2000.

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pendently within the skunk population remains unclear. The establishment of an independent cycle of rabies in the skunk population may have serious consequences for rabies vaccine baiting programs because the current oral vaccine for raccoons is not as effective in skunks (10).

The epizootiology of raccoon rabies in the eastern United States has been investigated in several states, including Virginia (11,12), Connecticut (13), and Maryland (14,15). Models have been developed to describe the spatial and temporal patterns of raccoon rabies epizootics (16–18). Several studies have also described the behavior of skunk rabies epizootics in western North America (19–21), Texas (22), and Canada (23). The existing raccoon and skunk rabies studies show that epizootic patterns appear to differ between skunks and raccoons, possibly because of differences between the species, rabies virus variants, or environmental factors. However, no documented studies exist on the relatively recent increase of rabies in skunks caused by the raccoon rabies virus variant in the eastern United States. In light of the recent efforts to implement rabies control programs for raccoons in the eastern United States, the epizootiology of raccoon rabies virus variant occurring in skunks in this part of the country needs to be better understood.

The objectives of this study were to describe the epizootiology of skunk rabies in the eastern United States, determine if skunk and raccoon rabies epizootics are associated spatially and temporally, and introduce methods to assess evidence of spillover of rabies from raccoons to skunks compared with independent cycling of the virus within the skunk population.

Materials and Methods

Surveillance Data

Rabies case data for raccoons and skunks for each county by month from 11 states (Connecticut, Delaware, Maryland, Massachusetts, New Jersey, New York, North Carolina, Pennsylvania, Rhode Island, Virginia, and West Virginia) were used for analysis. Only counts of rabid animals per county were used because not all counties reported total numbers of animals submitted for testing. The observation period for each county started when the first case of raccoon or skunk rabies was reported, with a maximum study interval of 20 years (1981–2000) and a minimum of 11 years (1990–2000). The lengthy study interval reduced the variability of reporting within a county that may be observed when an epizootic arrives. The unit of analysis was the number of laboratory-confirmed rabies cases in raccoons and skunks reported per month by county. To identify counties that had an appreciable number of skunks infected with rabies, analysis was restricted to counties that reported at least 12 rabid skunks within 12 months of first detecting rabies in skunks. This average of one rabid skunk per month corresponded to the 90th percentile of all counties reporting at least one rabid skunk, and 36 counties met this criterion. Upon examination, one county was excluded because of geographic isolation in the western part of Maryland (Garrett County) that would have severely biased the spatial analysis, and three counties in New York (Clinton, Franklin, and Oswego) were excluded because rabies found in skunks was the result of spillover from a red fox epizootic emerging from Canada (24).

Descriptive Analysis

Rabies epizootics among skunks and raccoons in the 32 counties used for our analyses were identified by using the following algorithm: an epizootic began when the monthly number of rabid animals reported was greater than the county's monthly median for two consecutive months and ended when this number was less than or equal to the county median for two consecutive months (16). Additionally, an epizootic had to be at least 5 months in duration. In calculating a county's monthly median number of rabid animals, months occurring before the appearance of the first rabid animal were excluded. For example, if rabid skunks first appeared in a county on June 1, 1994, then the months before were excluded for calculation of the skunk median. In that same county, if rabid raccoons appeared on December 1, 1993, then the months before were excluded from calculation of the raccoon median. The size and length of epizootics were compared between species by a Wilcoxon rank sum test. The Kolmogorov-Smirnov two-sample (KS) test was used to assess seasonal differences in the number of rabies cases by species.

Temporal Analysis

A series of Poisson regression models were used to further explore the relationship between the number of rabid skunks and rabid raccoons. The outcome variable was defined as the log number of rabid skunks. The predictor variables were the number of rabid raccoons, time (continuous, 1–140 months), and calendar month of report. The time variables started at 1 with the appearance of the first rabid animal (skunk or raccoon) and continued for up to a total of 140 months (maximum number of months of observation). All counties had at least 72 months of follow-up, and 50% had more than 107 months of follow-up. To smooth each time series, a moving average of the number of rabid animals was calculated on the basis of the present and previous month's observations for both species and used for subsequent analyses. A time-squared term, an interaction term of time by number of rabid raccoons and indicator variables for county and calendar month of report, was included in the model. The effect of repeated

measures by county was controlled in the analysis by using a generalized estimating equation (25). Lag periods of 0 to 5 months for the number of rabid raccoons were introduced and assessed to identify any improved fit in the Poisson regression model, as determined by comparing the log likelihood values. The model with the highest (less negative) log likelihood value was chosen as the best fitting model.

The full Poisson regression model can be represented as

$$\text{Log (\# of skunks)} = \beta_0 + \beta_1(\text{\# of raccoons}_{t-i}) + \beta_2(t) + \beta_3(t*t) + \beta_4(t*\text{\# of raccoons}_{t-i}) + \beta_j(\text{county}_j) + \beta_k(\text{month}_k) + E$$

where
t = time in months (starts at 1 with first appearance of rabid skunk or rabid raccoon in each county and ends with a maximum value of 140)
i = 0–5 lag time in months
j = 31 indicator variables representing the 32 counties used in the analysis
k = 11 indicator variables representing months, with December being the reference group
E = residual error.

Spatial Analysis

To determine if skunk and raccoon epizootics were associated spatially from 1990 through 2000, the mean center of the counties reporting a rabies case was determined for successive years, 1990–2000, for each species (Crimestat, Department of Justice). The standard deviational ellipse was also calculated, showing the dispersion in two dimensions (Crimestat) of the mean centers. The distance between mean centers of successive years by species was calculated by the Pythagorean theorem. The direction between mean centers was calculated by converting latitudinal and longitudinal coordinates into the Universal Transverse Mercator projections of eastings and northings. On dividing the difference in eastings by the difference in northings, the arctangent was calculated (26), yielding the angle (degrees) between the mean centers. The angle was then converted to degrees from the reference angle of 0° (true north). The resulting series of vectors (Figure 2) was used to determine if the mean centers by year for each species were moving in a similar direction. The Watson-Williams test (27) was applied to test for a difference in the angle of rotation between the mean centers of each species from 1990 to 2000. An F test was used to determine if the epizootic direction of spread differed between the species. The cumulative mean direction (rotational angle that summarizes a series of vectors through successive years) and circular variance of the mean centers were also calculated (Crimestat).

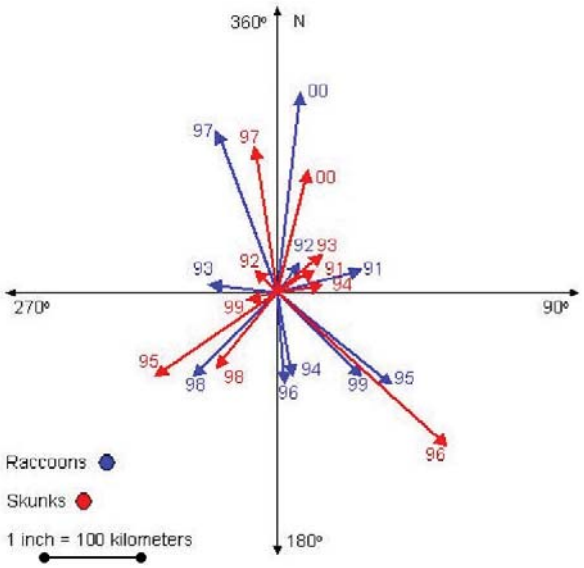

Figure 2. Magnitude and direction (vectors) of successive mean centers of counties from 11 mid-Atlantic states reporting rabies from 1990 to 2000 for raccoons and skunks.

Results

Descriptive Analysis

Of 495 total counties, 344 (69.5%) reported at least one rabid skunk, and 421 (85.1%) reported at least one rabid raccoon (Table 1). The median number of reported rabid raccoons was greater than that for skunks ($p<0.0001$). Three hundred thirty-nine counties (68.5%) reported rabies in both skunks and raccoons. Within these counties, rabid raccoons preceded rabid skunks in 297 counties (87.6%), rabid skunks preceded rabid raccoons in 30 counties (8.8%), and rabid skunks and raccoons were first reported in the same month in 12 counties (3.5%). The median interval between the initial appearance of rabid raccoons and skunks was 14 months and ranged from –108 months (i.e., rabid skunks preceding rabid raccoons) to 177 months.

Of 344 counties with at least one reported rabid skunk, 36 counties had at least 12 rabid skunks appearing in the first 12 months after the first rabid skunk appeared. Four counties were omitted for reasons described in the Methods section. In these 32 counties used for more detailed analysis, rabid raccoons preceded skunks in 30 (93.8%) counties, rabid skunks preceded raccoons in 1 (3.1%) county, and both skunks and raccoons appeared in the same month in one county (3.1%). The median interval between the appearance of rabies in raccoons and skunks was 5 months, and ranged from –2 months to 13 months. In the four omitted counties, rabid raccoons preceded skunks in two counties, and rabid skunks preceded raccoons in the remaining two counties.

Table 1. Characteristics of counties within 11 mid-Atlantic states reporting skunk and raccoon rabies, 1990–2000

	Median no. of rabid animals per county (min, max)		No. of counties with at least one rabid animal (% of total counties in state)	
State	Skunks	Raccoons	Skunks	Raccoons
Connecticut	77 (12,209)	380 (225,918)	8 (100)	8 (100)
Delaware	52 (30,97)	269 (168,372)	3 (100)	3 (100)
Massachusetts	80 (14,160)	170 (36,387)	11 (78.6)	11 (78.6)
Maryland	13 (2,82)	226 (105,1075)	23 (95.8)	24 (100)
North Carolina	5 (1,55)	25 (1,166)	41 (41)	86 (100)
New Jersey	31 (4,78)	131 (4,350)	21 (100)	21 (100)
New York	20 (3,202)	127 (2,1294)	55 (88.7)	58 (93.5)
Pennsylvania	11 (1,72)	37 (2,343)	64 (95.5)	66 (98.5)
Rhode Island	56 (9,123)	67 (23,173)	5 (100)	5 (100)
Virginia	7 (1,87)	20 (1,1100)	92 (67.6)	109 (80.1)
West Virginia	8 (1,42)	17 (1,101)	21 (38.2)	30 (54.5)
Total or median	13 (1,209)	42 (1,1294)	344 (69.5)	421 (85.1)

For all 32 counties, the peak number of rabid raccoons reported was reached by 21 months; the median interval from the first case to the peak number of cases was 10.5 months. In contrast, the interval from the first to the peak number of skunks ranged from 6 to 90 months, with a median interval of 16.5 months ($p<0.001$). The calendar month when the peak was reached did not exhibit a pattern for rabid raccoons, whereas for rabid skunks there was a strong tendency for the peak to be reached in the last quarter of the year.

Analysis of epizootic characteristics found differences between the first epizootics of each species (Table 2). The first raccoon epizootic was significantly larger (median=126; range 9–494, $p<0.0001$) than subsequent epizootics among raccoons and also significantly greater than the first skunk epizootic (median=16; range 4–85, $p<0.0001$). However, after the first epizootic, the epizootics converged and characteristics did not differ, with the exception of the third epizootic, in which the duration and magnitude were lower for skunks than for raccoons. In general, the size of subsequent epizootics among raccoons showed damped oscillations, while skunk epizootics appeared uniform.

Temporal Analysis

Overall, a significant relationship existed between the number of rabid raccoons (RACCOON) and the number of rabid skunks (SKUNK) (Figure 3, Table 3). Specifically, a significant interaction existed between time and RACCOON on SKUNK with the effect of RACCOON on SKUNK increasing with increasing time. The fit of the models improved significantly when a 1- or 2-month lag for RACCOON was used to predict SKUNK; however, the lag of 1 month provided the best fit. The time-squared term was not significant and was dropped from subsequent models. A month by RACCOON interaction was also tested and did not significantly improve the model fit. At the beginning of the time series, the peak in SKUNK coincided with the larger peak in RACCOON. In the period of approximately 25 months to approximately 50 months, the sharp reduction in RACCOON and coincident reduction in SKUNK was well below model predictions. A second peak in SKUNK at approximately 55 months and 70 months coincided with the increase in RACCOON associated with a second epizootic among raccoons. The model predictions reflected this increase, but the predicted SKUNK fell below the actual SKUNK for the peak months. In addition to a positive correlation with RACCOON over time, SKUNK displayed a strong seasonal component with annual peaks occurring in the fall months (Figure 4). These strong seasonal peaks were unique to skunks, and not present in raccoons ($KS=10.6$; $p<0.0001$).

Among the 32 counties used for Poisson regression analysis, four counties in Massachusetts exhibited a gener-

Table 2. Characteristics of raccoon and skunk epizootics in 32 counties with at least 12 rabid skunks during the first 12 months after the appearance of the first rabid skunk

	Epizootic 1		Epizootic 2		Epizootic 3		Epizootic 4		Epizootic 5	
Characteristic	Raccoon	Skunk	Raccoon	Skunk	Raccoon	Skunk	Raccoon	Skunk	Raccoon	Skunk
No. of counties with epizootics	32	31	22	19	10	12	2	6	0	2
Duration of epizootics-median no. of mon. (min,max)	18.5 (6,26)	8 (5,24)[a]	8.5 (5,23)	8 (5,10)	8 (6,12)	6 (5,10)[b]	11.5 (11,12)	8 (5,13)	—	7.5 (5,10)
Size of epizootics-median no. of animals (min,max)	126 (9,494)	16 (4,85)[a]	19 (5,138)	18 (5,39)	19 (9,43)	13 (4,32)[b]	53 (28,78)	18 (6,37)	—	13 (12,14)

[a] $p<0.0001$.
[b] $0.01<p<0.05$.

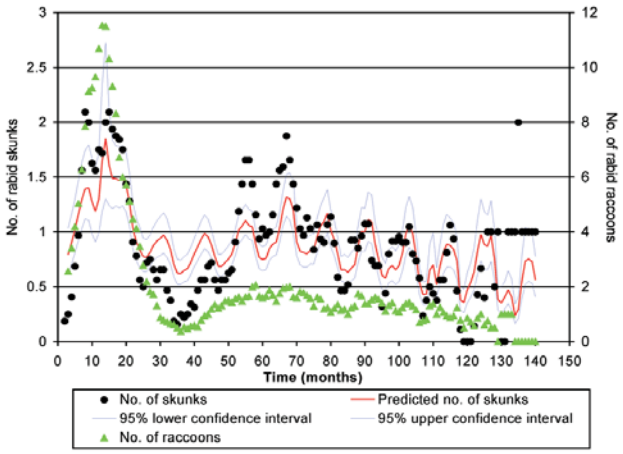

Figure 3. Fitted line resulting from Poisson regression analysis of 32 counties with at least 12 rabid skunks in first 12 months. logY= 0.2835 + 0.0262(RACCOON$_{t-1}$) – 0.0021(time) +0.0020 (RACCOON$_{t-1}$*time) + β_i(countyi) + β_j(monthj)

al increase in the number of skunks over time (Essex, Middlesex, Norfolk, and Plymouth). A separate model was fit to determine potential differences between these counties and the 28 other counties that did not exhibit a significant increase in rabid skunks over time. The modeling results did not differ among these counties compared with those for the other 28 counties.

Spatial Analysis

As determined by previously described methods that used vectors (Figure 2), the mean centers of the counties first reporting rabies in both species were in Maryland in 1990 (Figure 5a), and Virginia/West Virginia in 2000 (Figure 5b). The mean direction and distance traveled of the skunk and raccoon epizootics were similar. The mean centers of the epizootics from 1990 to 2000 moved an average of 339.3 km for skunks and 368.2 km for raccoons in a southwesterly direction. Application of the Watson-Williams test resulted in no significant difference between the angles of rotation of successive epizootics [$F_{1,18}$= 0.11($F_{1,18;0.95}$<4.41, n.s.)], indicating that the mean centers of the skunk and raccoon epizootics were moving in a similar direction. The cumulative mean directions of the epizootics from 1990 to 2000 were 42.06° ± 0.23 for skunks, and 47.76° ± 0.28 for raccoons.

Discussion

This study examined the relationship between the occurrence of rabies in skunks and raccoons in the eastern United States. The present analysis indicated epizootic cycles of 4–5 years for raccoons and skunks, consistent with previous studies of rabies in raccoons (16,17) in this region. In comparison, studies of the epidemiology of skunk-variant rabies among skunks from the Midwest reported epizootic cycles with periods ranging from 4 to 5 years (21) to 6 to 8 years (20,28). If raccoon-variant rabies virus becomes established in eastern skunk populations, the periods of epizootic cycles in skunks may subsequently decouple from those of raccoons so that independent cycles among skunks may be observable. However, differences in the periodicity of cycles between these species may be caused by many factors, including differences between the variants, resulting in changes in incubation period, transmission potential, and duration of disease.

The spatial analysis performed in this study indicated that skunk rabies epizootics in the eastern United States are closely coupled to epizootics in raccoons. These epizootics moved in similar directions and traveled similar distances as they progressed upwards along the eastern seaboard. The mean centers of epizootics in each species originated near Maryland and are now situated near the Virginia/West Virginia border as of 2000 (Figure 5a,b). The southwesterly movement is of concern as the raccoon epizootic encroaches areas in the Midwest, where the skunk virus variant predominates.

The Poisson regression analysis showed a statistically significant association between the number of rabid skunks and raccoons through time. The association was weakest during the first months, apparently due to the large number of rabid raccoons that are characteristic of initial rabies epizootics in raccoons (16). After the initial peak in numbers at approximately 15 months, both species exhibited a secondary peak at 60 months, consistent with the 4–5 year cycle (16,17) for raccoon epizootics in the eastern United States. After 80 months, the number of peaks in both species diminished in size and increased in frequen-

Table 3. Summary of Poisson regression analysis of number of rabid raccoons and skunks in 11 mid-Atlantic states

Parameter	Estimate	Standard error	p value
Intercept	0.2835	0.1433	0.0479
Raccoon[a]	0.0262	0.0087	0.0025
Time[b]	-0.0021	0.0023	0.3748
Raccoon* time	0.002	0.0004	<0.0001
County	[c]	-	-
Month	[d]	-	-

[a]Raccoon = no. of rabid raccoons lagged 1 month.
[b]Time = time in months (starting at 1 with appearance of first rabid raccoon or skunk in each county to a maximum value of 140).
[c]Parameter estimates for each county (Worcester, MA, referent group): –0.6529, Berkshire, MA; 0.3297, Bristol, MA; –1.0655, Burlington, NJ; –0.9563, Caroline, MD; –1.1583, Columbia, NY; –1.1351, Dorchester, MD; –0.6038, Dutchess, NY; 0.4234, Essex, MA; –0.2474, Fairfield, CT; –1.0079, Gloucester, NJ; –0.2611, Hartford, CT; –0.3519, Kent, DE; –0.5922, Litchfield, CT; 0.2036, Middlesex, MA; –0.8831, Middlesex, NJ; –1.2675, Monmouth, NJ; –0.5668, Monroe, PA; 0.0172, New Haven, CT; 0.3402, Newport, RI; 0.3887, Norfolk, MA; –0.4269, Orange, NY; –0.0079, Plymouth, MA; 0.3419, Providence, RI; –1.1506, Putnam, RI; –1.0282, Somerset, NJ; –0.3285, Sussex, NJ; –0.3688, Ulster, NY; –1.2583, Union, NJ; –0.3556, Washington, RI; 0.0306, Westchester, NY; –0.6928, Wicomico, MD.
[d]Parameter estimates for each month (December referent group): –0.4074, January; –0.7792, February; –0.6313, March; –0.4830, April; –0.6289, May; –0.8763, June; –0.6548, July; –0.0262, August; 0.2592, September; 0.2851, October; 0.2555, November.

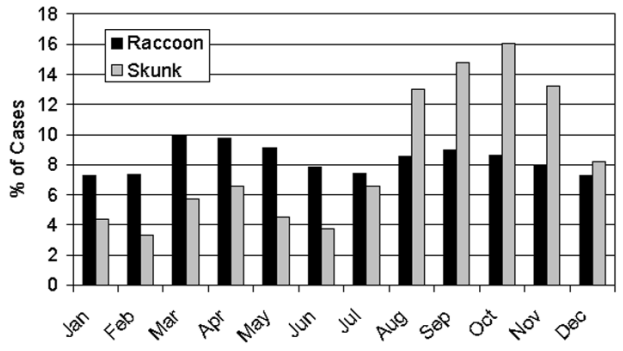

Figure 4. Proportion of rabies cases by month for each species, 1990–2000.

cy, with the rabies cases in skunks maintaining a strong seasonal component.

The comparison of epizootic characteristics by species also found that the size and duration of epizootics in both species converged after the first epizootic. Of note, however, were the four counties in which rabies cases in skunks were outnumbering those in raccoons near the end of our study period. In these counties, rabies cases in skunks became less sporadic: cases were regularly reported throughout the year but the annual peaks in the fall months remained. As surveillance continues for these four counties, current observations suggest that skunks may be acting as important secondary hosts of the raccoon rabies virus variant in certain geographic areas of the eastern United States and that the potential for independent cycles to emerge exists.

Although we varied the time variable between raccoon and skunk rabies from 0 to 5 months, the best fitting regression model resulted from using a 1-month lag time. This lag time is consistent with the generally accepted incubation period for rabies of 3–8 weeks (29), which would permit at least one cycle of virus multiplication among raccoons before transmission from raccoons to skunks. The regression model also showed that reports of rabid raccoons remain fairly constant by month throughout the year. In contrast, the number of rabid skunks showed an independent seasonal pattern that consistently peaked during the fall months (Figure 4). In the Midwest, where rabies is endemic in skunks, the major peak is in late winter and early spring, with a smaller peak in the fall (20,28). The peaks in winter and late spring have been attributable to the breeding season, and the fall peak to dispersal of juveniles (23). Why a dominant fall peak is apparent in the eastern states is not clear at this time. However, during dispersal, skunks may have increased contact with more raccoons, thereby increasing the risk for transmission of rabies. The absence of a spring peak may indicate little to no transmission between skunks in communal winter dens and during the breeding season.

Skunks and raccoons coexist within the same geographic areas in different ecologic niches. Raccoons are social animals that are capable of existing in fairly high densities in close proximity to human habitation and prefer forested habitats (4,5,15). Skunks are rather solitary animals and are found in lower densities than raccoons (23). Skunks prefer grasslands (21), agricultural areas (30), and interfaces between agricultural and nonagricultural lands (22). These characteristics would suggest that contact between the two species should occur less frequently than among those of the same species. However, since rabies affects the central nervous system, rabid animals may exhibit aberrant behaviors, leading to increased contact between the species and cross-species transmission of the virus.

Monitoring rabies among skunks in regions where the raccoon rabies virus variant circulates has important implications for public health intervention programs. To control the spread of the raccoon rabies epizootic, an oral rabies vaccine-baiting program has been implemented in several states (7–9,31) after the successful development of a vaccinia virus recombinant vaccine expressing the rabies virus glycoprotein gene (V-RG) for raccoons (6,32). The oral vaccine has been effective in raccoons. However, as formulated for raccoons, it has not been proven to be as effective for preventing rabies infection in skunks (10). Administration of intramuscular rabies vaccines has been

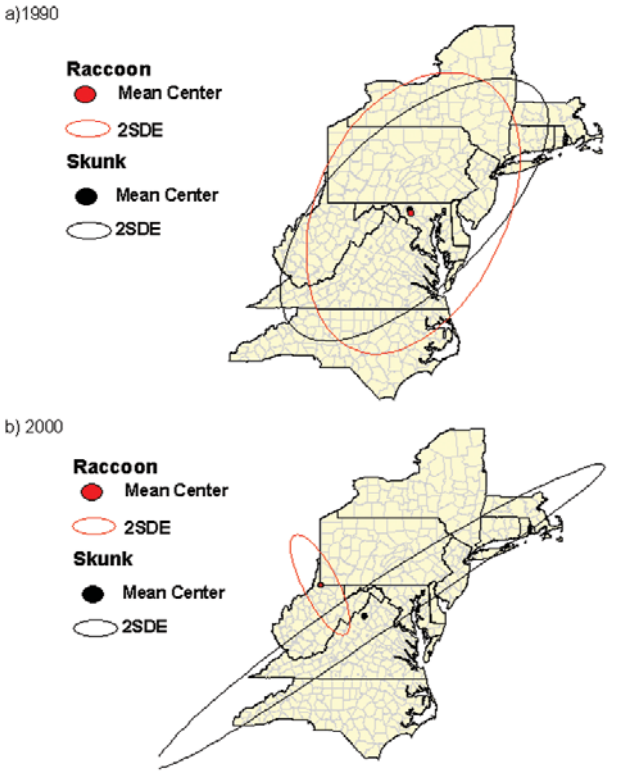

Figure 5. The mean centers and standard deviational ellipses (SDE) of counties reporting rabies in skunks and raccoons in the mid-Atlantic States.

shown to be effective in controlling rabies in skunks (5), but this method is labor-intensive and cost-prohibitive. The emergence of independent maintenance or cycling of raccoon-associated rabies virus within skunks would necessitate the development of alternative strategies to control rabies within wildlife populations. At least one vaccine candidate (33) designed for skunks has been identified but will require further development for this species and prevent spillover of rabies back into the raccoon population.

Currently, we have no evidence that the raccoon rabies virus variant is cycling independently in the skunk population of the eastern United States or that the variant has undergone any genetic adaptations among skunks. However, epizootic rabies in skunks was first reported in 1990 and, with expected epizootics cycling every 4–5 to 6–8 years, it may be too soon to detect decoupling of rabies cycles in skunks and raccoons. Surveillance and monitoring must continue through several cycles to further evaluate additional epizootics for changes in patterns. Additionally, scant information exists on the population densities and behavior patterns of skunks and raccoons in the eastern United States. Field investigations to assess the incidence of rabies in wildlife populations have rarely been conducted. Further research is needed to evaluate environmental factors that can affect the population density and structure, the behavior of both raccoons and skunks, and factors influencing interactions between them. Finally, the genetics of the raccoon rabies virus variant should be monitored for changes that might indicate cross-species adaptation after spillover into skunks. Assessment of these changes and continued surveillance can provide important guidelines to ensure the success of oral rabies vaccination programs for the control of rabies in wildlife and to decrease the risk of acquiring rabies among the human and domestic animal populations.

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Tick-borne Relapsing Fever Caused by *Borrelia hermsii*, Montana

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Five persons contracted tick-borne relapsing fever after staying in a cabin in western Montana. *Borrelia hermsii* was isolated from the blood of two patients, and *Ornithodoros hermsi* ticks were collected from the cabin, the first demonstration of this bacterium and tick in Montana. Relapsing fever should be considered when patients who reside or have vacationed in western Montana exhibit a recurring febrile illness.

Tick-borne relapsing fever, caused by *Borrelia hermsii*, is endemic in the higher elevations and coniferous forests of the western United States and southern British Columbia, Canada (1). Although many multicase outbreaks of relapsing fever associated with *B. hermsii* and its tick vector, *Ornithodoros hermsi*, have been reported (2–6), none has been documented in Montana. Patients usually become ill after they have slept in cabins infested with spirochete-infected ticks that feed quickly during the night. The illness has an incubation period of 4 to ≥18 days and is characterized by recurring episodes of fever accompanied by a variety of other manifestations, including headache, myalgia, arthralgia, chills, vomiting, and abdominal pain (1). Relapsing fever is confirmed by the microscopic detection of spirochetes in the patient's blood (Figure 1) (7).

In 1927, relapsing fever was diagnosed in a a 33-year-old man in Walla Walla, Washington, although his possible site of exposure was Montana (8). A specific location was not given, however, and spirochetes causing the illness were not identified. *Ornithodoros parkeri*, another tick vector of relapsing fever spirochetes in western United States, was collected during 1936 in Beaverhead County in southwestern Montana, and an undisclosed number of these ticks transmitted *Borrelia parkeri* to one mouse in the laboratory (9). If relapsing fever were to occur in Montana, *B. parkeri* transmitted by *O. parkeri* was suspected as being the likely etiologic agent (9,10).

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In summer 2002, a multicase outbreak of relapsing fever associated with a privately owned cabin occurred in western Montana. Spirochetes were isolated from two patients and identified as *B. hermsii*, and this spirochete's tick vector, *O. hermsi*, was collected from the cabin where the patients slept. This is the first multicase outbreak of tick-borne relapsing fever in Montana and the first report of *B. hermsii* and *O. hermsi* in the state, thereby documenting the risk of this infection beyond the geographic range known previously within the United States.

The Study

From July 30 to August 20, 2002, a total of 5 persons in a group of 20 became ill with symptoms consistent with tick-borne relapsing fever during or following their visit to western Montana (Table). The common site of exposure was a cabin on the south shore of Wild Horse Island (47°50'30" N; 114°12'30" W) in southwest Flathead Lake, Lake County, Montana. The 875-hectare island became a state park in 1978, although 56 privately owned properties exist, many of which have cabins. No one lives permanently on the island, and camping overnight (by day visitors to the island) is not allowed. The island is approximately 4.6 km wide from east to west and 3.2 km wide from north to south; its elevation varies from 881 m at the shoreline to its highest point of 1,141 m. The island is separated from the mainland by 2.0 km to the south and 2.4 km to the north. The habitats include Ponderosa Pine and Douglas Fir forests, native grassland, and steep rocky outcroppings. Red squirrels (*Tamiasciurus hudsonicus*) and deer mice (*Peromyscus maniculatus*) are abundant.

On July 22, the first of four related families arrived at the cabin, and on July 25, a 54-year-old man (case 1, Table)

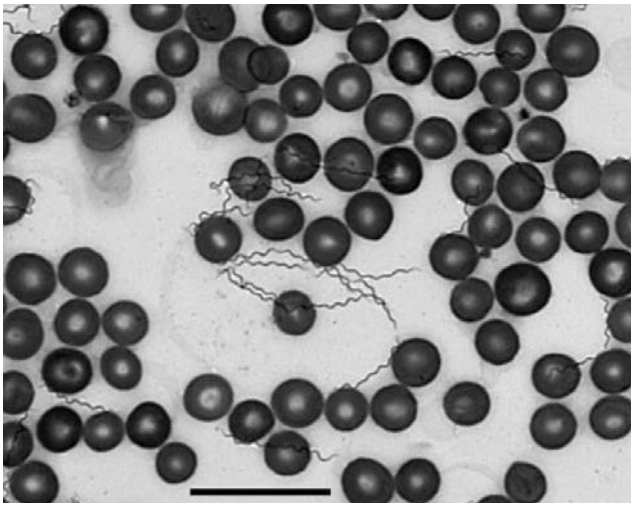

Figure 1. *Borrelia hermsii* in a thin smear of mouse blood stained with Wright-Giemsa stain and visualized with oil immersion bright-field microscopy (X600) for the confirmation of infection with relapsing fever spirochetes in humans and other animals. Scale bar = 20 μm.

Table. Summary of relapsing fever patients exposed in western Montana during July–August, 2002

Case no.	Patient age (y)[a]	Sex	Onset	Signs/Symptoms	Initial blood smear	Final laboratory results
1	54	M	July 30[b]	38.9°C–39.4°C temperature, rash, headache, myalgia, arthralgia	Spirochete negative	Seropositive by IFA[c] and Western blot
2	5	F	August 10	40.6°C temperature., vomiting, diarrhea, headache, myalgia	Spirochete positive	Mouse inoculation positive; isolation of *Borrelia hermsii*
3	43	M	August 16	Fever, headache, myalgia, arthralgia	Spirochete positive	Mouse inoculation positive; isolation of *B. hermsii*
4	43	F	August 30	38.9°C–39.4°C temperature, vomiting, diarrhea, headache	Spirochete negative	Mouse inoculation negative; no isolation
5	13	M	August 11	40.6°C temperataure, vomiting, headache	Spirochete positive	Mouse inoculation and isolation not attempted

[a]Confirmed cases with demonstration of spirochetes in blood; presumptive cases with appropriate manifestations but no spirochetes detected.
[b]This patient relapsed on August 6.
[c]IFA, immunofluorescence assay.

entered the east end of the attic and removed nest material that had accumulated there. He slept at night and napped during the day in one of two bedrooms located immediately under the area of the attic where the nest material had been partially removed. On July 30, he became ill with fever, headache, arthralgia, myalgia, and rash, and 2 days later he visited the emergency room of a local hospital but a diagnosis was not made. Over the next several days he improved, and on August 6, he and his family began driving back to their home in Seattle, Washington. During the trip, he relapsed with another febrile episode. That evening, he was taken to the emergency room of a Seattle hospital and admitted early the next morning. On the basis of his history, a diagnosis of relapsing fever was considered, although spirochetes were not detected in the blood.

Three additional families (17 persons) arrived at the cabin on July 31 and on August 5 and departed on August 8 and 9. One family of five returned to their home in Seattle, and three of them became ill on August 12, 17, and 20 (cases 2–4). Relapsing fever was suspected immediately, and spirochetes were detected in Wright-stained blood smears from two patients (cases 2, 3). On August 10, a family of six returned to St. Louis, Missouri, where a 13-year-old boy (case 5) became ill the next day. On August 12, he was taken to an emergency room and to his pediatrician the following day. His mother communicated with the family in Seattle, where a young girl (case 2) was ill, and spirochetes had been detected in her blood. This discovery led to the detection of spirochetes in a blood smear from the boy. All patients had fever and other clinical manifestations consistent with tick-borne relapsing fever (Table). They were all treated with doxycycline, and all recovered with no subsequent relapses.

Blood smears from three of the Seattle patients (cases 2–4) were prepared and stained separately with monoclonal antibodies H9724, which recognizes all known species of *Borrelia* (11), and H9826, which is specific for *B. hermsii* (12), and rabbit hyperimmune serum to *B. hermsii* (Figure 2A). Indirect immunofluorescence assays (IFA) and microscopic analysis demonstrated spirochetes from two patients (cases 2, 3) that were reactive with all antibodies, which identified these bacteria as *B. hermsii*. Blood from the third patient (case 4) was negative for spirochetes with all antibodies. EDTA-treated whole-blood samples from these patients were injected intraperitoneally into mice, and the two samples positive by microscopic examination also produced detectable levels of spirochetemia in mice. Whole blood obtained from the infected mice was injected into modified Kelly's medium (BSK-H supplemented with 12% rabbit serum; Sigma-Aldrich Corp., St. Louis, MO), and spirochetes that originated from two patients were isolated.

A convalescent-phase serum sample from the first case-patient (case 1) was collected 55 days after the onset of his illness. This sample was examined by IFA with whole cells of *B. hermsii* (13) and by immunoblot with a whole-cell lysate of *B. hermsii* and recombinant GlpQ (13). The patient's IFA titer to *B. hermsii* was positive at 1:1,024, and the sample was positive by immunoblot at 1:100 dilution.

The five persons with confirmed or presumptive relapsing fever slept in two adjacent bedrooms in the east end of the cabin under the attic where animal nest material had been partially removed. People who slept only on the outside porch or in other bedrooms did not become ill. On August 24, 2002, the two east bedrooms were examined for ticks, but none were found. The remaining nest material was collected from the attic and taken to Rocky Mountain Laboratories. During the next several weeks, the material was processed with two small Berlese extraction funnels, which separate live arthropods from nonliving debris. Fourteen *O. hermsi* were recovered, including 1 larva, 10 nymphs, 2 males, and 1 female (Figure 2B). The postlarval stages of *O. hermsi* are very similar to those of *O. sparnus,* which parasitizes woodrats and deer mice in Utah and Arizona, but the latter species is an incompetent vector of *B. hermsii* (14,15). The larva collected from the cabin displayed morphologic characteristics consistent with *O. hermsi*. Voucher specimens (one nymph, one larva) of *O. hermsi* collected at the study site were deposited in the U.S. National Tick Collection, Georgia Southern

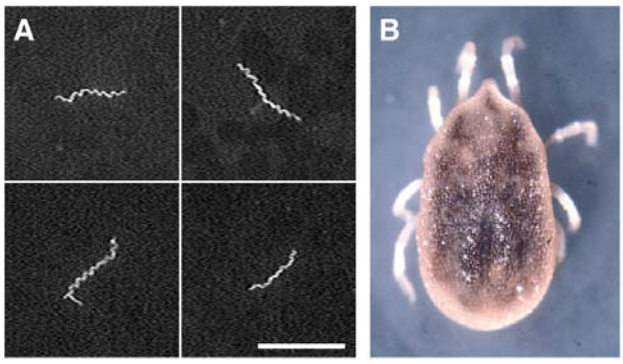

Figure 2. (A) *Borrelia hermsii* in the blood of one patient (case 3) stained with rabbit hyperimmune serum and anti-rabbit fluorescein isothiocyanate. Scale bar = 20 μm. (B) An *Ornithodoros hermsi* nymph collected from the attic of the cabin. The length of the tick is 3.0 mm, excluding the legs.

University, under accession number RML 123385. The 12 remaining ticks were allowed to feed on a laboratory mouse to determine whether they were infectious. The blood of the mouse did not become spirochetemic during the 10 days after tick bite. These ticks were not examined for infection by other methods and were kept alive to establish a laboratory colony.

On June 21, 2003, the attic, utility room, and bedrooms where the infected persons slept were treated with an over-the-counter insecticide-acaricide (Ortho Indoor Insect Fogger, The Ortho Group, Columbus, OH). Sentinel *O. hermsi* ticks (late stage nymphs and adults) from a laboratory colony were confined in open flasks in one treated bedroom (46 m^3) and a family room that was not treated to examine the efficacy of treatment. After the 4-hour application with two 141-gm cans of fogger, all 54 ticks in the treated bedroom were dead, whereas all 52 ticks in the untreated room were alive.

Discussion

Tick-borne relapsing fever caused by *B. hermsii* is acquired only within the geographic range of its specific tick vector, *O. hermsi*. This tick has been found in southern British Columbia, Washington, Idaho, Oregon, California, Nevada, Colorado, and the northern regions of Arizona and New Mexico (2,4,16). As this and other outbreaks demonstrate, patients often become ill after they leave disease-endemic areas where they were bitten by infectious ticks (2,6). One patient (case 1) remained untreated early in his illness in spite of seeking medical attention at a hospital near the site of exposure.

The cabin where the patients were infected has been owned by the same family for nearly 40 years. None of the members of the four related families questioned recalled any prior illnesses consistent with what they experienced with this outbreak of relapsing fever. The event that appears to have instigated this outbreak was the partial removal and disturbance of animal nest material in the east end of the attic. Some ticks presumably fell through the spaces between the ceiling boards to the two bedrooms below. The boy (case 5) slept all but part of one night on the porch, but during the night of August 6 a thunderstorm forced him indoors, and he moved to the front east bedroom. His onset of illness in St. Louis was on the afternoon of August 11, which equates to an incubation period of approximately 4.5 days. The incubation periods for the others were estimated at 5 to 15 days.

The animals that maintained the enzootic cycle with *B. hermsii* and *O. hermsi* in the cabin are unknown. Red squirrels are highly susceptible to infection with *B. hermsii* (17), are important hosts for these ticks (1), and were abundant in the forest surrounding the cabin. However, no evidence of squirrels was found in the cabin. Deer mice were routinely in the cabin, and the owners used poison bait stations to control the indoor population. One dead mouse was found near the cabin, and two carcasses were in the attic material that had been removed on July 25. American robins (*Turdus migratorius*) had nested in the attic, and two dead robin chicks were found in the material collected from the attic on August 24. Recently, a *B. hermsii*–like spirochete was implicated in the death of a northern spotted owl (*Strix occidentalis*) in Kittitas County, Washington (18), and many years ago, 26 *O. hermsi* were collected from the nest of a bluebird (either *Sialia mexicana* or *S. currucoides*) in Summerland, British Columbia (19). The role of birds in perpetuating relapsing fever spirochetes and their tick vectors in nature is worthy of further investigation. A serologic survey of red squirrels and deer mice in the vicinity the cabin for immunologic evidence of exposure to *B. hermsii* might also help explain the enzootic involvement of these rodents.

This outbreak demonstrated for the first time that *B. hermsii* and its tick vector *O. hermsi* exist in Montana and caused multiple cases of relapsing fever. Owners of cabins in the vicinity where the outbreak occurred met with the Montana state epidemiologist and received information regarding the epidemiology and prevention of tick-borne relapsing fever. Although the outbreak was localized, a large area of western Montana has the appropriate ecologic parameters to support enzootic cycles that provide the potential for relapsing fever caused by *B. hermsii* to occur. A diagnosis of relapsing fever should therefore be considered when patients who have resided or vacationed in western Montana seek treatment for a recurrent febrile illness.

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Paecilomyces lilacinus Vaginitis in an Immunocompetent Patient

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Paecilomyces lilacinus, an environmental mold found in soil and vegetation, rarely causes human infection. We report the first case of *P. lilacinus* isolated from a vaginal culture in a patient with vaginitis.

Paecilomyces lilacinus, a saprobic filamentous fungus, found in soil, decaying vegetation, saunas, and laboratories (as an airborne contaminant), is an infrequent cause of human disease (1,2). Most cases of disease caused by the genus *Paecilomyces* occur in patients who have compromised immune systems, indwelling foreign devices, or intraocular lens implants (2,3). Rarely has disease been reported in immunocompetent hosts without any identifiable risk factor.

We describe the first case of *P. lilacinus* isolated from a vaginal culture in a patient with vaginitis and review the published literature addressing *P. lilacinus* infections in immunocompetent patients. Our review demonstrates that the reports of *P. lilacinus* infections in immunocompetent hosts have become more frequent in the last several years. This trend indicates that *P. lilacinus* may be an emerging pathogen.

Case Report

A 48-year-old woman reported vaginal itching and discharge of 5 months' duration. Her symptoms had been recalcitrant to several courses of therapy for a presumptive diagnosis of candidal vaginitis. She had been treated initially with fluconazole, then sequentially with topical clotrimazole, ticoconazole ointment, and intravaginal boric acid gel. Her medical history was notable for mild gastritis (treated with omeprazole) and irregular uterine bleeding, controlled with hormone replacement therapy (a transdermal estrogen/progesterone combination). The patient was in a monogamous relationship with her husband but reported abstinence for several months because of the severity of her vaginal symptoms.

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On physical examination, vaginal erythema with a white liquid vaginal discharge was observed. Although a potassium hydroxide (KOH) preparation was not obtained at baseline, the discharge grew *P. lilacinus* in pure culture.

The patient was treated empirically with itraconazole, 200 mg orally twice a day for 3 weeks. At the end of therapy, she reported complete resolution of her vaginal discharge and a significant decrease in her vaginal pruritus. A repeat vaginal culture was not obtained at her first follow-up appointment after completion of itraconazole therapy because the vaginal vault contained a large amount of blood. At an appointment 6 months later, she remained free of vaginal discharge; a vaginal fungal culture and KOH preparation performed at that time were negative.

The results of laboratory studies, including serum protein electrophoresis (with immunoglobulin [Ig] G, IgA, IgM) C3, C4, erythrocyte sedimentation rate, a complete blood count, CD4 cell count, and CD8 cell count were all within normal limits. Results of a test for antibodies to HIV were negative. An anergy panel (with *Candida* and *Trichophytin* used as controls) was reactive. A purified protein derivative was not placed because the patient had a history of a positive test result.

The patient's isolate was forwarded to the Fungus Testing Laboratory, Department of Pathology, University of Texas Health Science Center at San Antonio, Texas, for confirmation of the identity and antifungal susceptibility testing, and accessioned into the stock collection as UTHSC 01-872. The isolate was initially subcultured onto potato flakes agar (PFA, prepared in house), which was prepared in-house, at 25°C, 30°C, 35°C, and 40°C (ambient air with alternating daylight and darkness). The isolate was subsequently plated onto carnation leaf agar (CLA [prepared in-house]) and malt agar (Remel, Lenexa, KS) at 25°C. Temperature studies were repeated after initial observations.

The case isolate was evaluated for susceptibility to antifungal agents by using the National Committee for Clinical Laboratory Standards broth macrodilution method M38-P (4). Briefly, the case isolate and the *P. variotii* control organism, UTHSC 90-450, were grown on PFA for 7 to 10 days at 25°C to induce conidial formation. The mature PFA isolate and control slants were overlaid with sterile distilled water, and suspensions were made by gently scraping the colonies with the tip of a Pasteur pipette. Heavy hyphal fragments were allowed to settle, and the upper, homogeneous conidial suspensions were removed. Conidia were counted with a hemacytometer, and the inoculum was standardized to 1.0×10^5 CFU/mL. Conidial suspensions were further diluted 1:10 in medium for a final inoculum concentration of 1.0×10^4 CFU/mL. Final drug concentrations were 0.03–16 μg/mL for amphotericin B (Bristol-Myers Squibb, Princeton, NJ), ketoconazole

(Janssen Pharmaceutica, Titusville, NJ) and clotrimazole (Schering-Plough, Kenilworth, NJ), 0.125–64 µg/mL for 5-flucytosine (Roche Laboratories, Nutley, NJ), fluconazole, voriconazole (Pfizer, Inc., New York, NY), and terconazole (Ortho-McNeil Pharmaceuticals, Inc., Raritan, NJ), and 0.015–8 µg/mL for itraconazole (Janssen Pharmaceutica) and posaconazole (Schering-Plough).

Results

Growth of the isolate on PFA produced a buff-colored to slightly lavender, somewhat granular colony after 7 days' incubation at 25°C. Repeat subcultures with extended incubation (up to 2 weeks) yielded colonies which were more definitely mauve-colored, consistent with those typically seen with *P. lilacinus* (Figure 1). The isolate failed to produce sporodochia on carnation leaf agar (CLA, prepared in-house), a feature seen with *Fusarium* species (many of which are lavender) and failed to produce a diffusing yellow pigment on malt agar, a characteristic seen with the closely related *P. marquandii.* The conidiogenous cells from the initial slide culture, held 7 days and prepared from a PFA block, consisted predominately of single, long, tapering phialides, somewhat atypical for the species. Repeat PFA slide cultures from subcultures displaying a more typical macroscopic structure yielded complex fruiting heads with verticillate conidiophores and divergent phialides, typical for *P. lilacinus* (Figure 2). Conidiophore roughness, a feature described for *P. lilacinus*, was not observed, however, even after repeated subculturing and examinations. Smooth-walled, elliptical conidia occurred in long, tangled chains and measured approximately 2.0 X 2.5 µm. No chlamydospores were observed on any of the media examined. Temperature studies performed on two separate occasions (PFA) indicated 4+ growth at 25°C and 30°C, 2+ growth at 35°C, and no growth at 40°C.

Species of *Paecilomyces* known to produce pinkish to purplish colonies include *P. javanicus, P. fimetarius, P. fumosoroseus, P. lilacinus,* and *P. marquandii.* The first three species were excluded from consideration on the basis of the size of the conidia, as well as the lack of synnematal production for *P. fumosoroseus* (5). *P. marquandii* differs from *P. lilacinus* by the production of an intense yellow diffusible pigment, smooth-walled hyaline conidiophores, and the production of chlamydospores. Although the isolate in this case did display smooth conidiophores, no yellow pigment or chlamydospores were observed. The existence of intergrading forms between *P. lilacinus* and *P. marquandii* has been described (6). In such strains, characteristics of both species may be observed. On the basis of the characteristics above, the isolate was identified as *P. lilacinus*.

In vitro 48-hour to 72-hour MIC data in µm/mL for the isolate were as follows: amphotericin B, >16; 5-flucytosine (5-FC), >64; ketoconazole, 0.5/0.5; fluconazole, 32/64; itraconazole, 0.5/0.5; clotrimazole, 0.06/0.25; voriconazole, 0.25/0.25; terconazole, 4/8; and posaconazole, 0.125/0.125.

Figure 1. Macroscopic structure of case isolate after 2 weeks' incubation at 25°C on potato flakes agar, prepared in house.

Conclusions

P. lilicanus rarely causes human infection. A MedLine review of English-language literature from 1966 to 2003 yielded approximately 60 reports of *P. lilacinus* infections in patients who were immunocompromised, had undergone ophthalmologic surgery, or had indwelling foreign devices (2,3). A Medline review in the same time period indicated only six cases of *P. lilacinus* infections among patients who lacked a readily identifiable risk factor. A review of the bibliographies of relevant articles yielded three additional reports, for a total of nine cases in apparently immunocompetent hosts. The salient features of these cases, as well as ours, are summarized in the Table.

The source of infection in most cases, including ours, is not easily identifiable. *P. lilacinus* has been isolated as a benign commensal organism on the toenails of immuno-

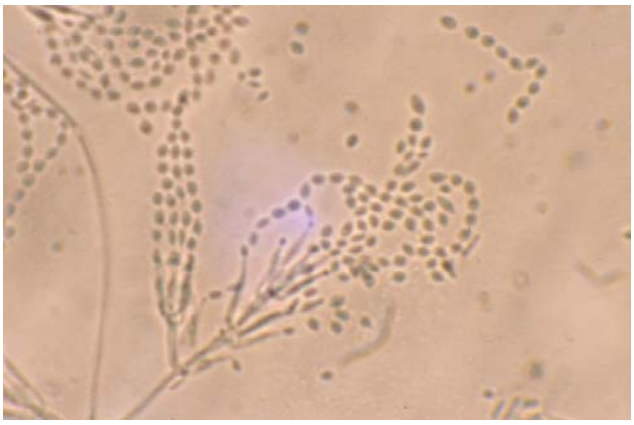

Figure 2. Divergent phialides and long, tangled chains of elliptical conidia borne from more complex fruiting structures characteristic of *Paecilomyces lilacinus*, 460X.

Table. Clinical features of cases of *Paecilomyces lilacinus* infections in immunocompetent hosts

Patient age, gender, and reference no.	Y	Type of infection	Treatment	Outcome
48-year-old woman (our case)	2002	Vaginitis	Iraconazole	Cure
36-year-old man (3)	1999	Cutaneous	Itraconazole	Cure
59-year-old woman (7)	2001	Cutaneous	Itraconazole	Cure
20-year-old woman (8)	1977	Cutaneous	Griseofulvin	Improvement, but not cure
19-year-old man (9)	1984	Cutaneous	Griseofulvin, then ketoconazole	Improvement
57-year-old man (10)	1999	Lung abscess	Surgery	Cure
20-year-old man (11)	1972	Pulmonary effusion	Amphotericin B	Cure
47-year-old woman (12)	1980	Sinusitis	Surgical debridement	Cure
34-year-old man (13)	1997	Endophthalmitis	Fluconazole, ketoconazole, itraconazole	Progression of disease
59-year-old woman (14)	1998	Onychomycosis	Terbinafine, various topical therapies, nail clipping	Progression of disease

competent hosts (15). In some cases, however, *P. lilacinus* has been pathogenic and has been implicated as a cause of onychomycosis in an immunocompetent adult (14). The low pathogenicity of this fungus in normal hosts is demonstrated by the indolent nature of two of the cutaneous infections listed in the Table (8,9), which were characterized by many years of chronic infection.

All isolates of the genus *Paecilomyces* should be tested for fungal susceptibility since clinical isolates of *P. lilacinus* frequently display considerable resistance. Isolates of *P. lilacinus*, for example, are usually resistant to amphotericin B and 5-flucytosine and susceptible to miconazole and ketoconazole, whereas isolates of the species *P. variotii* are usually susceptible to amphotericin B and 5-flucytosine (16). On the basis of breakpoints established for other fungi, the case isolate appeared resistant to amphotericin B, 5-flucytosine, fluconazole, and possibly terconazole. The approved azoles, itraconazole, ketoconazole, and clotrimazole, appeared susceptible, as did the investigational triazoles, voriconazole, and posaconazole.

Although the source isolate was susceptible to clotrimazole, the patient's symptoms did not resolve after clotrimazole treatment. However, the duration of therapy with this agent and the degree of the patient's adherence to the treatment regimen are unknown; one or both of these factors may have contributed to treatment failure.

Our review demonstrates that reports of *P. lilacinus* infections in immunocompetent hosts appear to be increasing. The four earliest cases occurred from 1972 to 1984, with one case reported every 3–5 years. The eight subsequent cases occurred between 1996 and 2002, for an average of slightly more than one new case per year.

We report the first case of *P. lilacinus* isolated from a vaginal culture in a patient with vaginitis, whose symptoms failed to improve after treatment with fluconazole. Her symptoms resolved after treatment with itraconazole, to which the case isolate was susceptible. *P. lilacinus* has been described as an emerging opportunistic pathogen in humans (17). In May 2002, the first case of disseminated *P. lilacinus* infection in an HIV-infected patient was reported (18). Our review suggests that *P. lilacinus* may be an emerging pathogen in immunocompetent adults as well.

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Fluoroquinolone and Macrolide Treatment Failure in Pneumococcal Pneumonia and Selection of Multidrug-Resistant Isolates

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Streptococcus pneumoniae serotype 3, isolated from a penicillin-allergic patient and initially susceptible to fluoroquinolones, macrolides, lincosamides, quinupristin-dalfopristin, and telithromycin, became resistant to all these drugs during treatment. Mutations in the *parC* and *gyrA* and in the 23S rRNA and the ribosomal protein L22 genes were detected in the resistant isolates.

Macrolide antimicrobial drugs and new fluoroquinolones have become good therapeutic choices in the treatment of penicillin-resistant *Streptococcus pneumoniae* infections and in penicillin-allergic patients with pneumococcal pneumonia. Until now, clinical failures of fluoroquinolones during treatment of pneumococcal infections have rarely been reported (1–3) and development of resistance in *S. pneumoniae* to quinupristin-dalfopristin and telithromycin during or after treatment with a macrolide or a combination of macrolide and quinolone antibiotics has never been reported.

We describe failure of treatment of pneumococcal pneumonia in a 71-year-old man, who was allergic to penicillin and had a history of chronic obstructive pulmonary disease. During treatment, isolates that were susceptible to levofloxacin, clarithromycin, clindamycin, quinupristin-dalfopristin, and telithromycin became resistant.

The Study

S. pneumoniae were identified to the species level by their colony morphology, optochin sensitivity, and the bile solubility tests. Serotyping was performed by the Quellung reaction (Quellung antisera, Statens Seruminstitut, Copenhagen). MICs of the antibiotics and criteria of susceptibility and resistance, otherwise not indicated, were those of the broth microdilution procedure described by the National Committee for Clinical Laboratory Standards (NCCLS) (4). The agar dilution method (5) and the E-test, as referred to in Table 1, were performed to expand the range of dilutions available in the broth microdilution trays. No discordance was observed in the susceptibility results, which were obtained by using the broth microdilution, the agar dilution, or the elution E-test.

Molecular typing methods (pulsed-field gel electrophoresis [PFGE], BOX-polymerase chain reaction [PCR], and multilocus sequence typing) of the isolates were performed according to previously described protocols (6). Presence of the *mefA, ermB,* and *ermA* (*ermTR*) genes and point mutations at Ser-79 in the *parC* and at Ser-81 in the *gyrA* genes were detected as previously described (6). Fragments of the domains II and V of the 23S rRNA genes and of the genes encoding ribosomal proteins L4 and L22 were amplified by using the primers and conditions previously described (7,8). Amplification products were sequenced after purification.

Case Description

In January 2002, a 71-year-old man, who was allergic to penicillin and had a history of chronic obstructive pulmonary disease, was hospitalized due to pneumonia. The first *S. pneumoniae* strain was isolated from sputum obtained before antibiotic treatment with intravenous levofloxacin (500 mg once a day for 13 days) was begun. On day 4, intravenous clarithromycin (500 mg twice a day) was added but withdrawn after 4 doses. On day 14, clinical and radiologic conditions had deteriorated, and treatment was changed to intravenous clarithromycin (500 mg) and intravenous ciprofloxacin (200 mg) twice a day for 7 days. On the same day, a second pneumococcal isolate resistant to levofloxacin and clarithromycin but susceptible to clindamycin was obtained (Table 1). The MIC of clarithromycin for this second isolate was 2 μg/mL; by the double-disk test (9) showed that the susceptibility of clindamycin was not modified after the erythromycin induction. Initially, this second isolate was incorrectly reported as clarithromycin susceptible because of an erroneous record of the result of the disk-diffusion method. On day 24, the patient was discharged with oral clarithromycin. Twenty-four hours later, the patient was readmitted with exacerbation of the respiratory infection and cor pulmonale, and two pneumococcal isolates resistant to levofloxacin, clarithromycin, and clindamycin were found within 6 hours. The patient received trimethoprim-sulfamethoxazole for 5 days; a fifth pneumococcal isolate was found from a pleural effusion specimen. The pneumonia completely resolved after 10 days of treatment with

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Table 1. In vitro antibiotic susceptibilities determined by agar dilution in *Streptococcus pneumoniae* isolates

Drug	MIC (μg/mL) for each isolate/specimen collected, by date				
	1st isolate/sputum, 1-26-2002	2nd isolate/sputum, 2-8-2002	3rd isolate/sputum, 2-20-2002	4th isolate/sputum, 2-20-2002	5th isolate/pleural fluid, 2-26-2002
Ciprofloxacin	2	32	16	16	16
Levofloxacin	2	16	16	16	16
Moxifloxacin	1	4	4	4	4
Gemifloxacin	0.06	0.5	0.5	0.5	0.5
Erythromycin (14-m macrolide)	≤0.25	2	>128	>128	>128
Clarithromycin (14-m macrolide)	≤0.25	2	>128	>128	>128
Azithromycin (15-m macrolide)	0.5	4	>128	>128	>128
Midekamycin (16-m macrolide)	≤0.5	4	128	64	16
Clindamycin	≤0.25	≤0.25	16	16	16
Quinupristin-dalfopristin [a]	1	32	>32	>32	2
Telithromycin	≤0.12	2	8	16	0.5

[a] Determined by E-test; m, membered.

vancomycin. The five *S. pneumoniae* serotype 3 isolates recovered over a 32-day period had the same PFGE, BOX-PCR patterns, and multilocus sequence typing (ST180) results.

All *S. pneumoniae* isolates were susceptible to penicillin (MIC ≤0.03 μg/mL), trimethoprim-sulfamethoxazole (MIC ≤0.5/9.5 μg/mL), tetracycline (MIC ≤2 μg/mL), chloramphenicol (MIC ≤2 μg/mL), and vancomycin (MIC=0.5 μg/mL). The first isolate was susceptible to both macrolides and fluoroquinolones. This isolate had a levofloxacin MIC of 2 μg/mL, confirmed by all susceptibility methods used (E-test, broth microdilution, and agar dilution), although it had a point mutation in the *gyrA* gene, as shown in Table 2.

For the second isolate, MICs of macrolides, quinupristin-dalfopristin, and telithromycin were higher than those for the first isolate, and a 18-base insert in the sequence of the gene encoding the ribosomal protein L22 was detected. The result, deduced from the corresponding ribosomal protein, was a six–amino acid (RTAHIT) insertion between amino acids T108 and V109 (GenBank accession no. AY140892). The third and fourth isolates, with resistance to macrolides, clindamycin, quinupristin-dalfopristin, and the highest telithromycin MICs of all the isolates, had an A2058G (*Escherichia coli* numbering) mutation in the sequence of the gene corresponding to the domain V of the 23S rRNA as well as the 6–amino acid insert in the ribosomal protein L22. The four alleles encoding the 23S rRNA gene had the A2058G mutation. The sequences of the fifth isolate, resistant to macrolide antibiotics and clindamycin, with an intermediate susceptibility to quinupristin-dalfopristin, indicated a mutation at position 2058 of domain V, but no insert was found in the ribosomal protein L22.

Conclusions

Since the introduction of antimicrobial drugs in therapy, *S. pneumoniae* has shown a strong ability to acquire resistance to the progressive introduction of new antibiotics to treat it.

Surveillance studies suggest that the levels of resistance to macrolide antibiotics in *S. pneumoniae* are high and are still rising (9,10). Ketolides, of which telithromycin is the first to be registered for clinical use, and quinupristin-dalfopristin are new compounds belonging to the macrolide-lincosamide-streptogramin B (MLSb) class of antimicrobial agents. One of the main advantages attributed to these two new families of antibiotics is their ability to retain activity against most resistant pneumococcal isolates (11). Recently, mutations in the 23S rRNA genes and in ribosomal proteins L4 and L22 have been identified in macrolide-resistant *S. pneumoniae,* although the predominant mechanisms of resistance are mediated by *ermB* or by *mefA* genes (12). The combination of the mutation in the

Table 2. Point mutations in fluoroquinolone targets and macrolide-lincosamide-streptogramin-resistance determinants and ribosomal mutations in *Streptococcus pneumoniae* isolates

Location	1st isolate	2nd isolate	3rd isolate	4th isolate	5th isolate
parC	-	Ser-79	Ser-79	Ser-79	Ser-79
gyrA	Ser-81	Ser-81	Ser-81	Ser-81	Ser-81
ermA, ermB and *mefA* genes	-	-	-	-	-
Ribosomal protein L4	-	-	-	-	-
Amino acids insert in ribosomal protein L22	-	RTAHIT[a]	RTAHIT	RTAHIT	-
23S rRNA gene (domain II)	-	-	-	-	-
23S rRNA gene (domain V)	-	-	A2058G	A2058G	A2058G

[a]Sequence data GenBank AY140892.

domain V of the 23S rRNA and the insertion in the L22 ribosomal protein gene has not been previously described in *S. pneumoniae* isolated in vivo or in vitro. In these strains, a high level of resistance to 14-, 15-, and 16-membered ring macrolides and to clindamycin and resistance to quinupristin-dalfopristin and telithromycin were observed. The continued use of clarithromycin in the presence of an isolate with an insertion in the L22 ribosomal protein gene may have led to the selection of the isolates with the double mutations, L22 and 23S rRNA genes, associated with combined resistance to telithromycin and quinupristin-dalfopristin, although neither of these antibiotics was used. The A2058G mutation in all four 23S rRNA genes alone slightly increased both quinupristin-dalfopristin and telithromycin MICs as seen in the fifth isolate. The L22 insertion alone, as observed in the second isolate, was enough to confer a high level of quinupristin-dalfopristin resistance and also increased the telithromycin MIC to 2 μg/mL. The combination of both mutations (L22 insertion and A2058G mutation in the 23S rRNA genes) led to high level of resistance to telithromycin and increased the quinupristin-dalfopristin MIC (third and fourth isolates).

The first isolate was susceptible to all antibiotics tested, and although it had a point mutation in the *gyrA* gene, it had no phenotypic expression. In mutants obtained in vitro, other authors observed point mutation in the *gyrA* gene without mutation in the *parC* gene with or without phenotypic expression of quinolone resistance (13). Nevertheless, using fluoroquinolones to treat a strain that had an existing, but unapparent, first-step mutation in the *gyrA* gene, probably favored the development of the high level of resistance to fluoroquinolones observed in the later isolates. Fluoroquinolone resistance in clinical isolates of *S. pneumoniae* is still infrequent, but in some places, the resistance has been increasing (9,14–16).

Until now, most erythromycin- or fluoroquinolone-resistant pneumococci had belonged to only a few serotypes. Finding an erythromycin-resistant serotype 3 was unusual, and the isolation of a fluoroquinolone-resistant serotype 3 *S. pneumoniae* was the exception, if ever reported. Penicillin or another appropriate β-lactam antibiotic could have been a valid therapeutic option in the absence of allergy to penicillin. Serotype 3 is considered the most virulent of *S. pneumoniae* serotypes, and it is commonly associated with invasive disease in adults. Most serotype 3 isolates have broad antibiotic susceptibility (17). A fatal infection associated with a multiply drug-resistant *S. pneumoniae* serotype 3 was first reported in 1988 (18). This strain was resistant to erythromycin, clindamycin, and tetracycline.

The therapeutic failure and selection of resistance to several antibiotics by *S. pneumoniae*, the emergence of new mechanisms of resistance to macrolides in clinical isolates of *S. pneumoniae,* and the appearance of multidrug resistance in a serotype 3 isolate (ST180) evoke concern.

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Microbiologic Characteristics, Serologic Responses, and Clinical Manifestations in Severe Acute Respiratory Syndrome, Taiwan[1]

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The genome of one Taiwanese severe acute respiratory syndrome-associated coronavirus (SARS-CoV) strain (TW1) was 29,729 nt in length. Viral RNA may persist for some time in patients who seroconvert, and some patients may lack an antibody response (immunoglobulin G) to SARS-CoV >21 days after illness onset. An upsurge of antibody response was associated with the aggravation of respiratory failure.

In November 2002, cases of a life-threatening and highly contagious febrile respiratory illness of unknown cause were reported from Guangdong Province in southern China, followed by reports from Vietnam, Hong Kong, Singapore, Canada, the United States, and other countries (1–4). This illness was identified as a new clinical entity and designated as severe acute respiratory syndrome (SARS) in late February 2003. This disease has a high propensity to spread to healthcare workers and household members and may cause outbreaks in the community (1–4). Recent reports have demonstrated that a novel coronavirus, SARS-associated coronavirus (SARS-CoV), is associated with the pathogenesis of SARS (5–7). Laboratory diagnostic tests to analyze clinical specimens for SARS-CoV include reverse-transcriptase polymerase chain reaction (RT-PCR) specific for RNA and detection of specific antibody by using indirect fluorescence antibody and enzyme-linked immunosorbent assays (8,9). However, data on the timing and intensity of serologic responses after illness onset and the association of these responses with clinical manifestations of the disease are lacking.

In Taiwan, the first case of SARS occurred in a businessman working in Guangdong who was admitted to National Taiwan University Hospital (NTUH) on March 8, 2003. As of May 18, 2003, a total of 308 probable cases of SARS were reported by the Center for Disease Control, Department of Health, Taiwan (10).

The Study

This study included seven Taiwanese patients, treated at the National Taiwan University Hospital from March 8 to May 3, 2003, whose illness met the recent Centers for Disease Control and Prevention (CDC) and World Health Organization (WHO) case definition for probable cases of SARS (11,12). The patients were 26–53 years of age, and six were men. The incubation period ranged from 2 to 12 days. Of the seven patients, four had recently returned from China: two patients (patients 1 and 7) from Guangdong Province and two (patients 5 and 6) from Beijing. In addition, two family members (patients 2 and 3), and one healthcare worker (patient 4) were from a cluster, which had household or healthcare contact with a SARS patient, and two patients (patient 5 and 6) were from another cluster, which had close contact with a SARS patient in an airplane.

All patients had fever (body temperature >38°C) and dry cough. Other symptoms included malaise (five patients), myalgia (five patients), and rigor (four patients). All but one patient (patient 7) had loose stools or diarrhea 2–10 days after febrile episodes, and five, including the four cluster A patients, had aggravating diarrhea 9–14 days after febrile episodes. The mean interval between onset of symptoms and hospitalization was 7.3 days (range 4–12 days).

Pneumonia developed in all seven patients, acute respiratory distress syndrome (ARDS) developed in four (patients 1, 2, 3, and 6), and ventilator support was given 10–12 days after the onset of illness. Pancytopenia compatible with hemophagocytosis syndrome developed in

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patient 2. Five patients (patients 2, 3, 4, 5, and 6) received ribavirin, intravenous corticosteroid (methylprednisolone, 2 mg/kg/d), and intravenous immunoglobulin (IVIG, 1 gm/kg/d for 2 days). Interstitial pneumonia developed in patient 7, who responded well to ribavirin and antibiotic treatment. All patients survived.

Urinary antigen detection for *S. pneumoniae* and *Legionella pneumophila* serogroup I was negative in all seven patients. Serum from patient 5 was positive for *Mycoplasma pneumoniae* immunoglobulin (Ig) M (enzyme-linked immunosorbent assay [ELISA]) antibody with a fourfold increase in complement fixation (CF) antibody titer in acute- (<1:40) and convalescent-phase sera (1:160). An elevated *Chlamydia pneumoniae* CF antibody (1:32) but negative reaction for *C. pneumoniae* IgM (ELISA) antibody was found in the acute-phase serum sample from patients 1 and 6 and in the acute- (1:32) and convalescent-phase serum (1:32) samples from patients 5 and 7. The antibody titers of acute- and convalescent-phase serum samples for *C. pneumoniae, C. trachomatis, C. psittaci,* and *L. pneumophila* in the other patients showed no significant increase. Five patients (patients 1, 2, 4, 5, and 6) had elevated CF antibody levels (≥1:16) against parainfluenzavirus 1, 2, or 3. Cultures for influenza virus, parainfluenzavirus, mumps, respiratory syncytial virus, adenovirus, enterovirus, herpes simplex virus, varicella-zoster virus, and cytomegalovirus were negative from various clinical samples of these patients.

Nucleic acid was extracted from the sputum and serum samples and the infected Vero E6 cells by using a viral RNA kit (QIAamp, Qiagen Inc., Valencia, CA). Reverse transcription polymerase chain reaction (RT-PCR) for SARS-CoV was performed with 3 sets of primers (IN-6 and IN-7; Cor-p-F1 and Cor-p-R2; and BNIinS and BNIAs) developed by CDC and WHO Network Laboratory. The RT-PCR products were analyzed, and the unique fragment was cloned and sequenced (6,11). RT-PCR test results for SARS-CoV were positive in oropharyngeal swabs from patients 6 and 7; sputum from patients 1, 2, 3, 4, and 5; and serum specimens from patients 1, 2, 3, 4, 5, and 7. Cultures of all oropharyngeal swabs and serum specimens were negative.

Cytopathic effects in the Vero E6 cells were first found between day 3 and day 4 after injection of serum specimens from patients 3 and 4. The initial cytopathic effect was focal**,** with cell rounding, and was followed by cell detachment. Similar cytopathic effects developed rapidly (between day 2 and day 3) after subculture.

Ultra-thin sections were prepared for electron microscopy by fixing a washed infected Vero E6 cell pellet with 2.5% glutaraldehyde and embedding in Spurr's resin. The SARS-CoV (range 60–80 nm in diameter) was identified by electron microscopy (Figure 1 A and B). RT-PCR from the infected Vero-E6 cells identified the same amplicon. Sequences of the amplicons from all patients were identical and were also identical to those from infected Vero E6 cells.

The genome of the SARS-CoV (TW-1) (GenBank accession no., AY291451) strain from patient 3 was 29,729 nt in length. A comparison of TW1 sequences to the sequences described previously is summarized in the Table. The number of nucleotide differences between this TW1 isolate and the Urbani (AY278741), TOR-2 (AY274119), HKU-39849 (AY278491), and CUHK-W1 (AY278554) strains was 6, 3, 12, and 10, respectively.

IgG antibody to the SARS-CoV was detected by a standard indirect fluorescence antibody assay (IFA) with serial serum specimens from the seven patients. Spot slides for IFA were prepared by applying the suspension mixed with SARS-CoV–infected Vero E6 cells from one patient (patient 4) and uninfected cells. Slides were dried and fixed in acetone. The conjugates used were goat antihuman IgG conjugated to fluorescein isothiocyanate (Organon Teknika-Cappel, Turnhout, Belgium). The starting dilution of serum specimens was 1:25 (5). Ten serum samples obtained from 10 pregnant women during routine prelabor check-ups were used as control sera. Two IVIG products, one domestic (from Taiwanese donors) and one imported (Bayer, Leverkusen, Germany), were also tested for the presence of antibody.

All serum samples from the 10 pregnant women and the two IVIG products were negative for IgG antibody (<1:25) to SARS-CoV. Six patients had detectable IgG antibody to SARS-CoV during the course of illness, and all of them

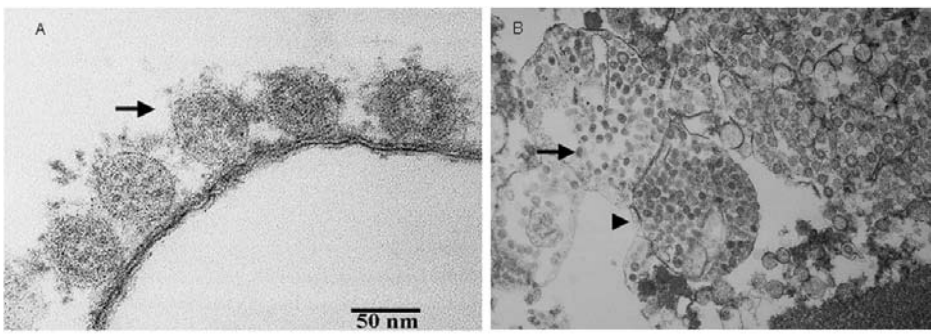

Figure 1. Thin-section electron micrograph of severe acute respiratory syndrome-associated coronavirus grown in Vero E6 cells. Panel A shows extracellular viral particles (arrow) lining the surface of the plasma membrane. Some spikes projecting from the envelope of the virus are seen. Panel B shows numerous spherical coronavirus particles (arrow) within dilated cytoplasmic vacuoles (arrowhead).

Table. Nucleotide base differences among the TW-1, TOR-2, HKU-39849, CUHK-W1, and the Urbani sequences of SARS-CoV

	SARS-associated coronavirus sequence					
Base	TW-1	TOR-2	HKU-39849	CUHK-W1	Urbani	A. a. change[b]
						TW1/Urbani
2,601	T	T	C	T	T	Val/Val
3,165	G	A	A	A	A	Ser/Ser
7,746	G	G	T	T	G	Pro/Pro
7,919	C	C	C	C	T	Ala/Val
9,404	T	T	C	C	T	Val/Ala
9,479	T	T	C	C	T	Val/Ala
16,622	C	C	C	C	T	Ala/Ala
17,564	T	T	G	G	T	Asp/Glu
17,846	C	C	T	T	C	Arg/Arg
19,064	A	A	G	G	G	Glu/Glu
21,721	G	G	A	A	G	Gly/Asp
22,222	T	T	C	C	T	Ile/Thr
23,220	T	G	T	T	T	Ser/Ala
24,872	T	T	T	T	C	Leu/Leu
25,298	G	A	G	G	G	Gly/Arg
26867	T	T	T	T	C	Ser/Pro
27,827	T	T	C	C	T	Cys/Arg

[a]SARS, severe acute respiratory syndrome.
[b]Indicates a base difference resulting in an amino acid change.

had at least fourfold elevation of antibody levels in acute- and convalescent-phase serum samples (peak levels range 1:400– ≥1:1600) (Figure 2). Antibody titers (>1:25) of these six patients could be detected 9–18 days (mean 12.3 days) after the onset of illness. The antibody titer increased to a plateau 4–10 days after the appearance of antibody. The high antibody levels might last for 1 to >2 months after onset of illness (Figure 2). One previously healthy patient (patient 7) with positive SARS-CoV RNA by RT-PCR from both sputum and serum specimens had no detectable antibody to SARS-CoV in serum specimens obtained 7, 10, 14, and 24 days after illness onset. Although the antibody levels reached a plateau in all patients, viral RNA persisted in the serum samples from patients 1 and 2 and sputum from patients 1 and 4 for 19 to 29 days after onset of their illness.

Although four patients had received ribavirin, corticosteroid, and IVIG treatment in the early stage of the disease, antibody was detected as early as 10–12 days after the onset of illness. The peak level of antibody was 1:400 in patients 2 and 6, 1:800 in patient 3, and ≥1:1600 in patient 1.

Conclusions

Serologic study indicated that the antibody to SARS-CoV appeared as early as 9 days after disease onset and that a high level of antibody could last for 1–2 months after disease onset. Previous reports indicated that the mean time for IgG seroconversion was 20 days and may start as early as 9–10 days. Our finding supported the results of Peiris et al. (7,12). Levels and appearance of antibody to SARS-CoV did not seem to be influenced by the use of ribavirin and immunosuppressive or immunomodulatory agents (corticosteroid or IVIG, a blood product prepared from the serum of 1,000 to 15,000 donors per batch) (13).

Third, the long-term persistence (19–29 days after illness onset) of viral RNA in the serum and sputum specimens of the SARS-CoV-specific IgG seroconverters is an important finding. Prolonged shedding of viral RNA in respiratory secretions (11 days after illness onset), plasma (up to 9 days), and stool specimens (25 days) was documented previously (6). Further studies are needed to determine whether the viable viral particles existed in body fluids in the presence of high antibody to the virus. Finally, one SARS patient, who did not have an underlying coexisting condition and did not receive any immunosuppressive agents during hospitalization, did not have detectable antibody to SARS-CoV 24 days (>21 days) after illness onset. The serum and sputum RT-PCR for SARS-CoV were positive in this patient, and the sequence was confirmed. Whether the patient was anergic to SARS-CoV infection is unknown. A later serum sample taken in the convalescent stage should be tested to determine whether this patient subsequently seroconverts (7).

The upsurge of IgG antibody to SARS-CoV and its correlation with the progression of ARDS, necessitating ventilator support in four of the seven patients, was evident. Previous study suggested that an overexuberant host response rather than uncontrolled viral replication, contributed to severe clinical symptoms and progressive lung damage (12). Whether the addition of SARS-CoV–specific antibody in SARS patients further aggravated the preexisting overactive immune-mediated deterioration was unclear.

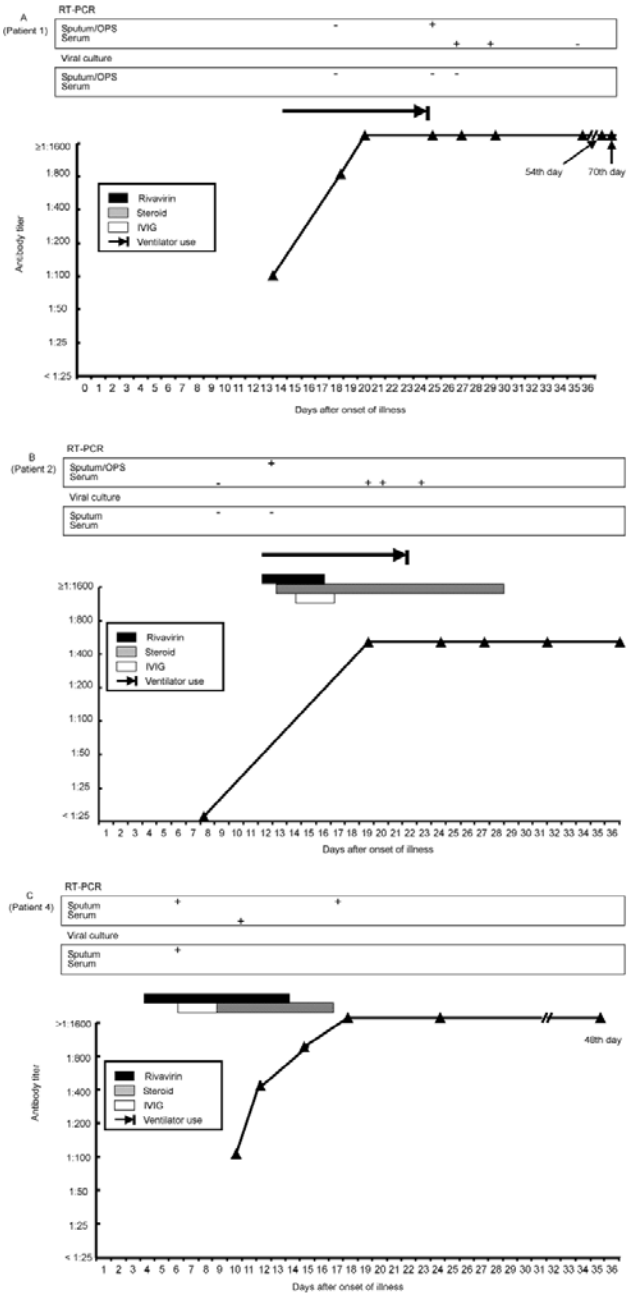

Figure 2. Timelines of positive reverse transcription polymerase chain reaction, antibody responses and treatment regimens (ribavirin, corticosteroid, and intravenous immunoglobulin) after onset of disease in seven patients with severe acute respiratory syndrome. Panels A–C indicate patients 1, 2, and 4.

High concentrations of viral RNA, up to 100 million molecules per milliliter, were detected in a sputum sample from an index patient on day 9 (6). In the present series, a physician contracted the infection from a patient (patient 2) 12 days after the onset of symptoms, indicating that shedding of the virus from the respiratory tract of symptomatic SARS patients may last for ≥12 days. Viral RNA in the sputum samples of patient 2 collected 12 days after the onset of symptoms supports this clinical finding.

Dual infection caused by *M. pneumoniae* and SARS-CoV was found in patient 5. No evidence of *M. pneumoniae* infection existed in patient 6 from the same cluster. This finding is similar to a previous report (6). Four of our patients had elevated IgG antibody titers for *C. pneumoniae,* and five had elevated antibody titers against parainfluenzavirus 1, 2, or 3 in acute-phase serum samples without a fourfold rise of titers in convalescent-phase serum samples. Whether the antibody responses of these patients reflected past infections from *C. pneumoniae,* parainfluenzavirus, or both, or merely a cross-reaction with antibody against SARS-CoV virus remains unclear.

As of May 16 2003, data of complete genomic sequences for 13 SARS-CoV strains isolated from Hong Kong, Singapore, China, Canada, Vietnam, and Taiwan were available in GenBank. The number of nucleotides ranged from 29,705 (SIN2677 strain) to 29,751 (TOR2) (14,15). Since February 2003, at least three different clusters of SARS outbreaks occurred in different parts of Taiwan, and five strains were identified from patients in these clusters. The availability of the sequence data of different strains in a given geographic area will have an immediate impact on the effort to trace the origins and transmission of SARS-CoV and develop novel rapid diagnostic tests and a vaccine.

In summary, analysis of these seven patients with virologically or serologically documented infections caused by SARS-CoV in Taiwan not only extended the knowledge of this emerging novel disease but also provided microbiologic and immunologic clues for the physicians caring for patients suspected of having this disorder. Viral RNA may persist for some time in patients who seroconvert, and some patients may lack an antibody response to SARS-CoV >21 days after illness onset. An upsurge of antibody response is associated with the aggravation of respiratory failure that required ventilator support.

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HEALTH ALERT NOTICE
Health Alert Notice for International Travelers Arriving in the United States from China, Vietnam, and Singapore

TO THE TRAVELER: During your recent travel, you may have been exposed to cases of severe acute respiratory disease syndrome (SARS). You should monitor your health for at least 10 days. If you become ill with fever, cough, or difficulty in breathing, you should consult a physician. In advance of your visit to the physician, tell him or her about your recent travel to these regions and whether you were in contact with someone who had these symptoms. Please save this card and give it to your physician if you become ill.

TO THE PHYSICIAN: The patient presenting this card may have recently traveled to China, Vietnam, or Singapore, where cases of SARS have been identified. If you suspect that this patient may have SARS, please contact your city, county, or state health officer (see http://www.cdc.gov or call the CDC Emergency Operations Center at 770-488-7100).

English

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医生请注意：父给您本卡片的患者最近可能曾前往中国、越南和新加坡旅行。这些地区已发现SARS（即严重急性呼吸道综合症）病例。如果您认为此患者可能患有SARS，请与市、县、或州保健官员联系（联系方法请查阅http://www.cdc.gov，或拨电CDC Emergency Operations Center，770-488-7100）。

Simplified Chinese

KHUYẾN CÁO Y TẾ
THÔNG BÁO QUÍ HÀNH KHÁCH QUỐC TẾ ĐI ĐẾN HOA KỲ TỪ TRUNG-QUỐC, VIỆT-NAM VÀ SINGAPORE.

THÔNG BÁO QUÍ HÀNH KHÁCH: Trong chuyến du hành vừa mới đây, quí vị có thể đã bị truyền nhiễm chứng bệnh hô hấp cấp tính nghiêm trọng (SARS). Quí vị nên theo dõi kỹ tình trạng sức khỏe của mình ít nhất là 10 ngày. Nếu quí vị bị mắc bệnh lên cơn sốt kèm theo ho hoặc khó thở, thì nên tham khảo với bác sĩ ngay. Trước khi đến bác sĩ, quí vị nên cho bác sĩ biết về những nơi mà quí vị đã du hành vừa mới đây tại những khu vực này, và cho bác sĩ biết nếu quí vị đã có chung đụng với những người có triệu chứng bệnh này. Xin quí vị hãy giữ phiếu khuyến cáo này để trình cho bác sĩ nếu quí vị bị mắc bệnh.

THÔNG BÁO QUÍ BÁC SĨ: Bệnh nhân trình phiếu khuyến cáo này có thể vừa mới đây đã du hành đến Trung-Quốc,Việt-Nam, hoặc Singapore nơi đã phát hiện bệnh SARS. Nếu quí vị nghi ngờ rằng khách đã mắc phải bệnh SARS, xin liên lạc với cán bộ y tế của thành phố, quận, hoặc tiểu bang, (xem http://www.cdc.gov/ hoặc gọi cho Trung Tâm Kiểm Soát Bệnh Dịch, Phòng Cấp Báo CDC (CDC Emergency Operation Center), điện thoại 770-488-7100).

Vietnamese

HEALTH ALERT NOTICE
건강 경보 공지사항
KHUYẾN CÁO Y TẾ
健康に関する注意喚起
AVIS D'ALERTE MÉDICALE
AVISO DE ALERTA DE SALUD
緊急保健通告
紧急保健通告

DEPARTMENT OF HEALTH AND HUMAN SERVICES

CDC

健康に関する注意喚起
中国、ベトナム、およびシンガポールから来国に渡者の旅行者へ

旅行者の皆さんへ：最近なさった旅行において、重症急性呼吸器症候群（SARS）にさらされた可能性があります。最低10日間、ご自分の健康状態をよく観察してください。高熱、咳、あるいは呼吸困難などの症状を伴う病気になった場合、医師の診察を受けてください。その際は前もって、これらの地域へ渡航したこと、またこのような症状のある人と接触したかどうかを医師に伝えてください。このカードは保存し、これらの症状が出た場合には医師に提示してください。

医師の皆さんへ：このカードを提示した患者は、重症急性呼吸器症候群（SARS）が発生している中国、ベトナム、またはシンガポールへ、最近旅行したと思われます。患者が重症急性呼吸器症候群（SARS）にかかっていると思われる場合は、市、郡、または州の衛生官へ連絡してください。（詳細は、http://www.cdc.gov をご覧になるか、またはCDC救急対策センター（電話 770-488-7100）に、お問い合わせください。）

Japanese

AVIS D'ALERTE MÉDICALE
aux voyageurs internationaux arrivant aux États-Unis en provenance de la Chine, du Vietnam et de Singapour

AU VOYAGEUR : Au cours de votre séjour réalisé récemment dans les régions susmentionnées, vous avez peut-être été en contact avec des personnes atteintes du syndrome respiratoire aigu sévère (SRAS). Par conséquent, vous devriez surveiller votre état de santé pendant au moins dix (10) jours. Consultez un médecin si vous présentez l'un des symptômes du SRAS (fièvre, toux, difficulté à respirer). Appelez le bureau du médecin avant de vous rendre. Informez-le de votre voyage dans ces régions et dites-lui si vous avez été en contact avec des personnes qui manifestaient ces symptômes. Conservez cette carte et présentez-la à votre médecin si vous devenez malade.

AU MÉDECIN : La personne qui vous présente cette carte a peut-être visité récemment la Chine, le Vietnam ou Singapour, soit des régions touchées par le SRAS. Si vous croyez que cette personne peut être atteinte du SRAS, contactez les autorités de la santé publique de votre ville ou de votre région. (Visitez le site http://www.cdc.gov ou appelez le Centre des opérations d'urgence du Centers for Disease Control and Prevention au (770) 488-7100).

French

건강 경보 공지사항
중국, 베트남, 싱가폴로부터
미국으로 도착하는
국제 여행객을 건강 경보 공지사항

여행객에게: 최근 여행기간 동안 급성 호흡기 증후군(SARS)에 노출되었을 수 있습니다. 적어도 10일 동안 건강을 주시하여야 합니다. 열, 기침 또는 호흡 곤란 등의 병이 발생하는 경우 의사의 진찰을 받아 보아야 합니다. 병원을 방문하기에 앞서, 상기 지역으로의 여행 사실과 위의 증상을 앓고 있는 환자와의 접촉 여부를 의사에게 알려 주십시오. 이 카드를 보관하였다가 아플 경우 담당의사에게 주십시오.

의료인에게: 본 카드를 제출하는 환자는 급성 호흡기 증후군의 발생이 확인된 중국, 베트남 또는 싱가폴로 최근 여행하였을 수 있습니다. 급성 호흡기 증후군의 의심되는 경우, 시, 군, 또는 주 보건 담당자에게 연락하십시오. (http://www.cdc.gov 를 참조하거나, CDC 비상 대책 센터 770-488-7100 으로 전화 주십시오.)

Korean

As part of Centers for Disease Control and Prevntion's response to the severe acute respiratory syndrome (SARS) outbreak, from March 15 through July 17, 2003, quarantine officials at U.S. ports of entry distributed 2,721,965 health alert notices to passengers arriving from areas with SARS. The notices, translated into eight languages, advised travelers of SARS symptoms and provided physicians with reporting information.

Co-trimoxazole–Sensitive, Methicillin-Resistant *Staphylococcus aureus*, Israel, 1988–1997

Jihad Bishara,* Silvio Pitlik,* Zmira Samra,* Itzhak Levy,† Mical Paul,* and Leonard Leibovici*

Among bloodstream methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from adult patients in a single hospital, susceptibility to co-trimoxazole increased progressively from 31% in 1988 to 92% in 1997 (p<0.0001). If also observed in other institutions, these findings should encourage the performance of a clinical trial of sufficient size to compare co-trimoxazole to vancomycin in treating MRSA infections.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a growing medical concern. During the last 2 decades, the rates of infections caused by MRSA increased among hospitalized patients in most developed countries (1). The aim of this study was to examine trends in antibiotic resistance of hospital-acquired bloodstream MRSA isolates from 1988 to 1997 in our institution.

The Study

Included in the analysis were all patients >18 years of age who had hospital-acquired bacteremia caused by *S. aureus.* The study took place at Rabin Medical Center, Beilinson Campus, Petach-Tikva, Israel, a 900-bed university hospital. Our center serves an urban population of approximately 1 million persons as both a first-line and tertiary facility. A prospective surveillance of all bacteremic episodes occurring at our medical center is performed continuously and, since 1988, has been incorporated into a computerized database for bacteremia. Episodes of bacteremia are detected by daily surveillance of the microbiology laboratory records, with an annual range of 700 to 900 episodes.

Antibiotic susceptibility was tested by using the disk diffusion technique on Mueller-Hinton agar, according to the procedures established by the National Committee for Clinical Laboratory Standards (NCCLS) (2). Plates were incubated at 30ºC for 18 h and 40 h for methicillin (5 µg/disk) and at 37°C for 18 h for other antibiotics. Bacteremia was considered to be hospital-acquired if it appeared 48 h after admission.

During the study period, a total of 944 episodes of *S. aureus* bacteremia were documented. We found 598 (63%) hospital-acquired episodes, with an annual number of episodes ranging from 35 to 121. Among the hospital-acquired episodes, 270 (45%) were due to MRSA strains. During the recent decade, rates of resistance to methicillin were high but stable among the hospital-acquired isolates, ranging from 25% to 57%. Rates of susceptibility to co-trimoxazole among patients with hospital-acquired MRSA increased significantly from 31% in 1988 to 92% in 1997 (p=0.0001) (Figure).

The hospital-acquired MRSA isolates were persistently highly resistant to chloramphenicol (69% in 1988 and 100% in 1997; p=NS), gentamicin (89% in 1988 to 94% in 1997; p=NS), and ciprofloxacin (87% in 1988 to 96% in 1997; p=NS). The resistance to clindamycin (62% in 1988 to 92% in 1997; p=0.04), fusidic acid (6% in 1988 to 14% in 1997; p=0.03), and rifampicin (21% in 1988 to 76% in 1997; p=0.02) increased significantly. All isolates were sensitive to vancomycin.

Conclusions

Our study shows that 92% of nosocomial MRSA strains were sensitive to co-trimoxazole in 1997 as compared with 31% in 1988. Several factors may have influenced the emergence of co-trimoxazole–sensitive MRSA, including the reduced usage of this drug in our institution. According to the pharmacy records, usage of co-trimoxazole in our institution decreased progressively from 28 daily doses per 1,000 hospital days in 1990 to 17 daily doses per 1,000

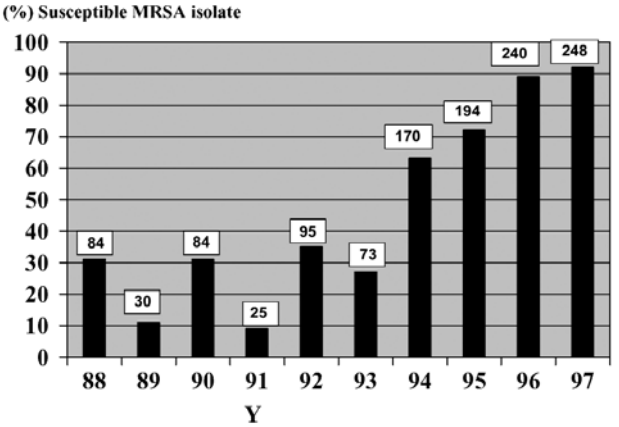

Figure. Co-trimoxazole susceptibility among methicillin-resistant *Staphylococcus aureus*. Columns indicate percentage of hospital-acquired methicillin-resistant *S. aureus* (MRSA) susceptible to co-trimoxazole. Numbers on top of the columns are absolute numbers of hospital-acquired MRSA susceptible to co-trimoxazole.

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hospital days in 1997 (3). A recent multicenter report from several Belgian hospitals showed an increase in co-trimoxazole susceptibility among MRSA isolates (4). These findings are in contrast with trends of increasing resistance of *S. aureus* to a variety of anti-staphylococcal drugs other than co-trimoxazole, since the beginning of the antibiotic era. These trends had culminated recently with the appearance of glycopeptide resistance in hospitals and methicillin resistance in the community (5). Whether our findings reflect an increase of co-trimoxazole–sensitive MRSA clone/s in our institution needs further exploration. In settings where co-trimoxazole is extensively used, a substantial increase of MRSA resistance to co-trimoxazole has been observed. For example, Martin et al. described a serial cross-sectional study of resistance to co-trimoxazole among all clinical isolates of *S. aureus* and other *Enterobacteriaceae* during a 16-year period at San Francisco General Hospital (6). In this study, resistance to co-trimoxazole increased from 0% to 48% in *S. aureus* isolates obtained from HIV-infected patients. The authors explained this increase of resistance to co-trimoxazole by the extensive use of this drug as prophylaxis against *Pneumocystis carinii* pneumonia.

Eventually, our data may favor the use of co-trimoxazole as a potentially cost-effective antimicrobial drug for treating MRSA infections. Co-trimoxazole has been shown to be effective against MRSA both in vitro and in vivo in mice (7), as well as in clinical reports on meningitis, septicemia, and endocarditis (8–9). A controlled comparative trial of intravenous co-trimoxazole versus intravenous vancomycin in 101 cases of severe *S. aureus* infections in intravenous drug users was conducted by Markowitz et al. (10) in 1992. The authors reported 100% cure rates for either drug in MRSA infections, including bacteremia. More recently, Stein et al. showed varying degrees of success in treating with co-trimoxazole orthopedic implant infections caused by *S. aureus* (11). Unfortunately, this study did not distinguish MRSA from methicillin-sensitive *S. aureus* strains.

Recent in vitro data have shown good activity of co-trimoxazole against clinical isolates of vancomycin-intermediate *S. aureus* (12–13) and vancomycin-resistant *S. aureus* (14). In some of these cases, co-trimoxazole in combination with surgical debridement and other anti-staphylococcal drugs has been used successfully (12,14). In clinical practice, cyclical usage of co-trimoxazole and vancomycin and possible other newer anti-MRSA drugs such as oxazolidinones and streptogramins may prove of value in slowing down rates of development of antibiotic resistance in MRSA. The in vitro–presented results, if confirmed in other institutions, in conjunction with anecdotal clinical data, should encourage the performance of a clinical trial of sufficient size to compare co-trimoxazole to vancomycin in treating MRSA infections.

Dr. Bishara is a specialist in internal medicine and infectious diseases, and he serves as a senior physician and infectious disease consultant at the Rabin Medical Center, Israel. His major research interests are infective endocarditis and cardiovascular and nosocomial infections.

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Enteroaggregative *Escherichia coli* Serotype O126:H27, Israel

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Enteroaggregative *Escherichia coli* (EAEC) is a newly diarrheagenic agent wherein several predominant serotypes are reported. We studied the association between those serotypes, as clonal indicators, and the trait of enteroaggregative adherence to host cells, tested by polymerase chain reaction. We also evaluated the clinical manifestations of infection in 17 hospitalized children by our most common EAEC serotype, O126:H27.

Enteroaggregative *Escherichia coli* (EAEC) is an emerging pathogen that causes diarrhea in many parts of the world, including children from Israel (1) and Jordan (2). This group of *E. coli* was preliminarily defined by its aggregative pattern of adherence (AA) to HEp-2 cells (3). Their identification was facilitated by a DNA probe developed from a plasmid (pCVC432 syn. pAA) necessary for expressing the aggregative phenotype (4). Based on that probe, a polymerase chain reaction (PCR) test was developed for screening EAEC strains (5). This test, which we used in this research, and another test using the same DNA probe (6), have been better indicators of diarrheagenic strains than the phenotypic Hep-2 cells test.

EAEC is a divergent group in terms of the organisms' ability to induce diarrhea (7), the factors involved in attachment to host cells (8), and kinds of serotypes (8,9). Since certain EAEC serotypes were already prevalent throughout the world, we studied whether those strains could be found in our isolates of diarrheagenic EAEC. To simplify the detection of EAEC, we selected a bacteriophage active specifically on our clinically evaluated EAEC strains of *E. coli* O126:H27. EAEC have been rarely evaluated clinically in Israel. Here we address that problem by reporting clinical and microbiologic findings of children hospitalized with gastroenteritis in which our most common EAEC serotype, O126:H27, was found.

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The Study

Clinical signs and the laboratory findings were evaluated for 17 children <2 years of age, hospitalized in four pediatric wards in different areas of Israel. All these children had gastroenteritis attributable to EAEC or enterotoxigenic *E. coli* (ETEC) of serotype O126:H27 (Table 1).

Serotyping was performed (10). To determine O-antigen, cultures were heated to 120°C for 1 h, then checked for agglutination with specific O-antisera at 50°C overnight. For determination of H-antigen, motile cultures were grown overnight in nutrient broth, treated with 0.5% formaldehyde, and investigated for agglutination with specific H-sera at 50°C for 2 h.

To detect EAEC, we used PCR primers specific for a short sequence of the plasmid pAA of EAEC, which is necessary for adherence. Analysis for the presence of pCVD432 sequences was performed at the Institute of Hygiene and Microbiology, the University of Wuerzburg, and the Institute of Medical Microbiology and Hygiene, Technical University of Dresden, Germany. Briefly, *E. coli* were isolates grown overnight on L-agar, and a single colony was suspended in 50 µL of phosphate-buffered saline (PBS). Amplification was carried out in a total volume of 50 µL containing each nucleotide triphosphate at 200 µm, 30 pmol of each primer, 5 µL of 10-fold concentrated AmpliTaq DNA polymerase synthesis buffer, 1.5 mM $MgCL_2$, 2.5 U AmpliTaq DNA polymerase (Applied Biosystems Applera, Weiterstadt, Germany), and 5 µL of template Oligonucleotides pCVD432/start (5′-CTG GCG AAA GAC TGT ATC AT-3′) and pCVD432/stop (5′-AAT GTA TAG AAA TCC GCT GT-3′) were purchased from Sigma-ARK GmbH (Darmstadt, Germany) (5). The PCR protocol comprises 30 rounds of amplification, each consisting of 30 s at 94°C, 60 s at 52°C, and 60 s at 72°C. The first cycle was preceded by a denaturation step of 10 min at 94°C, and the last extension cycle was followed by a final extension step of 10 min at 72°C.

Enterotoxins were determined by the Asialoganglioside-GM_1 enzyme-linked immunosorbent assay (GM_1-ELISA) method using the direct plate cultures technique. Heat-labile toxin (LT) was determined by GM1-ELISA using monoclonal antibodies against LT (11). Heat-stable toxin (ST) was determined in parallel in the same cultures by an inhibition-GM_1-ELISA that used monoclonal anti-ST (12). The test was performed in two 96-well polystyrene microplates A&B (Nunc A/S Roskilde, Denmark) and comprises several steps. The plates were coated with GM_1 by adding 0.1 mL of 0.3 nmol GM_1 (Sigma, Rehovot, Israel) in 0.1 M PBS, pH=7.2, to each well. After the plates incubated overnight at room temperature, they were washed three times with PBS, blocked with 0.1% bovine

[1]Drs. Shazberg and Wolk contributed equally to this paper.

Table 1. Bacteriologic parameters and clinical signs of children with *Escherichia coli* O126:H27

No.	Age (mo)	EAEC-PCR	Phage sensitivity	ST	Diarrhea (days)	Dehydration	IV fluid	Vomit	Fever	Concomitant clinical findings
1	6	+	+	-	+ (12)	+	+	+	+ 39	
2	1.5	+	+	-	+ (40)	+	+	-	-	Malabsorption, prolonged diarrhea
3	5	+	+	-	+ (4)	+	+	+	+ 40	
4	2	+	+	-	+ (4)	+	+	+	-	
5	15	+	+	-	+ (2)	+	+	+	+ 40	
6	21	+	+	-	+ (4)	+	+	+	+ 39	Tonsillitis
7	18	+	+	-	+ (1)	-	-	-	-	
8	16	+	+	-	+(9)	+	+	-	+39	Otitis
9	11	+	+	-	+ (8)	+	+	-	+40	Tonsillitis
10	16	+	+	-	+ (9)	+	+	+	+40	
11	15	+	+	-	+ (7)	-	+	-	+39	UTI
12	1	+	+	+	+(2)	+	+	-	-	
13	15	+	+	+	+(6)	-	+	-	+40	Tonsillitis leukocytosis
14	1.5	+	NT	+	+ (5)	-	+	-	+38.7	Meningitis
15	6	+	NT	+	+(1)	+	+	+	+40	
16	1 week	-	-	+	+(6)	+	+	-	-	
17	9	-	-	+	+ (5)	+	+	+	+40	

[a]EAEC, enteroaggregative *Escherichia coli*; PCR, polymerase chain reaction; ST, heat-stable toxin; UTI, urinary tract infections; NT, not tested.

serum albumin (BSA) in PBS for 30 min at 37°C, and finally washed once with PBS. To each of the GM_1-coated wells in plate A was added 0.2 mL LB broth, Lennox medium, adjusted to 45 µg lincomycin/mL and 2.5 mg glucose/mL. From each bacterial isolate, five colonies grown on McConkey agar were transferred directly into five separate wells. The cultures were grown for 24 h at 37°C with moderate shaking. Plate B (without the bacterial cultures) was processed after step 1 in a different way to determine ST. Briefly, plate B was coated with ST-CTB (consisting of the B-subunit of cholera toxin conjugated to ST) by adding 0.1 mL of ST-CTB in 0.1% BSA–PBS to each well and incubation of the plate at room temperature for 60 min. Then the plate was washed three times with PBS. To each well in plate B, 0.05 mL of culture medium from plate A was added (presumed to contain ST); immediately thereafter, 0.05 mL of the monoclonal antibody against ST (anti-ST) was added, and the plate was gently shaken. The plate was incubated for 90 min at room temperature and then washed three times with 0.05% PBS-Tween. After the culture medium was disposed of, plate A was washed three times with PBS-Tween. To each well, 0.1 mL of monoclonal antibody against LT (anti-LT) in PBS-BSA-Tween was then added. The plate was incubated for 90 min at room temperature and then washed three times with PBS-Tween. To each well of plates A and B we added 0.1 mL of goat anti-mouse immunoglobulin G–horseradish perioxidase (Jackson Immuno-Research Laboratories, West Grove, Pennsylvania) in PBS-BSA-Tween. The plates were incubated for 90 min at room temperature and then washed three times with PBS-Tween. Substrate was prepared by dissolving 10 mg of ortophenylene diamine (Sigma) in 10 mL of 0.1 M sodium citrate buffer (pH=4.5) to which 4 µL of 30% $H_2O_2$was added. To each well in plates A and B, 0.1 mL of this substrate solution was added. After 20 min, the plates were read at 450 nm in a Micro ELISA Auto Reader spectrophotometer (Dynatech Inc., Alexandria, VA).

When the optical density (OD) decreased >50% as compared with the OD of anti-ST mixed with ST negative control culture, which run in parallel to the experimental wells, the result was considered ST positive. When the OD value at 450 was ≥0.100 above the background, the result was considered LT positive. Since serotype O126:H27 was prevalent in our EAEC cultures, we tried to isolate bacteriophages specific to the EAEC of this serotype from sewage water. Five unrelated strains of EAEC serotype O126:H27 were used. One milliliter of an early logarithmic broth culture of each strain was seeded in a bottle of 50-mL nutrient broth. After incubation of 3 h at 37°C, 5 mL of sewage water was added to each bottle. After a new incubation of 6 h, cultures were killed by adding 1 mL of chloroform, followed by intensive shaking. The next day the supernatant of each bottle was tested for activity on the respective strain. The isolated phages were then diluted and purified twice by single plaque isolation (13). The five phages were active on EAEC strains of serotype O126:H27.

From July 1999 to December 2001, we collected and characterized 1,368 isolates of diarrheagenic *E. coli.* Of these isolates, 88 (6.4%) belonged to one of the five most common EAEC serotypes, i.e., serotype O126:H27 (n=48), O111:H21 (n=16), O125 (n=11), O44:H18 (n=11), O?:H10 (n=2) (Table 2). The percentages of EAEC

Table 2. Serotypes of enteroaggregative *Escherichia coli* (EAEC) evaluated by polymerase chain reaction

E. coli serotype	O126:H27	O111:H21	O125:Hx	O44:H18	O?:H10	Totals
No. positive	35 (73%)	12 (75%)	H9=5 H49=1 6(54.5%)	2 (18%)	1	56
No. negative	13 (27%)	4 (25%)	H49=3 H6=2 5(45.5%)	9 (82%)	1	32
Totals	48	16	11	11	2	88

PCR–positive strains (Table 2) were as follows: 73% in *E. coli* O126:H27 and 75% in *E. coli* O111:H21. In *E. coli* O125, the percentage was approximately 50%, and in *E. coli* O44:H18, unlike reported elsewhere (14), this percentage was low.

To determine if the isolated phages were specific for the enteroaggregative strains of serotype O126:H27, the five phages were tested by spot test at routine test dilution on our EAEC and non–EAEC cultures of this serotype. Four phages were active on both kinds of strains. Only phage no. 4 was active on 33 of the 34 EAEC cultures and on 1 of 12 non-EAEC cultures (Table 3). The sensitivity of this phage was 97%, and its specificity was 91%. This phage could therefore be used as an indicator for AA in this *E. coli* serotype.

E. coli O126: H27 was found in stools from 17 children in four pediatric wards in various areas in Israel (Table 1). The stools were watery; no mucus or blood was seen. Most of the children were dehydrated and needed IV treatment with fluids and electrolytes. Some children vomited several times. All 17 patients had a normal leukocyte count for age. Twelve of them had high fever (38.7°C–40°C). Three of these 12 children had diarrhea concomitant with other diseases (patients 11, 13, and 14). Stool cultures of these three children were taken as part of an investigation of febrile disease. The same three children received antibiotic treatment; all others recovered without antibiotics. The length of hospitalization was 2–8 days. The duration of diarrhea was 1–40 days (median 5 days) starting, in some cases, before hospitalization. ST was produced in six patients (nos. 12–17), while LT was not produced in any. Five patients (nos. 1, 2, 8, 9, 10) had prolonged diarrhea of >1 week, characteristic of EAEC (15).

Conclusions

In our patients, EAEC serotype O126:H27 appears to be a pathogenic agent of young children who require hospitalization and dehydration treatment. This same serotype has been reported as a common cause of diarrhea in children from England (16), Japan (17), and Bangladesh (9). However, we were not able to associate that serotype exclusively with the enteroaggregative pathotype, since nonaggregative Ec O126;H27 strains from hospitalized children (patients 16 and 17 in Table 1) produced ST and might therefore belong to the pathotype of ETEC.

Table 3. Phage sensitivity *of Escherichia coli* O126:H27 compared to EAEC-PCR[a]

Phage sensitivity[b]	EAEC-PCR positive	EAEC-PCR negative
Positive	33 isolates	1 isolate
Negative	1 isolate	11 isolates

[a]EAEC PCR, enteroaggregative *Escherichia coli* polymerase chain reaction.
[b]Sensitivity = 97%; specificity = 91%.

However, ST was apparently not the main diarrheagenic factor, since in the five children with prolonged diarrhea no ST was produced. Some other kind of toxin was probably involved in these cases. In strains from some patients (Table 1, numbers 12–15) we found both traits of EAEC and ETEC in the same organism. A simple test to identify EAEC in routine laboratory work is needed. A possible solution is to use a phage sensitivity test in addition to serotyping, such as we used here for EAEC O126:H27. Preliminary results suggest that this test (Table 3) is a reliable indicator. If this fact is confirmed on a large number of strains, specific phages might also be selected for EAEC of other serotypes.

The obvious accumulation of pCVD432-positive *E. coli* of serotype O126:H27 suggests that we found a clone that spread in Israel and probably has a selective advantage.

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Cryptosporidium muris, a Rodent Pathogen, Recovered from a Human in Perú

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Cryptosporidium muris, predominantly a rodent species of *Cryptosporidium*, is not normally considered a human pathogen. Recently, isolated human infections have been reported from Indonesia, Thailand, France, and Kenya. We report the first case of *C. muris* in a human in the Western Hemisphere. This species may be an emerging zoonotic pathogen capable of infecting humans.

Cryptosporidiosis can be a debilitating diarrheal disease. While infections are normally acute and self-limiting in immunocompetent persons, cryptosporidiosis can be life threatening in those with compromised immune systems. In humans, cryptosporidiosis is caused predominantly by *Cryptosporidium parvum* or *C. hominis* (the latter was previously known as the *C. parvum* human genotype), and major outbreaks of the disease have been clearly associated with contaminated drinking water (1).

Recently, another species of *Cryptosporidium*, *C. muris*, has been suggested to be of concern to human health. *C. muris* is a parasite first identified in the gastric glands of mice (2). Experimental transmission studies have shown that the parasite readily infects multiple nonrodent hosts including dogs, rabbits, lambs, and cats (3). *C. muris*–like organisms have also been reported as opportunistic infectious agents in immunocompromised nonhuman primates (4). In the past 2 years, five cases of infections with *C. muris* or *C. muris*–like parasites have been reported from HIV-positive and healthy persons in Kenya (5), France (6), Thailand (7), and Indonesia (8). In this paper, we report on the first documented case of *C. muris* in a human in the Western Hemisphere. The parasite was recovered during the summer of 2002 in stools of an HIV-positive Peruvian woman with severe diarrhea. This finding was confirmed by light microscopy, polymerase chain reaction (PCR)–restriction fragment length polymorphism (RFLP), and DNA sequencing.

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The Study

In 2002, we conducted a year-long collaborative study on the epidemiology of *Cyclospora cayetanensis* infections in Perú. As part of that study, we collected approximately 100 stool samples in 2.5% potassium dichromate solution from persons in Lima and Iquitos with *Cyclospora* infection. Fecal samples were initially identified as *Cyclospora*-positive in Lima, and then transported to the United States for additional confirmation using wet mount and Nomarski interference contrast microscopy.

Two stool samples, which were taken on two sequential days from an HIV-positive woman who was 31 years of age, contained oocysts that appeared, based on morphology, to be *Cryptosporidium muris*. Low numbers of *Cyclospora cayetanensis* and *Blastocystis hominis* oocysts were also identified in the stool samples. The *Cryptosporidium muris* infection was initially identified by using wet mount microscopy with oocysts (n=25) averaging 6.1 (± 0.3) x 8.4 (± 0.3) µM (range 5.6–6.4 x 8.0–9.0) and a shape index (length/width) 1.38 (1.25–1.61) (Figure 1). Numbers of oocysts were determined semiquantitatively in each sample by hemacytometer, with an estimated 737,000 and 510,000 oocysts/g recovered from the submitted samples on day 1 and day 2, respectively. The diagnosis of *C. muris* was later confirmed through DNA analysis.

HIV was first diagnosed in the patient in November 2000 by using enzyme-linked immunosorbent assay and Western blot (immunoblot). She arrived at the hospital clinic in June 2002 with fever and reported that she had been experiencing diarrhea for >3 months. The patient reported that she had lost approximately 25 lbs. in the past 7 months, consistent with HIV-wasting syndrome. Her chest x-ray was abnormal, but four direct sputum examinations for acid-fast bacteria using Ziehl-Neelsen staining were negative, as were efforts at culturing *Mycobacterium tuberculosis*.

Other laboratory values for this patient at the time of stool sample collection were as follows: CD4 cell count 66/µL; hematocrit 36%; leukocytes 4,100/µL with 4% bands, 55% neutrophils, 27% lymphocytes, and 0% eosinophils; urine examination normal; creatine 0.8 mg/dL; urea 21 mg/dL; glucose 105 mg/dL; serum glutamic oxalacetic transaminase 30 IU/L; serum glutamic pyruvic transaminase 46 IU/L; and bilirubin 0.9 mg/dL.

The diagnosis of *Cryptosporidium* in the patient's samples was confirmed by a small subunit rRNA-based nested

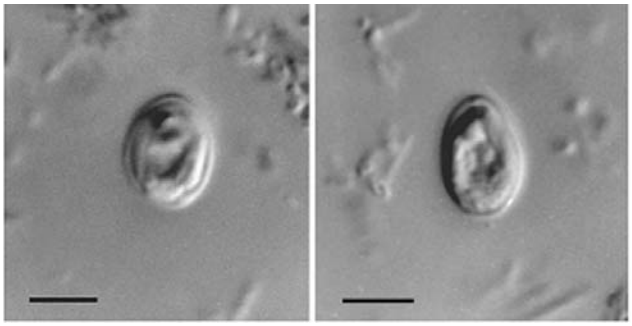

Figure 1. Nomarski interference contrast photomicrographs of *Cryptosporidium muris* from the feces of an HIV-positive human. Scale bars = 5 μm.

PCR, which amplified a portion of the rRNA gene (830 bp). *Cryptosporidium* spp. was determined by the banding patterns of restriction digestions of PCR products with *Ssp*I, *Vsp*I, and *Dde*I (9). Diagnosis was confirmed by DNA sequencing of three independent PCR products from each sample in both directions on an ABI PRISM 3100 (Applied Biosystems, Foster City, CA) instrument. Figure 2 shows the RFLP analysis of three PCR products from each sample with restriction enzymes *Ssp*I and *Vsp*I; these results suggest that these PCR products belonged to either *C. muris* or *Cryptosporidium andersoni* (10). Further RFLP analysis with *Dde*I showed banding patterns identical to *C. muris* (9; Figure 2). All DNA sequences obtained from the six PCR products were identical to those previously reported by Xiao et al. (10,11) from *C. muris* from a Bactrian camel, a rock hyrax, and mice (GenBank accession nos. AF093997 and AF093498) and another isolate recently found in an HIV patient in Kenya (5).

After the diagnosis of intestinal parasite infection, the patient was treated with TMP-SMX (trimethoprim 160 mg, sulfamethoxazole 800 mg) Forte twice a day for 1 week and then TMP-SMX once a day for *Pneumocystis carinii* pneumonia prophylaxis. The patient was also placed on AZT/3TC and nevirapine. The patient recovered with no further evidence of *Cyclospora*, *Blastocystis*, or *C. muris* in stool samples taken 2 months posttreatment. She became afebrile and had gained 5 kg as of 2 months' posttreatment. Molecular analysis of a stool sample collected 122 days after the initial diagnosis confirmed that the patient had recovered from the *C. muris* infection.

Conclusions

This report represents the third confirmed case of *C. muris* infection in humans. Previously, one case of *C. muris* infection was identified in an HIV-positive child in Thailand and in an HIV-positive adult in Kenya using microscopy and molecular analysis (5,7). *C. muris* and *C. andersoni*–like oocysts were found in two healthy Indonesian girls, but the diagnosis was not confirmed by molecular tools (8). One putative *C. muris* infection was reported in an immunocompromised patient in France based on sequence analysis of a small fragment of the SSU rRNA (6). However, the sequence presented was more similar to that of *C. andersoni* (2-bp differences in a 242-bp region) than to *C. muris* (8-bp differences in the region).

Although determining whether or not the *C. muris* contributed medical problems in this patient is not possible, detecting *C. muris* in her stool sample is an unexpected finding. A major difference between *C. parvum* or *C. hominis* and *C. muris,* is that *C. parvum* and *C. hominis* normally colonize the intestine, whereas *C. muris* is a gastric pathogen in cattle. Anderson (12) and Esteban and Anderson (13) reported that another gastric species, *C. andersoni,* infects only the glands of the cattle stomach (abomasum), where it retards acid production. These researchers postulated that this process may affect protein digestion in the abomasum and account for the fact that milk production in cows that are chronically infected with *C. muris* appears to be reduced by approximately 13%. Thus, an infection by *C. muris* may perhaps cause similar protein digestion problems in human infections, particularly in HIV-positive persons.

Even though only a few cases of *C. muris* infections have been identified so far in humans, gastric cryptosporidiosis occurs much more often than believed, especially in HIV-positive persons. Up to 40 % of cryptosporidiosis in HIV-infected persons includes gastric involvement (14). Although most gastric *Cryptosporidium* infections in HIV-positive persons are likely caused by *C. parvum* or *C. hominis* because of immunosuppression, the contribution of *C. muris* probably has been underestimated. Thus, molecular characterizations of stomach tissues from patients with gastric cryptosporidiosis may help us to understand the pathogenesis of human *Cryptosporidium* infection.

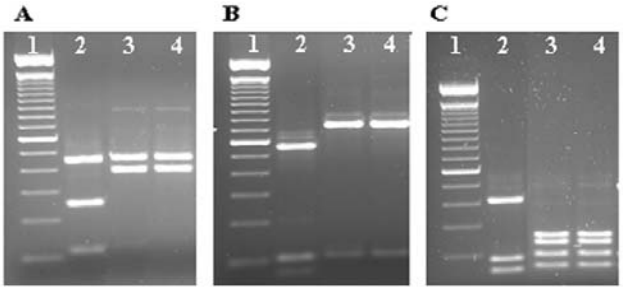

Figure 2. Identification of *Cryptosporidium muris* from two stool samples from a Peruvian patient using restriction fragment length polymorphism analysis of polymerase chain reaction products with *Ssp*I (A), *Vsp*I (B) and *Dde*I (C). Lane 1, 100-bp molecular markers; lane 2, *C. hominis* control; lanes 3 and 4, *C. muris* from the patient.

Our report expands the geographic range of suspect *C. muris* infections in humans and suggests that this species may be a global emerging zoonotic pathogen. This pathogen may be of particular importance to persons living in regions where rodents live in close proximity to humans and sanitation may be minimal. *C. muris* may also be more prevalent than currently recognized. The organism is nearly twice as large as *C. parvum* and closer in size to *Cyclospora cayetanensis*. Although *Cyclospora* autofluoresces while *Cryptosporidium* does not (15), *C. muris* could still be easily misdiagnosed, since few laboratory workers would be familiar with *C. muris* or its morphology.

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Malaria Clusters among Illegal Chinese Immigrants to Europe through Africa

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Between November 2002 and March 2003, 17 cases of malaria (1 fatal) were observed in illegal Chinese immigrants who traveled to Italy through Africa. A further cluster of 12 was reported in August, 2002. Several immigrants traveled by air, making the risk of introducing sudden acute respiratory syndrome a possibility should such illegal immigrations continue.

From November 2002 to March 2003, 17 cases of malaria were noted among illegal Chinese immigrants in seven hospitals across central and northern Italy (15 cases of *Plasmodium falciparum*, 1 case of *P. malariae*, and 1 mixed infection of *P. falciparum* and *P. malariae*). One patient died. Until recently, imported malaria in this group of illegal immigrants from China was not detected by malaria surveillance institutions within Europe (1). Although malaria is still endemic in parts of China, transmission in these regions is low-level (2); the predominant species is *P. vivax*. *P. falciparum* transmission is confined to provinces bordering Laos and Viet Nam. None of the patients reported coming from those areas. Investigating the cluster proved difficult because of language problems and reticence to provide detailed information of travel, since the patients were illegal immigrants (Table). The fatal case occurred in a general hospital in northern Italy. The 20-year-old woman (case 7) was admitted with a high fever, severe hemolytic anemia (hemoglobin 4.4 g/dL), and metabolic acidosis. After 48 hours, because of hypotension, seizures, and subsequent coma, she was transferred to the intensive-care unit of a referral hospital for infectious diseases. The blood film showed a 70% parasitemia with *P. falciparum*. The patient died 96 hours after admission, despite aggressive drug therapy and plasmapheresis.

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Discussion

Before 2000, no cases of *P. falciparum* had occurred in Chinese immigrants living in northern and central Italy, despite a large immigrant population. An initial cluster of 22 cases was described during summer 2000 in the Lombardy Region (3). A cluster of six cases was detected in Tuscany during the same period (4). In both outbreaks, the researchers described high rates of severe disease. All patients were exposed to malaria during a prolonged journey to Europe (3–9 months) through a number of Asian and African countries.

From 2000 to 2002, a total of 10 sporadic cases were reported to the Italian Ministry of Health in 2001 (L. Vellucci, Directorate for Prevention, Ministry of Health, Italy, pers. comm.). The 2003 cluster prompted us to examine hospital records from August 2002, where we identified an additional, previously undetected, cluster of 12 malaria cases in four of our study hospitals (data not included in the table). The Ministry of Health had 26 confirmed *P. falciparum* cases during 2002 (L. Vellucci, pers. comm.), suggesting an ongoing (and possibly increasing) influx of Chinese laborers. Some differences exist between the later cluster and the 2000 cluster. In the 2003 cluster, the proportion of severe cases was lower than in the previous reports, with a patient with a fatal case first admitted to a general hospital where diagnosis of malaria was not considered; in the others, awareness of the possibility of malaria had been raised by the earlier cluster (3,4) and led to prompt diagnosis and treatment, with favorable outcome. A single African country, Côte d'Ivoire, was the transit country for most of the patients. In previous cases, a number of other African countries were used for transit. Visa processing for entry to Europe was arranged by the courier organization in Côte d'Ivoire. The clustering of cases suggests that the illegal immigrants arrive in Europe in groups. Although Italy was the final destination, at least some immigrants entered through France, which also has had reports of *P. falciparum* cases in Chinese immigrants (F. Legros, Centre National de Référence de l'Epidémiologie du Paludisme, France, pers. comm.). As malaria is probably underreported in Europe, additional cases may well have occurred.

Use of clandestine travel by air to emigrate from China, where sudden acute respiratory syndrome (SARS) is present, poses a threat for the African countries, where the introduction of SARS virus could have devastating consequences on their health systems with a potential overlap with the HIV epidemic. Other diseases could be spread or

Table. Characteristics of 17 cases of malaria in illegal Chinese immigrants, Italy

Case	Sex, age[a]	Date first seen by physician	Country of transit	Time spent in country of transit	Mode of travel	Mode of travel to Europe	*Plasmodium* species	Clinical outcome
1	M, 21	11/05/02	Côte d'Ivoire	8 mo	Air	Air	*P. falciparum*	Recovered
2	M, 24	11/11/02	"Africa"	3 mo	Unknown	Air	*P. falciparum*	Recovered
3	F, 20	11/12/02	Côte d'Ivoire	22 d	Road/sea	Air	*P. falciparum*	Recovered
4	M, 22	11/15/02	Côte d'Ivoire	1 mo	Air	Air	*P. falciparum*	Recovered
5	M, 24	11/16/02	Côte d'Ivoire	14 d	Road/sea	Air	*P. falciparum*	Recovered
6	M, 28	01/09/03	Côte d'Ivoire	2 mo	Unknown	Air	*P. falciparum*	Recovered
7	F, 20	01/13/03	Côte d'Ivoire	Few days	Unknown	Air	*P. falciparum*	Died
8	M, 21	02/01/03	Côte d'Ivoire	Unknown	Unknown	Air	*P. falciparum*	Recovered
9	F, 32	02/02/03	Congo	Unknown	Unknown	Air	*P. falciparum*	Recovered
10	M, 22	02/03/03	Côte d'Ivoire	6 mo	Air	Air	*P. falciparum*	Recovered
11	M, 19	02/08/03	Côte d'Ivoire	Unknown	Unknown	Air	*P. falciparum*	Recovered
12	M, 34	02/13/03	Congo	2 mo	Road/sea	Air	*P. falciparum* and *P. malariae*	Recovered
13	F, 24	02/13/03	Côte d'Ivoire	50 d	Air	Air	*P. falciparum*	Recovered
14	M, 40	02/22/03	Côte d'Ivoire	Unknown	Road/sea	Air	*P. falciparum*	Recovered
15	M, 22	02/24/03	Côte d'Ivoire	2 mo	Road/sea	Air	*P. falciparum*	Recovered
16	M, 28	03/01/03	"Africa"	Unknown	Unknown	Air	*P. falciparum*	Recovered
17	M, 23	03/15/03	Côte d'Ivoire	50 d	Road/sea	Air	*P. malariae*	Recovered

[a]M, male; F, female.

acquired by the immigrants in the countries of transit. While curtailing the huge, illegal immigrant system to Europe is difficult, we cannot overemphasize the need for a sound surveillance on imported infectious diseases in this continent.

Both clusters of malaria were detected early through Salute Internazionale Regione Lombardia (SIRL), a network on imported diseases of the Lombardy Region, in conjunction with the European Network on Imported Infectious Disease Surveillance (TropNetEurop). Any physician in Europe who sees a Chinese patient with a history of recent travel and a high fever should exclude malaria, besides considering the possible diagnosis of SARS. Respiratory symptoms are also frequent in uncomplicated malaria (5,6), and acute respiratory distress syndrome has long been recognized as one of the main features of severe malaria (7,8).

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First European Case of Serotype A MAT*a* *Cryptococcus neoformans* Infection

To the Editor: *Cryptococcus neoformans* is an opportunistic fungus that causes meningoencephalitis, primarily in immunocompromised patients. However, *C. neoformans* can also cause illness in apparently normal hosts. The yeast is a heterothallic basidiomycete with two mating types, *MAT**a*** and *MAT*α identified in all the four serotypes, A, B, C, and D. However, the mating type ***a*** of serotype A is a rare and recent finding. One strain was isolated from a Tanzanian AIDS patient and a second from the Italian environment; the first was mating defective (1,2). We report the isolation of a serotype A *MAT**a*** strain of clinical origin that was characterized by mating at high frequency under standard laboratory conditions.

In August 1998, a 45-year-old Hungarian man was admitted to the Laszlo Hospital for Infectious Diseases in Budapest because of septic fever. The patient had a history of hematologic malignancy (Hodgkin disease), which was diagnosed in 1991. He had received several courses of chemotherapy and radiation. After 4 years when his cancer was in remission, in September 1995, the disease recurred (stage IVa) for which he received several more courses of chemotherapy, according to protocols BEAM (carmustine, etoposide, cytarabine, melphalan) and CEP (lomustine, etoposide, prednimustine). In February 1998, another relapse was diagnosed and the patient was given chemotherapy, according to protocol COPP (cyclophosphamide, vincristine, prednisolone, procarbazine) four times. In April 1998, he was hospitalized with herpes zoster infection and treated with acyclovir. At the last admission, in August 1998, he was pancytopenic and had septic fever. *Salmonella enteritidis* was cultured from his blood. The salmonella septicemia was successfully treated with ceftriaxone. As palliative treatment, he received 4x10 mg vinblastine for his residual disease. On September 30, he became febrile again. *Cryptococcus neoformans* was isolated from his blood, although cerebrospinal fluid culture and serologic tests were negative. On the right fossa cubitalis, cellulitis and a tender mass were present, although he did not have a history of recent central line or cytostatic treatment on this side. *Cryptococcus neoformans* was isolated from the sample taken from the mass. Antifungal treatment was started with 600 mg fluconazole per day and continued with amphotericin B, 1 mg/kg/day. The patient died 6 weeks after the isolation of *Cryptococcus*, probably because of his uncontrolled Hodgkin disease. As far as the physician was aware, the patient had not visited other countries.

The strain, isolated from the patient's blood during the European Confederation of Medical Mycology Cryptococcosis Survey, was sent for typing to the European Convenor. The isolate, IUM 99-3617, was identified as serotype A using Crypto Check serotyping kit from Iatron Laboratories (Tokyo, Japan) and genotyped as VN6 by multiplex polymerase chain reaction (PCR) (3) by using the primers previously described (4,5). The fungus was shown to be haploid by cytofluorimetric analysis (6). The strain's fertility was investigated, according to Kwon-Chung (7), by crossing the isolate with reference serotype A strains H99 (*MAT*α) and IUM 96-2828 (*MAT**a***), and with serotype D congenic strains JEC20 (*MAT**a***) and JEC21 (*MAT*α). When cocultured with *MAT*α strains (H99 and JEC21), IUM 99-3617 produced abundant basidiospores. On the contrary, the strain did not mate with JEC20 (*MAT**a*** *D*) or with IUM 96-2828 (*MAT**a*** *A*).

The genotypic and phenotypic characteristics of the fungus were then compared with those of serotype A (*MAT**a*** and *MAT*α) reference strains. The mating type was analyzed by using PCR amplification of *MF**a***, *MF*α genes, and *STE20**a***- and *STE20*α-specific genes for serotype A and serotype D. PCR reaction was performed as previously reported (4). The amplification product showed that IUM 99-3617, like IUM 96-2828, contains only serotype A *STE20**a*** and *MF**a*** genes.

To further confirm that IUM 99-3617 was *MAT**a*** in mating type, *MF**a*** and *STE 20**a*** genes were sequenced by an ABI PRISM 310 automatic sequencer using Big Dye Terminator (Applied Biosystems, Monza, Italy) and the primers, forward and reverse strands previously reported (4). The sequences were then aligned with the reported sequences of IUM 96-2828 (2,8), the Tanzanian isolate 295.1 (1), H 99, and the congenic JEC 20 and JEC 21 strains. The IUM 99-3617 sequences were found to be identical to those of IUM 96-2828 and of the Tanzanian isolate 295.1. The *MF**a***A and the *STE20**a***A sequence of IUM 99-3617 have been submitted to GenBank database (available from URL: www.ncbi.nlm. gov/Bankit/nhpbankit.cgi) under accession number AY182035 and AY182036, respectively.

Virulence studies in the mouse model demonstrate that, like IUM 96-2828, the strain is significantly less virulent than H99. The latter strain caused 100% deaths day 29, while IUM 99-3617 took until day 60 to kill 60% of mice (unpub. data). No difference was observed among the three serotype A strains when virulence factors such as capsule, melanin, phospholipase activity, and ability to grow at 37°C were tested.

The MAT***a*** of *C. neoformans* serotype A was long regarded as extinct or as existing in an undiscovered ecologic niche until the recent

finding of the clinical and the environmental isolate (1,2). The existence of MAT***a*** in nature is also supported by recent studies designed to established the origin of the serotype AD strains (4,5). These studies demonstrated that AD strains were diploid or aneuploid hybrids derived from a fusion of serotype A and D parents and that several of them were harboring a serotype A MAT***a*** locus. These hybrid strains have been found fairly often in Europe (9,10).

The finding of this isolate provides evidence of the pathogenic role of this rare mating type, emphasizes the critical function of molecular genetic tools in the characterization of *C. neoformans* populations, and represents an advance in knowledge of this fungal species whose genome is undergoing identification by a worldwide research team.

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Severe Acute Respiratory Syndrome: Relapse? Hospital Infection?

To the Editor: Severe acute respiratory syndrome (SARS) is an emerging infectious disease worldwide, and relapsing SARS is a major concern. We encountered a 60-year-old woman who was admitted to the Princess Margaret Hospital in Hong Kong on March 29, 2003, with a fever of 39°C, chills, cough, malaise, and sore throat for 2 days before admission. She had no history of travel within 2 weeks of admission. She also had no close contact with patients who had a diagnosis of suspected or confirmed SARS. Chest radiograph on admission indicated consolidation over the right middle zone. In accordance with the diagnostic criteria proposed by the World Health Organization (WHO), this patient's condition was diagnosed as SARS in view of her symptoms, temperature, and chest radiograph findings (1).

Standard microbiologic investigations to exclude common respiratory virus and bacterium for community-acquired pneumonia, including *Mycobacterium tuberculosis*, were negative in our patient. Reverse transcriptase–polymerase chain reaction (RT-PCR) of nasopharyngeal aspirate samples was negative for coronavirus twice. The coronavirus antibody titer was less than 1/25. The patient was initially treated with oral clarithromycin (500 mg twice a day) and intravenous amoxycillin-clavulanate combination (1.2 g three times a day). Despite the negative evidence for coronavirus infection, she was treated with intravenous ribavirin (24 mg/kg once a day) and hydrocortisone (10 mg/kg once a day) after 48 hours of antibiotics therapy (2). The patient's symptoms were relieved, and she remained afebrile 3 days after admission. Tolerance for medication was good except for a moderate degree of hemolytic anemia (her hemoglobin level dropped to 9.1 g/dL) and hypokalemia that developed during treatment. On day 15, the chest radiography was clear. The patient was discharged after 3 weeks of hospital stay.

The patient attended outpatient clinic on day 35, complaining of exertional dyspnea, low-grade fever, and malaise since her discharge. Her chest radiography showed extensive shadowing. Computer tomographic scan of the thorax indicated widespread ground-glass shadowing in both lung fields, which was especially prominent at left lower and lingular lobes.

Her hemoglobin level had dropped further to 8.4 g/dL. Sputum culture yielded substantial growth of methicillin-sensitive *Staphylococcus aureus* and *Pseudomonas aeruginosa*. RT-PCR results of throat and nasal swabs were positive twice for coronavirus, but coronavirus cultures from these areas were negative. One month after onset, her coronavirus antibody titer was 200. In view of possible relapse of SARS, she was treated with oral ribavirin (1,200 mg/day) and lopinavir (133.3 mg/day)/ritonavir (33.3 mg) combination (3 capsules twice a day) in addition to intravenous piperacillin/tazobactam combination. The patient was afebrile, and symptoms improved 3 days after admission. Serial chest radiograph showed gradual resolution of shadowing. Subsequent RT-PCR and sputum culture were negative.

This case illustrates several important issues regarding problems of infection control, diagnosis, and management of SARS. As the definition of SARS is nonspecific, patients with upper respiratory infection or community-acquired pneumonia could be mislabeled as having SARS. Accommodating confirmed SARS patients and patients mislabeled as having SARS in the same facility may be disastrous. Unfortunately, isolating every single case is impossible, particularly when a large number of patients are admitted. Our patient may have acquired the disease after admission since she was placed in the same ward with other patients confirmed to have SARS. For this reason, special cohorting of SARS patients with closely related signs and symptoms should be strictly implemented at admission. Since fever is the most common feature of SARS, isolating febrile cases with respiratory or gastrointestinal symptoms may be appropriate. Even patients with fever alone should be quarantined since the other symptoms of SARS may not be clinically obvious. Secondly, the sensitivity of diagnosing a coronavirus infection on admission is only 32% to 50% by nasopharyngeal RT-PCR test (3,4). Many infected cases will be missed as a result. Our patient may have had a relapse of disease during her second admission, although she had positive RT-PCR and antibody surge only 1 month after onset. However, we could not conclude whether the first RT-PCR on admission was a false negative or whether the patient acquired coronavirus infection in the hospital. Our study showed that sensitivity for diagnosing coronavirus infection could be increased by performing RT-PCR on samples from different parts of the body (4). Unfortunately, these samples were not taken from our patient. Furthermore, the chest infection with organisms recovered from her sputum could be the sole reason for her second admission, especially when her immune system was weakened by the administration of a high-dose steroid. The presence of genetic material for coronavirus from her nasal cavity and throat might not suggest that the virus is active. The absence of coronavirus growth in this patient might indicate that the virus is no longer viable, although the culture technique itself might not be sensitive enough to justify this claim. Therefore, further refinement of the diagnostic techniques for SARS is essential, especially for diagnosis during early onset. Thirdly, giving treatment to a patient without a legitimate diagnosis may be inappropriate, especially when the treatment carries substantial adverse effects, as illustrated in our patient, and a universally accepted therapy has not been available. Whether lopinavir/ritonavir combination is the key to a cure remains to be clarified, despite the satisfactory response that we observed, since the clinical and radiologic improvement in our patient might be the natural course of the disease.

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Remembering Jonathan M. Mann in a World Ajar

September 2003 marks the anniversary of the deaths of Jonathan M. Mann and his wife Mary Lou Clements aboard Swiss Air flight 111, which crashed off the shore of Peggy's Cove, Nova Scotia, 5 years ago. Although Jonathan and I were both members of the Council of State and

Territorial Epidemiologists in the early 1980s, when Jonathan served as state epidemiologist for New Mexico, our paths did not cross until years later in 1990. Jonathan had reluctantly resigned as director, Global AIDS Activities, World Health Organization, to become full professor at Harvard School of Public Health. I had taken a year's leave of absence from my position in Maine to enroll in Harvard's Master of Public Health program.

In a talk at the Centers for Disease Control and Prevention, Jonathan once outlined many of his hopes and fears for AIDS activities worldwide. Moved by his pleas for global commitment to the epidemic, I sought out Jonathan at the opening reception for new Harvard students. I shared his dreams for public health activism. We believed in inspiring others to careers in applied public health, so we initiated a brown bag lunch series for students and faculty to share experiences about work in public health (1). The common thread throughout these discussions was universal human rights and respect for human dignity.

Jonathan went on to establish the Francis Xavier Bagnoud Center for Health and Human Rights at the Harvard School of Public Health and used his position to promote health as the broad-based core of human values. His lectures on universal human rights centered on the idea that health transcends geographic, political, economic, and cultural barriers. Jonathan drew on his past experiences with the HIV epidemic to argue that the developing world would never achieve economic or political stability unless the health of its people improved. He maintained that, if not addressed, the health problems of the developing world would pose a global threat. "Public health," he wrote, "too often studies health without intruding upon larger, societal, inescapably laden issues…. If the public health mission is to assure the conditions in which people can achieve the highest attainable state of physical, mental and social well-being, and if these essential conditions predominantly are societal, then public health must work for societal transformation" (2).

Jonathan argued that discrimination and other violations of human rights were primary pathologic forces working against the improvement of public health and that if we ignored the plight of those whose rights were violated, we would be less than human ourselves. Jonathan very much admired Eleanor Roosevelt, chair, Declaration of Human Rights Drafting Committee, who on the 10th anniversary of the declaration asked, "Where, after all, do universal human rights begin? In small places, close to home—so close and so small that they cannot be seen on any map of the world. Such are the places where every man, woman and child seeks equal justice, equal opportunity and, equal dignity. Without concerted citizen action to uphold them close to home, we shall look in vain for progress in the larger world" (3).

On Jonathan's desk at Harvard, amidst family photographs, was a framed joker taken from an ordinary deck of cards. When I asked about its significance, he responded that, despite life's challenges, it remains important to smile. So smile we must at the memory of Jonathan and his many accomplishments. Each year, the Council of State and Territorial Epidemiologists remembers by holding a distinguished lecture named in honor of Jonathan M. Mann.

The public health practitioner must respond to the needs of people and yet be sensitive to world politics. In solving difficult issues, the practitioner must understand the interconnection of social values and scientific truths and work collaboratively with the medical community. Moved to the forefront by recent acts of terrorism, public health has achieved recognition as first responder and as integral part of planning for and responding to catastrophic health crises. We cannot promote safety and security if we fail to recognize, and advocate for, people around the globe who do not have access to basic health care, adequate living and working conditions, or education to enlighten their response to life's challenges. The anniversary of Dr. Mann's untimely death serves as reminder to the medical and public health communities of the ongoing need to promote universal human rights and to focus energies and resources on a global approach to public health.

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Mild Severe Acute Respiratory Syndrome

To the Editor: Severe acute respiratory syndrome (SARS) is a recently recognized infectious disease caused by a novel human coronavirus

(SARS-CoV) (1). The first case of SARS, diagnosed as communicable atypical pneumonia, occurred in Guangdong Province, China, in November 2002. Thousands of patients with SARS have been reported in over 30 countries and districts since February 2003.

SARS is clinically characterized by fever, dry cough, myalgia, dyspnea, lymphopenia, and abnormal chest radiograph results (1–3). According to the World Health Organization (WHO) (4), the criteria to define a suspected case of SARS include fever (>38°C), respiratory symptoms, and possible exposure during 10 days before the onset of symptoms; a probable case is defined as a suspected case with chest radiographic findings of pneumonia and other positive evidence.

Although most reported patients with SARS met the WHO criteria, we found two SARS case-patients who did not exhibit typical clinical features. Case 1 was in a 28-year-old physician. He had close contact with three SARS patients on February 1, 2003. After 10 days, he had mild myalgia and malaise with a fever of 37.3°C. He had no cough and no other symptoms. Leukocyte and lymphocyte counts were normal. The chest radiograph showed no abnormalities. He did not receive any treatment except rest at home. His symptoms disappeared after 2 days. He completely recovered and returned to work 4 days after onset of symptoms. After 12 weeks, his serum was positive for immunoglobulin (Ig) G against SARS-CoV in an indirect enzyme-linked immunosorbent assay (ELISA) with inactivated intact SARS-CoV as the coated antigen.

Case 2 was in a 13-year-old boy whose mother had been confirmed to have SARS on February 4, 2003. Fever developed in the boy 20 days after his mother's onset of the disease. He did not come into contact with other confirmed SARS patients during this period. He had a mild headache and diarrhea with a fever from 37.2°C to 37.8°C for 3 days. No other symptoms and signs developed, and a chest radiograph showed no abnormalities. He completely recovered after 5 days. After 12 weeks, his serum was positive for IgG against SARS-CoV, detected with an ELISA.

In both case-patients, SARS had been initially excluded in spite of their close contacts with SARS patients because their symptoms could be explained as a common cold, and no specific diagnostic approaches were considered when they were sick since the causative agent of SARS was not identified until March 2003 (5). However, their serum specimens were positive for IgG against SARS-CoV by ELISA. Those results strongly indicate that both patients had been infected with SARS-CoV, although their signs and symptoms did not meet the criteria for the SARS case definition. Mild SARS-CoV infection may not easily be defined clinically, and such patients may potentially spread the disease if they are not isolated.

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Transmission of Severe Acute Respiratory Syndrome

To the Editor: The worldwide pattern of severe acute respiratory syndrome (SARS) transmission in 2003 suggests that transmission has occurred more frequently in communities that share certain social and cultural characteristics. Of 8,500 probable cases since March, >90% were reported from China (including mainland, Hong Kong, and Macau) and Taiwan. Of the other 27 countries reporting SARS occurrences, 23 reported <10 cases and the others 1–3 cases. The small number of transmissions in these other countries suggests that the close contact required for transmission did not occur, whereas in China, community-based transmission has continued. In contrast, the relatively large number of cases in Canada, the United States, Singapore, and Vietnam (which comprise 7% to 10% of the total SARS cases worldwide) is related to the fact that relatively prolonged contact occurred because of the patients' close cultural ties with China. Why does Japan still have no cases of SARS, despite its geographic proximity to the most affected areas? We suggest that transmission has not occurred because Japan remains a society mostly closed to non-Japanese persons and has a his-

tory of casual contact between its citizens and the travelers and noncitizens who reside there.

Hospitals have functioned as junctions for varied communities in spreading the SARS virus further. Because of SARS' likely place of origin, the initial "community" included Chinese persons who then kindled the chain of transmission to other communities throughout the world. Daily, close contact between SARS patients and hospital personnel led to an unusually large number of infections among medical staff members. Effective prevention measures such as vaccines are not available and may be a factor in the spread of the infection.

Even in the era of globalization and mass air transit, most persons live inside a relatively small circle of community, made up of others of similar ethnicity, religious beliefs, educational level, and social class who live in the same vicinity; this sort of small circle has been described as "mutual coexistence" by anthropologist Kinji Imanishi (1). Basically, the SARS-associated coronavirus began circulating among members of such a community. This theory does not suggest that certain ethnic groups are predisposed to be susceptible to SARS.

Why have few cases of SARS occurred in children? All age groups are susceptible to the SARS virus, which is new to humans. However, adults have more chance to become infected through contacts in their daily lives, whereas children do not. Rapid isolation of the adult patients contributed to reduced frequency of exposure for children in that household, which is in contrast to the usual infectious diseases of childhood (since children do not have immunity against many age-old microbes).

Some contradictions exist for our interpretation of the SARS transmission pattern. Investigations have shown that in Canada, Hong Kong and elsewhere, some casual brief contact caused the infection or that the link between the source and the case was not at all clear. We may have missed other important routes of transmission, or a totally unknown element may be involved. Without an answer for this discrepancy, we note that the clinical virology for SARS, such as pattern of virus shedding and host immune response, is still developing (2). For example, a total of 19 cases in China were identified as SARS by coronavirus isolation, polymerase chain reaction, or serologic tests. For two case-patients, the results of three tests were positive; 10 case-patients had negative test results; and in 14 case-patients, the virus was not isolated. Interpreting these results is difficult. In the United States, 97% of the probable cases were attributed to a recent history of international travel to SARS-affected areas. Antibodies to SARS-associated coronavirus were demonstrated for 8 of 41 probable case-patients in convalescent-phase serum, bringing the proportion of laboratory-confirmed cases to 20%, even in the probable cases, and 0% among the suspected cases in the United States so far (3). These results are the best available by laboratories with the current limited technical knowledge. We are not persuaded that casual contact with SARS patients in unfamiliar settings results in contracting the disease.

The winter of 2003 will be critical for observing how the virus behaves, whether the winter climate accelerates the transmission, and how we handle that acceleration. Despite current global efforts, thin lines of transmissions may remain in China; the virus may flare up again. Officials in China and sites of the outbreak must interrupt as many chains of transmission as possible before October. Surveillance should also be intensified. Ongoing study to improve laboratory diagnosis and clinical virology is key, so that effective isolation can be practiced; at present, these measures are the only ones known to interrupt the transmission of SARS. The group on which to focus should be the community in close contact with previous outbreak areas.

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Home-prepared Hamburger and Sporadic Hemolytic Uremic Syndrome, Argentina

To the Editor: Argentina has the highest incidence of hemolytic uremic syndrome (HUS) in the world, and 10.4 cases per 100,000 children <5 years of age were reported in 2001. HUS is the leading cause of acute renal failure in children (1); in 20% to 35% chronic renal failure develops, ranging from mild to serious, and HUS is the second leading cause of chronic renal failure (2,3) in Argentina. Recently, evidence of

Shiga toxin–producing *Escherichia coli* (STEC) infection was found in 59% of Argentine HUS cases; O157:H7 was the predominant serotype isolated (4). Although outbreaks of *E. coli* O157:H7 have been linked to eating contaminated ground beef (5), the organism is rarely isolated from the implicated meat, and the sources of infection for sporadic cases have rarely been identified. We report a sporadic HUS case linked to the consumption of home-prepared hamburger contaminated with *E. coli* O157.

A 2-year-old girl was brought to the emergency room of the Hospital Nacional de Pediatría "Prof. Dr. Juan Garrahan" in Buenos Aires on April 26, 2002, with a 1-day history of bloody diarrhea. Results of a physical examination were normal, and a stool culture was requested. The patient was sent home with dietary and general instructions. As watery diarrhea persisted with vomiting and fever, the girl was brought in again 3 days later. At that time, she exhibited moderate dehydration, pallor, drowsiness, and a generalized seizure of 10 to 15 min duration, tachycardia, tender and tense abdominal wall, and a history of oligoanuria for the last 48 h. Blood pressure was 128/67 mm Hg. The child was hospitalized with a presumptive diagnosis of HUS and anuric renal failure.

Initial laboratory findings included the following: hematocrit, 26%; hemoglobin level, 8.8 g/dL; leukocyte count, 34,800/mm^3; segmented neutrophil count, 29,928/mm^3; platelet count, 91,000/mm^3; serum glucose, 160 mg/dL; blood urea nitrogen (BUN), 268 mg/dL; serum creatinine, 6.3 mg/dL; albumin, 1.7 g/dL; uric acid, 14.8 mg/dL; calcium, 6.9 mg/dL; phosphorus, 6.7 mg/dL; magnesium, 2.0 mg/dL; sodium, 113 mEq/L; potassium, 7.6 mEq/L; pH 7.28; bicarbonate, 10 mmol/L; base excess, –14.9 mmol/L. Chest x-ray findings were normal with a cardiothoracic index of 0.5; results of an abdominal sonogram were normal. A sonogram of the renal system also showed that the kidneys were of normal shape and size and had increased echogenicity. Results of a brain scan showed nonspecific brain atrophy.

The clinical findings and the laboratory features of microangiopathic hemolytic anemia, thrombocytopenia, and acute renal failure were consistent with the diagnosis of HUS. The patient remained anuric for 17 days, required 17 peritoneal dialysis procedures, and six infusions of packed red blood cells. One month after the acute period, she had elevated BUN and serum creatinine levels and massive proteinuria.

The rectal swab sample collected on April 26 was routinely cultured for *E. coli*, *Salmonella*, *Shigella*, *Yersinia*, *Aeromonas*, *Plesiomonas*, *Vibrio,* and *Campylobacter* species. Sorbitol nonfermenting colonies were recovered on sorbitol-MacConkey (SMAC) agar (Difco Laboratories, Detroit, MI) and SMAC supplemented with cefixime (50 ng/mL) and potassium tellurite (25 mg/mL) (CT-SMAC). The bacterial confluent growth zones of both SMAC and CT-SMAC were positive for *stx*2 and *rfb*O157 genes by multiplex polymerase chain reaction (PCR) using the primers described by Pollard et al. (6) and Paton et al. (7), respectively. The *E. coli* O157 isolates were identified by standard biochemical methods and serologic tests by using specific O157 antiserum (INPB-ANLIS "Dr. Carlos G. Malbrán") and sent to the Servicio Fisiopatogenia as National Reference Laboratory (NRL) for further characterization.

As part of the case-control study conducted in the pediatric hospital to identify the risk factors associated with the STEC infection, parents of the 2-year-old girl were interviewed with a standardized questionnaire 8 days after onset of symptoms. Information was collected about her clinical illness, potential exposures in the 7 days before the onset of diarrhea, and demographic issues. Her parents reported that on April 25 the girl had eaten a home-prepared hamburger, made from ground beef purchased from a local market. No other family members reported diarrhea.

Three days after the interview, on May 6, a formal complaint was presented by the mother at the Division of Public Health of Lanús, in the southern area of Buenos Aires, where the family lives. The frozen leftover ground beef from the same package used to make the hamburgers was provided by the child's family and processed at the Laboratorio Central de Salud Pública.

A 65-g portion of the ground beef was incubated overnight at 42°C in 585 mL of modified *E. coli* medium broth containing novobiocin (final concentration, 20 µg/mL). The sample was positive using the *E. coli* O157 Visual Immunoassay (Tecra Internacional Pty. Ltd., French Forest NSW, Australia) (8). Immunomagnetic separation was performed with 1 mL of the culture, according to the instructions of the manufacturer (Dynal, Inc., Oslo, Norway). The concentrate sample was plated onto CT-SMAC and O157:H7 ID medium (bioMérieux, Marcy l'Etoile, France). Up to 20 sorbitol-nonfermenting colonies were selected, confirmed as *E. coli* O157, and sent to NRL.

At NRL, both clinical and ground beef O157 isolates were confirmed as *E. coli* O157:H7, susceptible to all of the antibiotics assayed, as previously described (9). Genotypic characterization showed that the isolates harbored *stx*2, *eae,* and EHEC-*hly*A genes.

To establish their clonal relatedness, the strains were assayed by subtyping methods (9). The identity of the strains was confirmed by the unique pulsed-field gel electrophoresis (PFGE) pattern with the restriction enzymes *Xba*I and *Avr*II, and the same phage type 4. In addition, both strains

were characterized as *stx*2/*stx*2vh-a by PCR-restriction fragment length polymorphism.

To our knowledge, this is the first HUS case in our country in which the source of infection was identified. No investigation was conducted to trace back the source of the ground beef. This study illustrates the importance of the surveillance of STEC infections and the usefulness of molecular subtyping techniques, such as PFGE and phage typing, to determine the relatedness of strains and assess epidemiologic associations.

The public should be made aware that hamburgers, even when prepared at home, can be a source of infection. A primary strategy for preventing infection with *E. coli* O157:H7 is reducing risk behaviors through consumer education (10).

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Q Fever in Thailand

To the Editor: *Coxiella burnetii,* a strict intracellular bacterium, is the etiologic agent of Q fever, a worldwide zoonosis. Humans are infected by inhaling contaminated aerosols from amniotic fluid or placenta or handling contaminated wool (1). The bacterium is highly infectious by the aerosol route. Two forms of the disease are typical: acute and chronic. Acute Q fever is the primary infection and in specific hosts may become chronic (1,2). The major clinical manifestations of acute Q fever are pneumonia and hepatitis. Less common clinical manifestations are aseptic meningitis and/or encephalitis, pancreatitis, lymphadenopathy that mimics lymphoma, erythrema nodosum, bone marrow necrosis, hemolytic anemia, and splenic rupture (2). The main clinical manifestation of the chronic form is culture-negative endocarditis, but infection of vascular grafts or aneurysms, hepatitis, osteomyelitis, and prolonged fever have also been described (1,2). Fluoroquinolones, co-trimoxazole, and doxycycline are active against C. *burnetii* in vitro, and ceftriaxone has been shown to have a bacteriostatic effect and could be effective in the phagolysosome of *C. burnetii*–infected cells (3). However, the treatment of choice for Q fever is doxycycline.

The incidence of this disease is largely unknown, especially in Asia. Q fever has been reported from Japan and China (1). Seroepidemiologic surveys have shown that subclinical infection is common worldwide. Large outbreaks of Q fever have also been reported in many countries in Europe (4). A case series of acute Q fever was diagnosed in a prospective study in patients with acute febrile illness who were admitted to four hospitals in northeastern Thailand: Udornthani Hospital, Udornthani Province; Maharat Nakhon Rtachasima Hospital, Nakornrachasema Pro-

vince; Loei Hospital, Loei Province; and Banmai Chaiyapod Hospital, Bureerum Province. Two serum samples were taken from these patients, on admission and at a 2- to 4-week outpatient follow-up visit, and stored at –20°C until serologic tests were performed at the Faculty of Medicine Siriraj Hospital, Mahidol University, and the National Research Institute of Health, Public Health Ministry of Thailand. All serum samples were tested for the serologic diagnosis of leptospirosis, scrub typhus, murine typhus, and dengue infection as previously described (5,6). After these serologic tests were performed, serum samples from patients with unknown diagnosis were sent for the serologic test for Q fever at Unité des Rickettsies, Faculté de Médecine, Marseille, France. The microimmunofluorescent antibody test, using a panel antigen of *C. burnetii, Rickettsial honei, R. helvetica, R. japonica, R. felis, R. typhi, Bartonella henselae, B. quintana, Anaplasma phagocytophila,* and *Orientia tsutsugamushi*, was used as described previously (6).

A total of 1,171 serum specimens from 678 patients were tested for Q fever. Nine patients (1.3%, eight male and one female) fulfilled the diagnosis of acute Q fever. The median age was 42 (range 15–62) years. All patients were rice farmers, and their farm animals were chicken and cattle. The median duration of fever was 3 (range 1–7) days before admission into the hospital. When initially seen, all patients had acute febrile illness, headache, and generalized myalgia (i.e., a flulike syndrome). Clinical manifestations of acute Q fever in these patients ranged from this flulike syndrome (three patients), pneumonitis (one patient), hepatitis (two patients), pneumonitis and renal dysfunction (one patient), hepatitis and renal dysfunction (one patient), to severe myocarditis and acute renal failure (one patient). An epidemic of leptospirosis has been occurring in Thailand since 1996 (7). All patients in this study received a diagnosis of either leptospirosis or acute fever of undefined cause; therefore, empirical therapy, including penicillin G sodium, doxycycline, and cefotaxime or ceftriaxone, was administered. The patient with hepatic and renal dysfunction was treated with co-trimoxazole. The patient who had severe myocarditis and acute renal failure was treated with a penicillin G sodium and doxycycline combination. He also received a dopamine infusion and hemodialysis. The median duration between admission and a reduction of fever was 3 days (range 1–7) in this case series.

Results of several seroprevalence studies, using the complement fixation test, conducted in both humans and animals suggest that *C. burnetii* infection has been widespread in Thailand since 1966 (8). The prevalence in asymptomatic persons varies from 0.4% to 2.6% (9), and studies in domestic animals show that the highest prevalence of this infection occurs in dogs (28.1%). The prevalence in goats, sheep, and cattle varies from 2.3% to 6.1% (9). However, this clinical case series of acute Q fever is the first diagnosed in this country. The disease was diagnosed in patients in four hospitals, situated in various parts of the northeastern region of Thailand. These data confirmed that Q fever is widespread in this country. The disease had been unrecognized previously because the specific serologic test was not widely available in Thailand.

A self-limited course was suspected in four cases in this series. However, severe cases, especially those with myocarditis, could be fatal. Therefore, doxycycline should be an empirical therapy for patients with acute febrile illness in areas where leptospirosis, scrub typhus, and acute Q fever are suspected, such as in rural Thailand. Further studies to investigate the epidemiology of Q fever in this country are needed.

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Correction, Vol. 9, No. 8

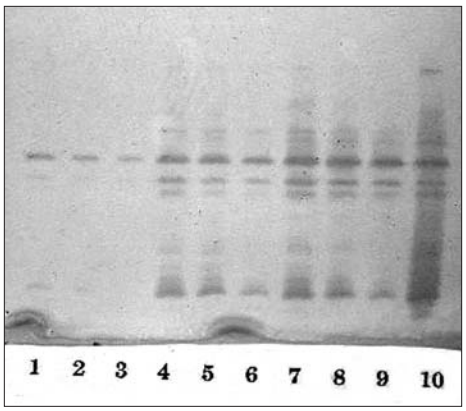
In "Emerging Pathogen of Wild Amphibians in Frogs (Rana catesbeiana) Farmed for International Trade," by Rolando Mazzoni et al., errors occurred in the figure legend on page 996.

The correct caption to the Figure appears below:

Figure. a and b, histopathologic findings from infected frogs. Characteristic sporangia (s) containing zoospores (z) are visible in the epidermis (asterisk, superficial epidermis; arrow, septum within an empty sporangium; bars, 10 µm). c, Skin smear from infected frog, stained with 1:1 cotton blue and 10% aqueous potassium hydroxide (aq KOH) (D, developing stages of *Batrachochytrium dendrobatidis*; arrow, septum within a sporangium; bar, 10 µm). d, Electron micrograph of an empty sporangium showing diagnostic septum (arrow) (bar, 2 µm).

Correction, Vol. 9, No. 8

In the article "NmcA Carbapenem-hydrolyzing Enzyme in *Enterobacter cloacae* in North America," by Sudha Pottumarthy et al., an error occurred the quality of the printing of Figure 3. A revised figure appears below. A color version is available from: URL: http:// www.cdc.gov/ncidod/EID/vol9no8/03-0096-G3.htm



We regret any confusion this error may have caused.

Emerging Infectious Diseases Policy on Corrections

The Emerging Infectious Diseases journal wishes error-free articles. To that end, we

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Expedition Medicine

(Revised Edition),
D. Warrell and S. Anderson, eds.
New York, Fitzroy Dearborn, 2003.
398 pp, hardbound. Price: $55.

Henry M. Stanley, in his second trans-Africa expedition of 1874–1877, lost 68% of his 356 men. Among the casualties, 58 died in battle or were murdered (several were cannibalized), 45 died of smallpox, 21 from dysentery, 14 drowned, and 1 was killed by a crocodile; several others died of starvation (all of this from the preface to this book). Modern-day expeditions—defined as organized and usually challenging journeys with a specific purpose of exploration, research, education, or discovery—are generally less dangerous than that experienced by Stanley. But they require extensive planning and preparation, by both leaders and expedition members, to reduce the frequencies of injury, illness, and death potentially associated with such adventures. This book is a compendium of information that will be useful to those who plan and participate in such journeys.

The editors have divided their book into three sections: expedition planning, field medicine, and specific environmental settings; each section comprises 7–14 chapters written by a total of 24 contributors. The section on planning includes advice on expedition risk assessment, assembling of medical kits, and first aid training. The second section addresses base camp hygiene, water purification, and care of various minor and serious conditions that may be encountered in the field; and the third addresses problems specific to high-altitude, polar, jungle, desert, and aquatic environments.

A major strength of this book is that, while targeted primarily to those (e.g, medical officers) who will be responsible for the health of expedition members, the writing is not highly technical. Hence, it is also suitable for paramedical personnel and other expedition members who may be interested (as they should be) in health issues specific to their expedition. In fact, this book is useful reading for those who may not have the background, time, or resources to join an expedition, but who simply enjoy wilderness experiences or ecotours for recreational purposes. As the editors point out, with the increasing availability of vacations in remote places offered by specialty tour companies, the boundary between such journeys and expeditions has become blurred. The book contains numerous tables and figures, which add to its readability. Inclusion of exotic subjects, such as treatment of bites by sea snakes and scorpions and attacks by large animals, makes for interesting reading.

The chapters vary somewhat in value to the reader. The chapter on commonly encountered ailments, such as gastrointestinal and respiratory illnesses, is very useful. The one on assessment of the injured patient is rather long; it is difficult to imagine wading through this chapter and recording various findings while managing the critically injured person in the field. The chapter on heat-related injuries fails to distinguish between heat exhaustion, heat stroke, and hyponatremia, conditions with different clinical presentations, management requirements, and prognosis. The chapter on medical aspects of survival is brief and not very useful.

Notwithstanding these minor shortcomings, this is a useful volume not only for those who plan and participate in expeditions but also for those of us who may aspire to join an expedition or who just dream of visiting exotic places. I recommend a copy for your bookshelf; better yet, for your backpack.

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The Global Threat of New and Reemerging Infectious Diseases: Reconciling U.S. National Security and Public Health Policy

Jennifer Brower and Peter Chalk
Rand Science and Technology
Santa Monica, California,
United States, 2003
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Brower and Chalk, authors of The Global Threat of New and Reemerging Infectious Diseases: Reconciling U.S. National Security and Public Health Policy, describe their book's purpose as examining "the changing nature of security" and focusing on "the threat of infectious diseases." There are many examples in today's world where the intersection of threats to public health and national security should direct the attention of policymakers, security and public health strategists, and the systems that support each toward an organized response.

The authors use two case studies: HIV/AIDS in South Africa and the U.S. public health response system. The first case, in South Africa, illustrates how a single microbial agent can undermine the economic, social,

and medical underpinnings of a developed country. The second study shows the negative effect of newly emerging diseases such as HIV/AIDS, Hantavirus infection, West Nile virus infection, Creutzfeldt-Jakob disease, and intentionally released agents (*Bacillus anthracis*). This study demonstrates how events can overload the public health response system and weaken public confidence in its government. The reader can easily conclude that the intersection of disease and national security can be dangerously destabilizing and seriously undermine a nation's social, economic, and political order. The recent outbreak of severe acute respiratory syndrome reinterates the global nature and warp speed of emerging infections.

In their summary and conclusions, the authors provide recommendations for policymakers addressing both public health and security issues. The thrust of the authors' conclusions is to push policymakers and strategists to actions that strengthen the infrastructure of a public health response system and broaden the traditional definition of national security to include the impact of naturally occurring and intentionally released microbial agents.

The authors present a compelling case study for HIV/AIDS in South Africa, where an emerging disease has gone unchecked and is having a devastating effect on a developed country. The case study of the U.S. public health response system is interesting and thoughtfully presented but lacks sufficient and carefully documented detail to aid the reader in drawing conclusions and formulating solutions. Unsubstantiated or incorrect examples also detract from the overall presentation of this case study. For instance, the contention that lack of good communications with area physicians and hospitals resulted in the deaths of postal workers in the fall 2001 anthrax crisis is not supported by the author's reference or by any other authoritative materials known to this reviewer.

In the public health response case study, the authors provide broad recommendations aimed at strengthening the public health infrastructure. Also included is an excellent summary of the current status of efforts begun in the mid-1990s in the United States to address the infrastructure of public health. The recommendations are presented in such a way that the shortcomings of the system can be addressed in critical areas, including a well-trained public health workforce; interagency coordination; private sector, hospital, and emergency response integration in public health; technical and educational interventions; and domestic and global investment in public health.

Brower and Chalk's book is a powerful and useful argument for the urgent need to integrate and streamline public health and national security strategies.

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Conference Summary

World Health Organization Global Conference on Severe Acute Respiratory Syndrome

On June 17–18, 2003, in Kuala Lumpur, Malaysia, the World Health Organization (WHO) sponsored a conference entitled SARS: Where Do We Go From Here? The purpose of the conference, which was attended by over 900 scientific and public health experts from 43 countries, was to review available knowledge and lessons learned and to identify key priorities for the future. Three overarching questions were addressed: Can severe acute respiratory syndrome (SARS) be eradicated? Are current control measures effective? Are current alert and response systems robust enough?

The first day included summaries of the history of the epidemic, global, and regional responses coordinated by WHO through its headquarters in Geneva and the Western Pacific Regional Office in Manila, respectively; and national responses in the People's Republic of China (PRC), including in the Hong Kong Special Administrative Region of PRC, Singapore, Vietnam, Canada, and United States. Nine presentations summarized scientific, clinical, public health, psychosocial, and communications aspects of the SARS outbreak. On the second day, breakout groups met and presented recommendations on the topics of epidemiology and public health, possible role of animals, environmental issues, modeling the epidemic, clinical diagnosis and management, reducing transmission in healthcare settings, blood safety, reducing community transmission, preventing international spread, surveillance and response coordination, effective communication, and preparedness. Background materials for the conference, slide presentations at the plenary sessions (including the breakout group reports), and the text of speeches by the Director General of WHO and other dignitaries are available on the Web (URL: www.who.int/csr/sars/conference).

Beginning in March 2003, after WHO recognized, through its Global Outbreak Alert and Response Network (GOARN), an outbreak of severe respiratory illness with high transmissibility in healthcare settings and international spread through airline travel, WHO issued a series of global alerts, travel advisories, and recommendations for diagnosis, clinical management, and prevention of transmission. Evolving information was discussed by virtual networks of experts, including virologists, clinicians, and epidemiologists. Field teams composed of staff from GOARN partners were quickly mobilized to assist affected countries in enhancing surveillance and containment measures, which included isolating cases, implementing strict infection control measures, identifying and following-up with contacts, and making recommendations to travelers to prevent international spread.

From a global perspective, the SARS epidemic demonstrated the importance of a worldwide surveillance and response capacity to address emerging microbial threats through timely reporting, rapid communication, and evidence-based action. The importance of international collaboration coordinated by WHO and the need for partnerships among clinical, laboratory, public health, and veterinary communities were emphasized. From the national perspective, lessons learned included the need for the following: strong political leadership at the highest levels to mobilize the entire society; speed of action; improved coordination between national and district levels in countries with federal systems; increased investment in public health; updated legislation regarding surveillance, isolation, and quarantine measures; and improved infection control in healthcare and long-term-care facilities and at borders.

Can SARS Be Eradicated?

The breakout groups concluded that it is too soon to tell if SARS can be eradicated, but substantial reasons for concern exist. Chains of person-to-person transmission can likely be terminated, provided no reservoir of asymptomatic carriers, chronic infection, or seeding of new settings (e.g., Africa) exists. But if an animal reservoir of the SARS coronavirus exists, as suggested by some studies, eradication would be very difficult. Fecal shedding of virus by infected persons and apparent virus stability in the environment could pose additional barriers to eradication, although these circumstances were not major modes of transmission in the recent epidemic. Research priorities include better understanding of the epidemiologic and virologic parameters of infection and transmission, including "superspreading events"; the possible role of animals, including host range and factors leading to emergence; the environment; and analysis of the effectiveness of specific interventions in controlling the epidemic. Additional priorities include standardization of diagnostic assays and reagents, development of a reliable front-line diagnostic test for use early in illness; facilitating the ability to ship diagnostic specimens; and development of animal models to improve understanding of pathogenesis and evolution of clinical disease and to use in vaccine development and antiviral drug testing.

Are Current Control Measures Effective?

Currently recommended measures to prevent transmission in healthcare settings are generally effective when applied, but require proper infrastruc-

ture, training, and consistent practice. Infection control capacity and practice in many healthcare settings need improvement. A minimum global level of safe practice (standard precautions, supplemented by risk-based precautions) should be established. Studies are needed to determine optimal protective measures (e.g., type of mask) and when they should be used. Appropriate protective measures (e.g., isolation facilities and masks fit-tested for individual workers) should be more widely available.

Measures to control community transmission (i.e., outside of healthcare settings) and prevent international spread require further evaluation. Such measures include public information campaigns, contact tracing and sometimes quarantine, hotlines to report fever, temperature screening in public places, recommendations to travelers, and entry and exit screening at borders with questionnaires and temperature checks. Control measures in the community would likely have the greatest yield if focused on links between healthcare settings and the wider community, with contact tracing prioritized according to the nature of exposure, but further evaluation is needed. Home or institutional quarantines, when used, should ensure financial and psychosocial support and daily needs of the affected persons. Stigmatization of affected persons and groups was identified as an important issue. In an attempt to reduce stigmatization, one country's president reportedly proclaimed quarantined persons to be "heroes in the nation's battle against SARS." Some participants stated that visible measures to control community and international spread were important in restoring public and business confidence and as deterrents, regardless of the yield of SARS cases detected.

Are Current Alert and Response Systems Robust Enough?

Current systems are robust in that SARS is being controlled, but many processes are not sustainable because of limited capacity. Surveillance priorities include developing a sensitive "alert" case definition in areas at greatest risk for recurrence, developing a front-line laboratory diagnostic test to identify patients with SARS coronavirus infection during periods of high incidence of other respiratory illnesses, improving laboratory diagnostic capacity and laboratory-based surveillance, and developing integrated information tools that allow real time analysis of clinical, epidemiologic, and laboratory data.

Response coordination priorities include development of contingency plans, including ensuring coordination and surge capacity at global, regional, and national levels; development of laboratory and information technology systems; and the ongoing revision of the international health regulations to focus on containing emerging infectious diseases.

Underlying any response is the need to communicate information in a transparent, accurate, and timely manner. Effective communication requires training, understanding, and use of a range of different media. Developing further the current communication systems and our understanding of risk communication is vital if future outbreaks are to be controlled quickly and effectively and the health, economic, and psychosocial effects of major health events are to be minimized.

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Upcoming Infectious Disease Conferences

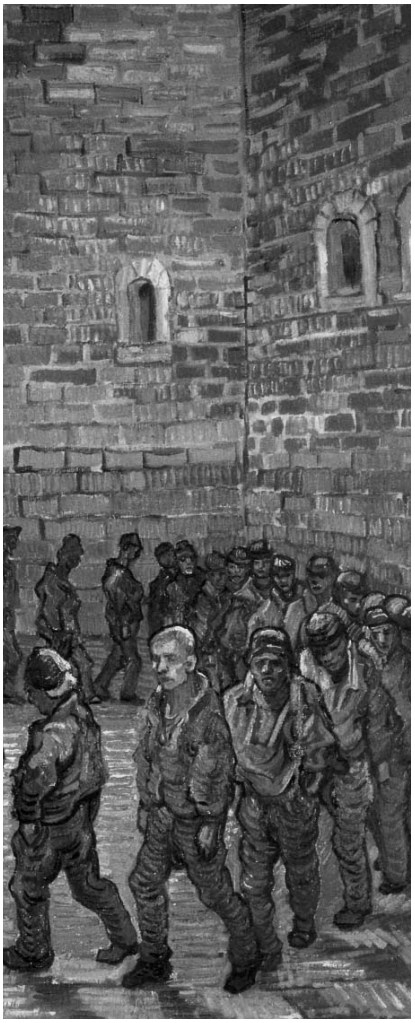
September 20–24, 2003
American Society for Microbiology (ASM) Conference on Salmonella: Pathogenesis, Epidemiology, and Vaccine Development
Porto Conte Research Center (Alghero)-Sardinia, Italy
Web site: http://www.asmusa.org/mtgsrc/salmonella.htm

October 7–8, 2003
Intensive Update Course in Clinical Tropical Medicine and Travelers' Health
American Society of Tropical Medicine and Hygiene
San Diego, CA
Contact: ASTMH
Phone: 847-480-9592
email: astmh@astmh.org
Web site: http://www.astmh.org

October 9–12, 2003
Infectious Diseases Society of America (IDSA)
San Diego, CA
Contact: 703-299-0200
email: info@idsociety.org
Web site: www.idsociety.org

October 26–29, 2003
Symposium on Bluetongue
Office International des Epizooties
Taormina, Italy
Contact: 33 (0)1 44 15 18 88
email: oie@oie.int
Web site: www.oie.int

November 9–13, 2003
6th OIE Seminar on Biotechnology and 11th International Symposium of the World Association of Veterinary Laboratory Diagnosticians
Office International des Epizooties
Bangkok, Thailand
Contact: 33 (0)1 44 15 18 88
email: oie@oie.int
Web site: www.oie.int



Vincent van Gogh (1853–1890). The Prison Courtyard (1890)
Oil on canvas, 80 cm x 64 cm. The State Pushkin Museum of Fine Arts, Moscow, Russia

Van Gogh painted The Prison Courtyard while "imprisoned" himself, in the Saint-Paul-de-Mausole asylum in Saint Rémy. He died 5 months later of a self-inflicted gunshot wound, the culmination of his long struggle with physical and mental illness (1).

The first Dutch master since the 17th century, van Gogh did not become an artist until 10 years before the end of his life. His early interests were literature and theology. The son of a protestant minister, he had a strong sense of mission, which was reinforced by his dislike of industrial society and his work as lay preacher among poor coal miners in Belgium. That experience with human misery influenced his art, which had an air of mysticism and austerity and, like that of his contemporary Honoré Daumier, often featured the oppressed and the downtrodden (e.g., The Potato Eaters, 1885) (2).

Van Gogh was influenced by Degas, Seurat, and other leading French artists. His use of pure color to define content places him ahead of his contemporaries as a forerunner of expressionism. "I will paint with red and with green the terrible passions of humanity," he wrote. "…Instead of trying to reproduce exactly what I see before my eyes, I use color more arbitrarily to express myself forcibly." As if he knew that his artistic career would be very brief, van Gogh painted with a sense of urgency, almost scarring the canvas with thick overlaid brush strokes that were distinct, deliberate, and intense. He wanted "to exaggerate the essential and to leave the obvious vague" (3,2). His paintings became vehicles of his emotional condition, shifting like his moods from the brightest landscapes (Sunflowers, Irises) to the darkest manifestations of personal symbolism (Starry Night, Wheat Field with Crows).

Van Gogh created his best work between 1888 and 1890, when he went to Arles in the south of France (3). There, in the Mediterranean countryside, he painted landscapes of almost pulsating light, as he tried to convey the spiritual meaning he believed animated all things. Exaggerating his figures with vibrant hues, he set them against thick, rough circles like halos, freeing them from static background and hurling them into infinity. Wishing to form an artists' colony in the region, he invited Paul Gauguin to join him "in this kingdom of light." The brief collaboration (less than 2 months) produced many noted works but ended abruptly when van Gogh became violent, attacked his friend with a straight razor, then in remorse cut off his own ear and offered it to a local prostitute.

Gauguin departed for Tahiti, while van Gogh, his health in a downward spiral, entered the asylum in Saint Rémy, where with the approval of his doctors he continued to paint. No one knows the cause of van Gogh's angst. His seizures, hallucinations, violent mood swings, and increasing anxiety have been variously diagnosed as neurologic disorder, depression, alcoholism, venereal disease, chemical or metabolic imbalance, and behavioral disorder possibly caused by a virus (4). While illness largely defined what the artist could and could not do (he only painted when he was lucid), art gave him reason to continue living. Even in confinement, his work extended far beyond his personal circumstance.

The Prison Courtyard on this month's cover of Emerging Infectious Diseases expresses the artist's hopelessness and despair. In the lower part of the painting, thirty-three inmates form a human corona, pacing heads down, in defeated rote and joyless resignation. In spite of the shared misery and monochrome prison garb, they are not uniformly anonymous; some faces can be

Walls (1896)

Without consideration, without pity,
without shame they have built big and
high walls around me.

And now I sit here despairing. I think
of nothing else: this fate gnaws at my
mind;

for I had many things to do outside.
Ah why didn't I observe them when
they were building the walls?

But I never heard the noise or the
sound of the builders. Imperceptibly
they shut me out of the world.

deciphered, particularly the one in the center, whose blond hair is lighted by an imperceptible sun's ray. That is van Gogh himself in what has been interpreted as a "metaphoric self-portrait" (1,5).

Merged with the pavement, the prison walls loom high above the inmates' heads, overpowering the canvas with finality and forcefulness. The harsh, impenetrable structure, so devoid of beauty, encages the inmates outside the common web of human interaction. These literal walls painted by van Gogh "in captivity" allude to the harsher metaphorical walls of his unknown illness and his spiritual isolation.

The causes of aberrant behavior that leads to imprisonment are largely unknown, as are the causes of many diseases and their consequent spiritual isolation. When an old microbe, a coronavirus, causes a new disease, severe acute respiratory syndrome, the unknown nature of the disease and the risk for contagion require containment to arrest spread of infection. To prevent and control physical illness, the exposed and the infected (in the case of SARS many healthcare workers) may also have to face spiritual isolation.

Polyxeni Potter

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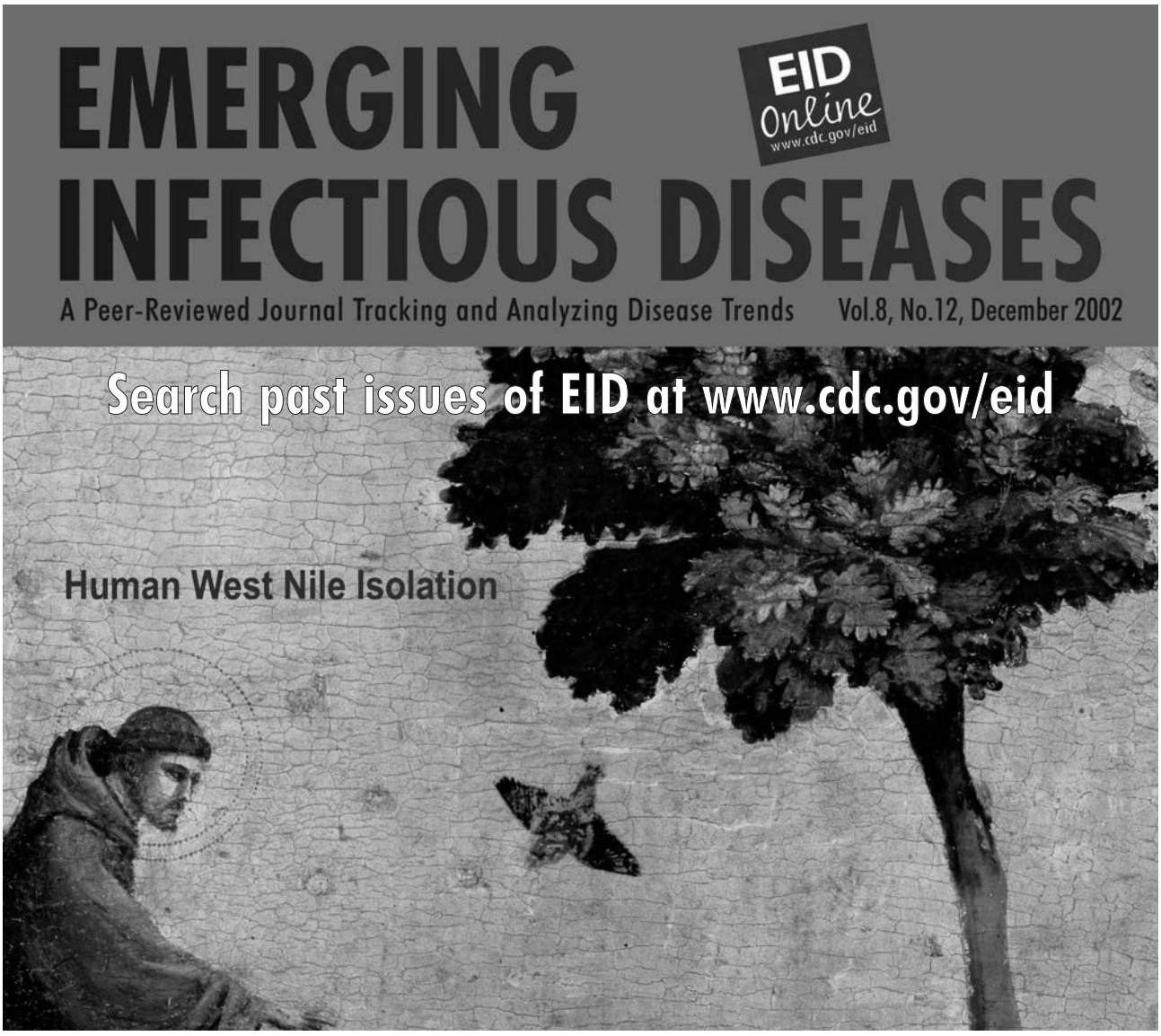
5. Zemel C. Van Gogh's progress: utopia, modernity, and late-nineteenth-century art. Berkley: University of California Press; 1997.



EMERGING INFECTIOUS DISEASES

A Peer-Reviewed Journal Tracking and Analyzing Disease Trends Vol.9, No.10, October, 2003

Upcoming Issue

For a complete list of articles included in the October issue, and for articles published online ahead of print publication, see http://www.cdc.gov/ncidod/eid/upcoming.htm

Look in the October issue for the following topics:

Syndromic Surveillance and Bioterrorism-related Epidemics

Superantigens and Streptococcal Toxic Shock Syndrome

Hazards of Healthy Living: Bottled Water and Salad Vegetables as Risk Factors for Campylobacter Infection

Multijurisdictional Approach to Biosurveillance, Kansas City

Toxoplasma gondii Infection in the United States, 1999-2000

Ebola in Northern Uganda

Mass Antibiotic Treatment for Group A Streptococcus in Two Long-term Care Facilities

Environmental Changes and Meningitis Epidemics in Africa

West Nile Virus Transmission in Resident Birds, Dominican Republic

Mycobacterium tuberculosis Beijing Genotype, the Netherlands

Small Colony Variants of *Staphylococcus aureus* and Pacemaker-related Infection

Severe Histoplasmosis in Travelers to Nicaragua

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

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A Peer-Reviewed Journal Tracking and Analyzing Disease Trends

Vol.9, No.8, August 2003

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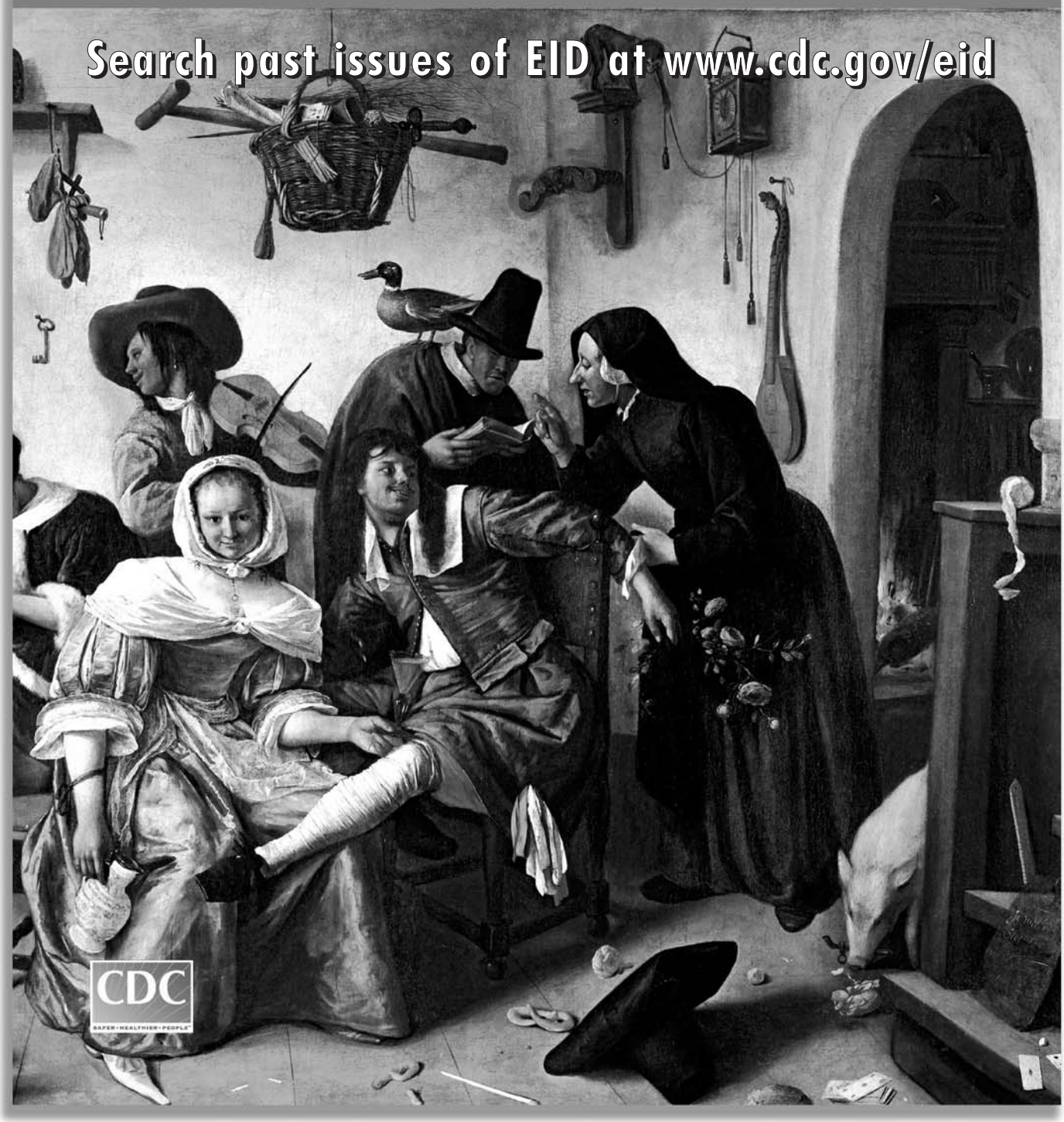

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Editorial Policy and Call for Articles

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 has an international scope and 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, demography, sociology, and other disciplines. Inquiries about the suitability of proposed articles may be directed to the Editor at 404-371-5329 (tel), 404-371-5449 (fax), or eideditor@cdc.gov (e-mail).

Emerging Infectious Diseases is published in English and features the following types of articles: Perspectives, Synopses, Research Studies, Policy and Historical Reviews, Dispatches, Commentaries, Another Dimension, Letters, Book Reviews, and News and Notes. The purpose and requirements of each type of article are described in detail below. To expedite publication of information, we post journal articles on the Internet as soon as they are cleared and edited.

Chinese, French, and Spanish translations of some articles can be accessed through the journal's home page at http://www.cdc.gov/eid.

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 e-mail 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. Printed manuscript should be single-sided, beginning with the title page. 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. To submit a manuscript, access Manuscript Central from the Emerging Infectious Diseases website (www.cdc.gov/eid).

Manuscript Types

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) 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) 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) and a brief biographical sketch of first author—both authors if only two. Report laboratory and epidemiologic results within a public health perspective. Although these reports may be written in the style of traditional research articles, they should 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) and a brief biographical sketch. Articles in this section include public health policy or historical reports that are based on research and analysis of emerging disease issues.

Dispatches. Articles should be 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 should not include 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. This section includes letters that present preliminary data or comment on published articles. Letters (500–1,000 words) should not be divided into sections, nor should they contain figures or tables. References (not more than 10) may be included.

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. (500–1,000 words) of emerging infectious disease conferences may provide references to a full report of conference activities and should focus on the meeting's content rather than on individual conference participants.